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TECHNICAL SERIES, No. 14.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ENTOMOLOGY.

L. O. HOWARD, Entomologist and Chief of Bureau.

THE BACTERIA OF THE APIARY,

WITH SPECIAL REFERENCE TO BEE DISEASES.

BY

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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ENTOMOLOGY,
Washington, D. C., September 24, 1906.

SIR: I have the honor to transmit the manuscript of a paper on the bacteria of the apiary, with special reference to bee diseases, by Dr. G. F. White, expert in animal bacteriology in the Biochemic Division of the Bureau of Animal Industry. This paper was prepared by Doctor White as a thesis in part fulfilment of the requirements for the degree of doctor of philosophy, at Cornell University, in June, 1905. The Bureau of Entomology considers itself fortunate in obtaining it for publication, since in this way a wider distribution can be made than would be possible were it published in a journal devoted exclusively to bacteriological investigations. It is hoped that the publication of these facts may help to clear up the confusion which now exists concerning the causes of the two most common diseases of the brood of bees. I recommend that the manuscript be published as Technical Series, No. 14, of this Bureau.

Doctor White wishes to acknowledge his indebtedness to Dr. Veranus A. Moore, professor of comparative pathology and bacteriology of Cornell University, under whose direction this work was done; to Dr. E. F. Phillips, acting in charge of apiculture, Bureau of Entomology, United States Department of Agriculture, for encouragement and assistance in the preparation of this manuscript; and to Messrs. Mortimer Stevens, Charles Stewart, N. D. West, and W. D. Wright, bee inspectors of the State of New York, for their interest in the work.

Respectfully,

L. O. HOWARD,
Entomologist and Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

PREFACE.

The spread of diseases of the brood of bees is to-day a great menace to the bee-keeping industry of the United States. It is therefore of great importance that all phases of these diseases should be investigated as thoroly as possible, and this paper, it is believed, will help in clearing up some disputed points in regard to the cause of the two most serious brood diseases.

Dr. G. F. White has offered this paper for publication as a bulletin in the Bureau of Entomology because in that way the statements herein contained may become more widely known than would be the case were it published in some journal devoted exclusively to bacteriological investigations. Obviously there are many points still unsettled, and it is hoped that some of these may be taken up for investigation in the near future, but the results so far obtained should by all means be made known to the persons practically engaged in bee keeping.

The necessity for the study of nonpathogenic bacteria found in the apiary may not be at first evident to the ordinary reader. When it is seen, however, that some of the investigators of bee diseases have apparently mistaken *Bacillus A* or some closely allied species for *Bacillus alvei* it will be evident that a study of nonpathogenic germs is necessary to a thoro investigation of the cause of these diseases and a full understanding of the confusion which has existed.

The names which should be used for the diseased conditions of brood was a matter which arose after this paper was offered for publication. It was desired that out of the chaos of names in use certain ones be chosen which would be distinctive and still clear to the bee keepers who are interested in work of this nature. Unfortunately, after a short investigation, Dr. W. R. Howard, of Fort Worth, Tex., gave the name "New York bee disease," or "black brood," to a disease which Cheshire and Cheyne described in 1885 as "foul brood." Since this is the disease in which *Bacillus alvei* is present, we can not drop the name "foul brood," and the word "European" is used to distinguish it from the other disease. The bee keepers of the United States have been taught that the type of brood disease characterized by ropiness of the dead brood is true foul brood,

but since *Bacillus alvei* is not found in this disease it obviously is not the same disease as that described by Cheyne. It would be well-nigh impossible, however, to change the name of this disease, and any effort in that direction would merely result in complicating laws now in force which control the infectious diseases of bees and would serve no good purpose. This disease is here designated "American foul brood." These names have been chosen only after consultation with some of the leading bee keepers of the United States, and these distinguishing terms were chosen by the majority of those consulted as indicating the place in which the diseases were first investigated in a thoroly scientific manner. Both diseases are found in Europe, as well as in America, so that the names indicate nothing concerning the geographical distribution of the maladies.

Strangely enough, certain writers for our American apicultural papers have seen fit to take exception to some of the statements made in this paper without having first found out the reasons for the decisions herein published. Apiculture will not be advanced to any appreciable extent by such eagerness to rush into print, especially when there is not a semblance of scientific investigation back of the criticism.

E. F. Phillips,
Acting in Charge of Apiculture.

CONTENTS.

THE BACTERIA OF THE APIARY WITH SPECIAL REFERENCE TO BEE DISEASES.

INTRODUCTION.

Since bacteriology is one of the youngest of the sciences, it is only natural that there should be many problems concerning which there is much confusion, and many others concerning which nothing is known. In a study of the saprophytic bacteria this is especially true; the exploration of this jungle of micro-organisms is scarcely begun. Comparatively few species have been studied and named, and a much less number can be identified. From studies that have been made one is led to believe that the species which might be classed under bacteria outnumber by far all the macroscopic plants known. Comparatively little is as yet known concerning the distribution of these minute organisms in nature, their needs for multiplication and growth, their power of endurance, their relations the one to the other, their relations to man and industries, and their relation to pathogenic species. Both from the standpoint of scientific interest and from the standpoint of practical economy these problems call for further investigation.

By far the greatest amount of work which has been done in the science of bacteriology has been prompted by the direct or indirect economic importance of the question. This is largely true of the present investigation, since honey bees suffer from a number of diseases, some of which are considered in Part II.

TECHNIQUE.

Obtaining Material for Study.

If necessary, bees may be conveniently shipped alive by mail in cages constructed for that purpose. Combs also may be sent by mail in small boxes. If combs, honey, pollen, or larvæ are desired, the hive must be entered. In case older adult bees are wanted it is not difficult to supply the needs from the entrance to the hive. To capture them one may stand at the entrance and catch the unwary toiler as she

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comes in loaded with pollen and honey. After the victim alights on the entrance board, by the aid of a pair of forceps, before she disappears within, one can easily lodge her safely in a petri dish. It is, however, an advantage to study the young adult bees as well as the older ones, and if young ones are desired they may be taken from the combs or from the front of the hive, near the entrance.

Obtaining Cultures.

(*a*) *From combs.*—With sterile forceps small pieces of the comb are put directly into gelatin or agar for plates or incubated in bouillon for 24 hours and then plated. Growing in bouillon and plating on gelatin is usually preferable.

(*b*) *From pollen.*—The same technique is used as for combs, but the direct inoculation of gelatin tubes for plates is generally preferable.

(*c*) *From honey.*—With sterile loops honey is taken from uncapped and capped cells. The caps are removed with sterile forceps and the honey is plated directly on gelatin or agar. Bouillon tubes are inoculated also with varying quantities of the honey.

(*d*) *From larvæ.*—The larva is carefully removed to a sterile dish, and with sterile scissors the body is opened and the contents plated directly, or bouillon cultures are first made and later plated, if a growth appears.

(*e*) *From parts of the adult bee.*—In studying the adult bee, a small piece of blotting paper wet with chloroform is slipt under the cover of the petri dish in which the insects have been placed, and in a short time the bees are under the influence of the anesthetic. Then with sterile scissors a leg, a wing, the head, the thorax, or the abdomen, the intestine being removed, is placed in bouillon and, after 24 hours incubation, plated, preferably on gelatin.

When it is desired to make a study of the bacteria of the intestine, the intestinal tract is removed and studied as follows: The bee is flamed and held in sterile forceps. With another sterile pair of forceps the tip of the abdomen is seized and, by pulling gently, the tip and the entire intestine are easily removed. This can then be plated directly. If gelatin, which is preferable, is used, the intestine itself must not be left in the gelatin or the medium will become liquefied by the presence of the tissue. If one desires to obtain cultures of the anaërobe, which is quite common in the intestine, it is most easily obtained in pure culture by the use of the deep glucose agar (Liborius's method). Cover glass preparations made direct from the walls of the intestine or its contents give one some idea of the great number of bacteria frequently present.

Differentiation and Identification of Bacteria.

These very low forms of plant life show a marked susceptibility to environmental conditions and those desirous of speculating on problems in evolution may find here food for thought and experimentation. On account of this susceptibility, various cultures which belong to the same species may possess slight variations in some one or more specific characters. Consequently one can not say that a species must possess certain definite characters and no others. It is convenient, then, to think of a species as more or less of a group of individuals whose characters approximate each other very closely.

In this paper are described a number of species each of which, in fact, represents a group, the individual cultures of which approximate each other so closely in character that the differences may be easily attributed to environmental conditions which are more or less recent.

Concerning the identification of species, the conditions have been well summed up by Chester. He says:

> Probably nine-tenths of the forms of bacteria already described might as well be forgotten or be given a respectful burial. This will then leave comparatively few well-defined species to form the nuclei of groups in one or another of which we shall be able to place all new sufficiently described forms.

The variations which occur and the very incomplete descriptions which can be found make it impossible to identify many species even to a more or less restricted group. For these reasons some of the cultures are not identified or named, but letters are used for convenience in this paper to represent the specific part. Migula's classification has been used.

The Cultures Which are Described.

Plate cultures were observed for some weeks, the different kinds of colonies which appeared being especially noted. Subcultures were then made in bouillon, and after 24 hours the subculture was replated. Subculturing and replating were then repeated. From this last plate the pure culture was made on agar for study. These were not studied culturally, as a rule, for some weeks, thus allowing time for the organism to eliminate any character due to recent environmental conditions (1).[a]

Morphology, Staining Properties, and Oxygen Requirements, with Suggestions on Variations.

(*a*) *Size.*—The length and thickness of a micro-organism often varies so much with its environmental conditions that certain re-

[a] Numbers in parentheses refer to papers in the bibliography at the end of Part I or that at the end of Part II.

corded dimensions should always be accompanied by facts concerning the medium, age, and temperature of incubation. The measurements recorded in this paper were all taken of organisms in preparations made from a 24-hour agar culture stained with carbol-fuchsin. The involution forms are not reckoned in the results.

(*b*) *Spores.*—The presence of spores was determined in each case by staining the various cultures at different ages. A check was made on their presence by means of the thermal death point.

(*c*) *Flagella.*—Loeffler's method, as modified by Johnson and Mack, was used for staining the flagella (2).

(*d*) *Motility.*—Motility may be present in cultures when first isolated, but after artificial cultivation appear to be entirely lost. The reverse of this also may be noted. No cultures should be recorded as nonmotile until cultures on various media at different temperatures and of different ages shall have been studied. Hanging-drop preparations were made from cultures on agar and bouillon, both incubated and not incubated, and on gelatin.

(*e*) *Staining properties.*—Basic carbol-fuchsin was the stain used almost exclusively. In the use of Gram's staining method, carbolic gentian violet (5 per cent carbolic acid 20 parts, saturated alcoholic solution gential violet 2 parts) was applied to a cover-glass preparation from a 24-hour culture on agar for 5 minutes, placed in Lugol's solution 2 minutes, and placed, without rinsing, in 95 per cent alcohol for 15 minutes, removed, washt in water, and allowed to dry.

(*f*) *Oxygen requirements.*—Determinations were made by observing whether a growth took place in the closed or open arm or both, of the fermentation tube containing glucose bouillon.

Media Employed and Suggestions as to the Description of Cultures.

(*a*) *Bouillon.*—All bouillon used was made from beef (meat 1 part, water 2 parts), to which infusion 1 per cent Witte's peptonum siccum and one-half per cent sodium chlorid were added. The reaction of the solution was then determined by titrating, and made +1.5 to phenolphthalein.

In describing a culture growing in bouillon as a medium, there is usually a more extended description given than in the case of sugar and sugar-free bouillons, since cultures in these media do not differ materially in gross appearance from those observed in the plain bouillon.

(*b*) *Sugar-free bouillon.*—This bouillon is made free from sugar by the use of *B. coli communis*, after which peptone and sodium chlorid (NaCl) were added as in bouillon.

(*c*) *Sugar bouillons.*—Five different sugars—glucose, lactose, saccharose, levulose, and maltose, as well as mannite—were used in the study. If a 1-per-cent solution of glucose in plain bouillon was fer-

mented with the production of gas, fermentation tubes were used for all the sugars and mannite. If no gas was formed in the glucose, the straight tubes were inoculated. The sugars and mannite were used in a 1-per-cent solution in sugar-free bouillon.

(*d*) *Reaction of media.*—The reaction of cultures is determined as it appears on the fifth day in the different media, unless otherwise stated. The medium in the open arm is used to determine the reaction in the fermentation tube. Beginning with a reaction of +1.5 to phenolphthalein, or slightly alkaline to litmus, the detection of an increase in acidity is not difficult. But inasmuch as the production of an alkali is very frequently small in degree, cultures are often in this paper recorded alkaline in reaction when probably the reaction has not changed.

(*e*) *Fermentation with the production of gas.*—Gas may be formed in such small quantities as not to be observed as such, but to be entirely absorbed by the medium. Whenever gas formation is mentioned as a character, visible gas is meant. The analysis of the gas was made in the usual manner by absorbing a portion with potassium hydrate (KOH) and testing the remainder with the flame. The amount absorbed by potassium hydrate (KOH) is referred to as carbon dioxid (CO_2) and the remainder, if an explosion is obtained, as hydrogen (H). This is, naturally, only approximately correct. Since the gas formula may vary from day to day, too much value must not be given to the exact proportion. It is well to observe whether the proportion of hydrogen to carbon dioxid is greater or less than 1.

(*f*) *Agar.*—One per cent agar is used. The description of the growth on this medium is made from the appearance as seen on the surface of an agar slant. The description is usually very brief, since it has, as a rule, little differential value.

(*g*) *Acid agar.*—This medium is made acid by titrating to +3 to phenolphthalein. The absence or presence, as well as the degree of growth, is noted.

(*h*) *Serum.*—The serum used is taken from the horse, sterilized at 55° C. and congealed at 80° C. Deep inoculations are made, and the surface of slanted serum is also inoculated. The degree of growth is usually noted. Cultures are observed for 6 weeks to 2 months. The presence or absence of liquefaction is the chief character sought for. Since room temperature varies so greatly, the time at which liquefaction begins varies, and little differential value, therefore, can be given to the exact time of this phenomenon.

(*i*) *Potato.*—The composition of potato varies so markedly that a description of a culture on this medium may differ materially from that which is observed on another tube of the same medium. It is the aim to omit for the most part the observed variations due to the composition of the different potatoes.

(*j*) *Potato water.*—To potatoes sliced very thin is added an equal amount of water by weight and the mixture is then boiled. This is strained and distributed in straight and fermentation tubes. The reaction of the solution was made +1.5 to phenolphthalein. If any of the micro-organisms ferment glucose with the production of gas, fermentation tubes are inoculated to test the fermentation of starch; if not, straight tubes are inoculated.

(*k*) *Milk.*—If a micro-organism breaks up glucose with the formation of gas, a fermentation tube of milk is inoculated with the culture; if not, straight tubes are used. Separator milk is used. The coagulation of the casein with or without liquefaction is the chief character noted. Very little stress is laid upon the time element in the coagulation of the casein and the other phenomena which are to be observed in milk. Different samples of milk and the different environmental conditions are factors which vary the length of time at which the different phenomena appear.

(*l*) *Litmus milk.*—The reaction as shown by the litmus and the discharging of the color are the chief points observed.

(*m*) *Gelatin.*—The color, degree of growth, the presence or absence of liquefaction, and the form of liquefaction are the chief points observed. The cultures are kept under observation 2 months or longer and, as in serum, the time given at which liquefaction takes place is only approximate.

(*n*) *Indol.*—The cultures are allowed to grow in sugar-free peptonized bouillon for 3 to 5 days, and are tested with potassium nitrite (KNO_2) and sulfuric acid (H_2SO_4) after the ring method. Too much stress may be placed upon the ability of an organism to form indol. This character has been shown to be a somewhat transient one (3).

(*o*) *Reduction of nitrates to nitrites.*—Cultures are cultivated 7 days in a solution of 1 gram of Witte's peptonum siccum and one-fifth gram of sodium nitrate in 1,000 c. c. of tap water. To such a culture and to a control tube are added a mixture of naphthylamine and sulfanilic acid (napthylamine, 1 part; distilled water, 1,000 parts: sulfanilic acid, one-half gram, dissolved in dilute acetic acid in the proportion of 1 part of acid to 16 parts of water). If nitrate is reduced to nitrite, a pink color develops. The control tube should remain clear, or slightly pink—owing to the absorption of a trace of nitrite from the atmosphere.

PART I. BACTERIA OF THE NORMAL APIARY.

Before studying the cause of a disease it is necessary that we know what bacteria are normally present, so that later, in studying diseased conditions, a consideration of these nonpathogenic species may be eliminated. In view of this necessity a bacteriological study

of the hives, combs, honey, pollen, larvæ, and adult bees was begun, to determine the bacteria normally present. It was not hoped that all the species isolated could be easily identified, or that all would merit a careful description, but it was hoped that those species which seemed to be localized in any part of the apiary, or upon or within the bees, might be studied and described with sufficient care to guarantee their identification upon being isolated again. The chance of variation in morphology, pathogenesis, and cultural characters due to environmental conditions to which these micro-organisms were being subjected at the time, or to which they had been subjected before isolation or study, has been carefully borne in mind.

BACTERIA FROM THE COMBS.

One might naturally suppose that very many species of bacteria would be present on combs, since these are exposed more or less to the contaminating influence of the air. The reverse, however, seems to be true. The number of different species isolated is comparatively small. Those which appear most often are described below. Some other species mentioned in this paper are found on combs, but inasmuch as they appear most frequently from other sources they are described there. One species of Saccharomyces from the comb, also, is described under the heading " Saccharomyces and fungi."

Bacillus A.

(*B. mesentericus?*)

Occurrence.—Found very frequently on combs, on scrapings from hives, and on the bodies of bees, both diseased and healthy.

Gelatin colonies.—Very young colonies show irregular edges, but very soon liquefaction takes place and the colony gives rise to a circular liquefied area, covered with a gray membrane, which later turns brown.

Agar colonies.—Superficial colonies present a very irregular margin consisting of outgrowths taking place in curves. Deep colonies show a filamentous growth having a moss-like appearance.

Morphology.—In the living condition the bacilli appear clear and often granular, arranged singly, in pairs, and in chains. The flagella are distributed over the body. The rods measure from 3μ to 4μ in length, and from 0.9μ to 1.2μ in thickness.

Motility.—The bacilli are only moderately motile.

Spores.—Spores are formed in the middle of the rod.

Gram's stain.—The bacilli take Gram's stain.

Oxygen requirements.—Aërobic and facultatively anaërobic.

Bouillon.—Luxuriant growth in 24 hours, with cloudiness of medium; a gray flocculent membrane is present. Later, the membrane sinks and the medium clears, leaving a heavy, white, flocculent sediment, with a growth of the organisms adhering to the glass at the surface of the medium. Reaction alkaline.

Glucose.—Luxuriant growth takes place in the bulb, with a moderate, flocculent growth in closed arm. The gradual settling of the organisms causes a

heavy white sediment to form in the bend of the tube. The reaction is at first slightly acid, but subsequently becomes alkaline. No gas is formed.

Lactose.—Reaction alkaline.

Saccharose.—Reaction alkaline.

Levulose.—Reaction acid.

Maltose.—Reaction acid.

Mannite.—Reaction alkaline.

Potato water.—Reaction alkaline.

Agar slant.—A luxuriant growth takes place on this medium. The growth gradually increases to a moist, glistening one, being then friable and of a grayish brown color.

Serum.—A luxuriant, brownish, glistening, friable growth spreads over the entire surface. No liquefaction is observed.

Potato.—An abundant fleshy growth of a brown color spreads over the entire surface. The water supports a heavy growth. The potato is slightly discolored.

Milk.—Precipitation takes place rapidly, followed by a gradual digestion of the casein, the medium changing from the top downward to a translucent liquid, becoming at last semi-transparent and viscid.

Litmus milk.—Precipitation of the casein takes place usually within 24 hours, followed by a gradual peptonization. Reduction of the litmus occurs rapidly, leaving the medium slightly brown; later the blue color will return on exposing the milk to the air by shaking. Reaction alkaline.

Gelatin.—An abundant growth takes place with rapid, infundibuliform liquefaction. A heavy, white, friable membrane is formed on the surface of the liquefied medium. A flocculent sediment lies at the bottom of the clear liquefied portion.

Acid agar.—Growth takes place.

Indol.—None has been observed.

Nitrate.—Reduction to nitrite is positive.

Bacterium acidiformans. (Sternberg, 1892.)

Occurrence.—Isolated from the scraping of propolis and wax from the hives and frames of healthy colonies.

Gelatin colonies.—The superficial colonies are friable, convex, opaque, and white with even border; when magnified they are finely granular, sometimes radiately marked. They are from 1 to 4 millimeters in diameter. The deep colonies are spherical or oblong and entire.

Morphology.—When taken from an agar slant 24 hours old, the rods are short, with rounded ends, singly and in pairs. Length about 1.6μ, thickness 0.8μ. They stain uniformly with carbol-fuchsin. Flagella are apparently absent.

Motility.—No motility has been observed in any medium.

Spores.—Spores are apparently absent.

Gram's stain.—The bacteria are decolorized by Gram's method.

Oxygen requirements.—Facultatively anaërobic.

Bouillon.—The medium becomes slightly clouded with a feeble ring of growth on the glass at the surface of the liquid. A moderate amount of white friable sediment is formed. Reaction alkaline.

Glucose.—Uniformly and slightly clouded. No gas is formed. Reaction acid.

Lactose.—Reaction acid.

Saccharose.—Reaction alkaline.

Levulose.—Reaction acid.

Maltose.—Reaction acid.

Mannite.—Reaction acid.

Potato water.—Reaction acid.

Agar slant.—A moderate, gray, glistening growth, confined to the area inoculated with the loop, is formed on the inclined surface.

Serum.—A feeble gray growth is formed only on the inoculated surface. No liquefaction takes place.

Potato.—A gray growth covers the inoculated surface.

Milk.—Heat causes a ready coagulation of the casein. Reaction acid.

Litmus milk.—Coagulation of casein occurs promptly on boiling a culture 2 weeks old. Reaction acid.

Gelatin.—Growth of spherical colonies appears along the line of inoculation, the surface growth being grayish and spreading slowly. No liquefaction takes place.

Acid agar.—Growth takes place.

Indol.—A trace was observed.

Nitrate.—No reduction to nitrite could be observed.

BACTERIA FROM POLLEN.

As in the case of the examination of the combs, the number of species of bacteria found in pollen is comparatively small. The following are often found to be present. Other species have been isolated, but their distribution in the pollen is not at all constant.

Bacillus B.

Occurrence.—Found frequently in pollen and in the intestine of healthy honey bees.

Gelatin colonies.—The colonies are egg-yellow with even border. Liquefaction takes place slowly. Surface colonies are about 1.5 millimeters in diameter, have coarsely granular center, finely granular margin, and clear and sharply defined border. A peculiar toruloid growth is often observed.

Morphology.—The organisms are short rods with rounded ends, which stain uniformly with carbol-fuchsin, and are 1μ to 2μ in length. Few short involution forms occur.

Motility.—The bacilli are actively motile in young cultures.

Spores.—No spores have been observed.

Gram's stain.—The bacilli are decolorized by Gram's stain.

Oxygen requirements.—Facultatively anaërobic.

Bouillon.—This medium becomes uniformly clouded, frequently with a scanty, friable membrane. Sometimes the organisms settle, clearing the medium and forming a viscid sediment. A growth of the culture adheres to the glass at the surface of the liquid. This, together with the membrane, is of a light egg-yellow color, which deepens somewhat with age. Reaction alkaline.

Glucose.—At first both arms of the fermentation tube are clouded slightly, and the cloudiness later increases. Sometimes a stronger growth occurs in the closed arm than in the open one. Reaction is at first acid, but slowly changes to alkaline.

Lactose.—Reaction alkaline.

Saccharose.—Reaction alkaline.

Levulose.—Reaction alkaline.

Maltose.—Reaction slightly acid.

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Mannite.—Reaction slightly acid, later alkaline.

Agar slant.—A moderate, slightly yellow, nonviscid glistening growth appears along the inoculated surface. This growth gradually spreads and deepens in color to an egg-yellow.

Potato.—A moderate, egg-yellow, nonviscid, glistening growth spreads over the entire surface. The potato is slightly discolored.

Milk.—The milk is covered by a yellow growth of the culture, resembling cream. Coagulation takes place on boiling.

Litmus milk.—Reaction alkaline.

Gelatin.—Growth takes place along the line of inoculation. Deep in the medium the colonies are white and spherical; the surface growth is yellow. After a few days liquefaction begins, and at the end of 2 weeks one-half the tube is liquefied. The liquefaction is infundibuliform. Liquefied gelatin is surmounted by a friable, egg-yellow pellicle. The growth in the liquefied portion is flocculent, which, on settling, forms a yellow sediment at the apex.

Indol.—None could be observed.

Nitrates.—No reduction to nitrites occurs.

BACTERIA IN HONEY AND NORMAL LARVÆ.

Comb honey from a large number of sources has been examined and found to be quite uniformly sterile. The healthy larvæ likewise are usually sterile.

BACTERIA UPON THE ADULT BEES.

On the external part of the bee we again find only a few different species. *Bacillus A*, described as found upon the combs, is frequently isolated from the bee. Other species which are found frequently are described below.

Bacterium cyaneus (Micrococcus cyaneus).

Occurrence.—Isolated from the body of a healthy honey bee and from pollen.

Gelatin colonies.—The colonies are lemon-yellow, with entire border, growth taking place readily on this medium. The superficial colonies, having well-defined border, are finely granular, and liquefy the medium within 3 to 6 days.

Morphology.—Short oval rods 0.8μ to 1.7μ in length, 0.7μ to 0.8μ in thickness. Short involution forms are present. The rods occur singly, paired, and in clumps. No flagella have been demonstrated.

Motility.—No motion has been demonstrated.

Spores.—No spores have been demonstrated.

Gram's stain.—The bacterium takes Gram's stain.

Oxygen requirements.—Aërobic.

Bouillon.—At first a slight cloudiness appears, the medium becoming turbid in old cultures. A heavy yellowish-white, slightly viscid ring forms on the tube at the surface of the medium. The sediment, and sometimes the medium, show marked viscidity. Reaction alkaline.

Glucose.—The growth of the culture is confined entirely to the open bulb, in which the medium becomes turbid. No gas is formed. Reaction alkaline.

Lactose.—Reaction alkaline.

Saccharose.—Reaction alkaline.

Levulose.—Reaction alkaline.

Maltose.—Reaction alkaline.

Mannite.—Reaction alkaline.

Potato water.—Reaction alkaline.

Agar slant.—On the surface of the agar there takes place an abundant growth, which is confined to the surface inoculated with the loop. The culture is fleshy, nonviscid, and lemon-yellow. It produces a soluble pigment that diffuses thru the agar, giving it a dark-pink color.

Serum.—Luxuriant growth takes place, accompanied by liquefaction.

Potato.—A lemon-yellow, fleshy, glistening growth spreads over the inclined surface of the potato.

Milk.—Precipitation followed by slow liquefaction of the casein occurs; later the medium becomes alkaline and very viscid.

Litmus milk.—The litmus is discharged and the casein is liquefied. Reaction alkaline.

Gelatin.—Infundibuliform liquefaction soon begins, which is followed by stratiform liquefaction. The liquefied gelatin is turbid and viscid.

Acid agar.—On this medium a moderate lemon-yellow growth is observed.

Indol.—None could be observed.

Nitrates.—No reduction of nitrates could be observed.

Micrococcus C.

Occurrence.—Isolated from the body of a healthy honey bee.

Gelatin colonies.—The surface colonies are round and slightly yellow. Liquefaction begins in from 2 to 4 days. The magnified colonies are finely granular, with sharply defined, entire border.

Morphology.—Cocci, about 0.8μ in diameter, occur in pairs and in small clusters.

Motility.—Nonmotile.

Spores.—Spores are apparently absent.

Gram's stain.—The coccus takes the Gram's stain.

Oxygen requirements.—Aërobic.

Bouillon.—This medium becomes uniformly clouded in 24 hours after inoculation, growth increases, and friable sediment forms. The liquid clears somewhat on standing. Reaction at first slightly acid; later returns to neutral.

Glucose.—The medium in the bulb becomes cloudy, while that in the closed arm remains clear. White friable sediment forms in bend of tube. Reaction acid. No gas is formed.

Lactose.—Reaction slowly becomes acid.

Saccharose.—Reaction acid.

Levulose.—Reaction acid.

Maltose.—Reaction acid.

Mannite.—Reaction acid.

Potato water.—Reaction acid.

Agar slant.—A grayish white, fleshy, nonviscid, glistening growth takes place along the inoculated surface. It does not spread, and retains a distinct boundary.

Serum.—A spreading growth takes place, accompanied by liquefaction.

Potato.—A gray, fleshy, glistening, nonviscid growth forms over the entire cut surface of the potato. The potato is slightly discolored.

Milk.—This medium becomes firmly coagulated and later the casein liquifies with the formation of a milky serum.

Litmus milk.—In this medium coagulation takes place, accompanied by reduction of the litmus. Reaction slightly acid.

Gelatin.—After a day or two infundibuliform liquefaction occurs, being followed by stratiform liquefaction; the liquefied gelatin is turbid. Growth below this portion is in the form of small spherical colonies.

Acid agar.—A white, fleshy, nonviscid growth is observed.

Indol.—A trace was observed.

Nitrates.—Reduced to nitrites.

BACTERIA OF THE INTESTINE OF THE HEALTHY HONEY BEE.

A great many investigations have been made in recent years on the bacteria found present in the intestines of vertebrates (4, 5, 6, 7, 8, 9), and striking similarities are noticed in the species found in many of them. In this investigation the intestinal contents of about 150 bees, mostly from one apiary, have been studied more or less thoroly. Several species which are found to be constant in many of the vertebrates are found in the intestine of the honey bee. Since the temperature of the bee approximates much of the time, especially when in the hive, that of the warm-blooded animals, many of the same species of bacteria inhabit the intestine of this insect as are found thriving in the same locality in man and other animals. A stained cover-glass preparation made directly from a healthy adult field bee reveals, almost without exception, a multitude of bacteria.

In a study of the bacterial flora stress has been placed upon the different species which were found to be more or less constant, rather than upon the actual number of bacteria or species in any quantity of material from a single bee. From the observations which have been made, it appears that the number of species in any individual is comparatively small, but the number of bacteria is in many cases very large. Sometimes, however, the plates show very few colonies, while cover-glass preparations show a very large number of bacteria. These organisms are probably the anaërobe, which is quite constant, as shown by cultures made direct from the intestine into glucose agar (Liborius's method).

When a loopful of the material from the intestine was used for the inoculation, the following data give the approximate findings:

Bee No. 1, 300 to 400 yellow colonies, probably alike.
Bee No. 2, a few colonies of fungi only.
Bee No. 3, 500 colonies, mostly yeast.
Bee No. 4, 100 or more colon-like colonies.
Bee No. 5, 2,000 or more, mostly yellow.
Bee No. 6, 20 or more colonies, mostly yeasts.
Bee No. 8, 400 or more yellow colonies.
Bee No. 9, 30 yeasts with a few fungi.
Bee No. 10, 50 yeast colonies with a few fungi.
Bee No. 11, no growth.
Bee No. 12, 300 colonies, slightly yellow.

Bee No. 13, 2,000 or more gray colonies.

Bee No. 14, yeast colonies and a few colonies of bacteria showing ground-glass appearance.

Bee No. 15, 2,000 or more colon-like colonies (*B. cloacæ*).

The following are the species which have been found to be most constant. The reader is referred also to the description of the yeast plant found very frequently in the intestine of the normal honey bee, described under "Saccharomyces and fungi."

Bacterium D.

Occurrence.—Frequent in the intestine of the healthy honey bee.

Agar colony.—Deep colonies when magnified are coarsely granular, showing a dark brown center, with a thin and ill-defined border.

Morphology.—A preparation made from a young culture taken from a glucose fermentation tube shows rods with rounded ends, occurring singly and in pairs, staining easily and uniformly with carbol-fuchsin, and measuring 0.7μ to 1.5μ in length and 0.5μ to 0.7μ in thickness.

Motility.—No motility could be observed.

Spores.—No spores could be demonstrated in young cultures. In old cultures their presence is questionable.

Oxygen requirements.—Strictly anaërobic.

Bouillon.—In straight tubes no growth occurs.

Glucose.—A moderate cloudiness can be seen in the closed arm, while the open bulb remains clear. No gas is produced. Reaction about neutral.

Glucose agar (Liborius's method).—Growth is rather slow. After 3 days a moderate growth may be observed; later, if cultures have recently been isolated from the bee's intestine, the growth imparts to the medium a diffused haziness or cloudiness. After many generations the culture loses this property.

Glucose gelatin (Liborius's method).—Very slow growth occurs in the depth of the medium. No liquefaction takes place.

Bacillus cloacæ.

Occurrence.—Found in the intestine of a large number of healthy honey bees.

Gelatin colonies.—Superficial colonies are thin and blue to gray in color; deep colonies, brown, regular, granular, and spherical to lenticular.

Agar colonies.—Superficial colonies are partially opaque, brown, finely granular, with well-defined margin; deep colonies are regular, spherical, or lenticular, with well-defined margin.

Morphology.—The rods from 24-hour agar cultures have rounded ends, varying in length from 1μ to 2μ and in width from 0.7μ to 0.9μ. They are usually found singly or in pairs. Involution forms are not uncommon. With carbol-fuchsin they stain uniformly. This species possesses a few peritrichic flagella.

Motility.—Active motility is observed in young cultures.

Spores.—No spores are formed.

Gram's stain.—The bacillus does not take Gram's stain.

Oxygen requirements.—Facultatively anaërobic.

Bouillon.—A uniform cloudiness appears in 24 hours. Growth continues until the medium becomes heavily clouded, followed by a gradual settling of many of the organisms, forming a viscid grayish-white sediment. A gray friable membrane, which adheres to the sides of the tube at the surface of the medium, is sometimes produced. Upon agitation this membrane breaks up and sinks to the

bottom, leaving a gray ring of the growth adhering to the glass. Reaction alkaline.

Glucose.—The medium in the bulb becomes turbid, while that in the closed arm is uniformly cloudy. A heavy grayish-white sediment is formed. The reaction is at first slightly acid, but in a few days becomes alkaline. Abundant and rapid gas formation takes place, filling usually from one-half to nine-tenths of the closed arm. The ratio of hydrogen to carbon dioxid is approximately 1 to 2; that is, the ratio of hydrogen to carbon dioxid is less than 1.

Lactose.—In this medium gas formation takes place more slowly than in glucose. At the end of 8 days one-fourth of the closed arm is filled with gas. The ratio of hydrogen to carbon dioxid is greater than 1. Reaction acid.

Saccharose.—Gas is formed abundantly and rapidly; more than one-half of the tube is usually filled with gas. The ratio of hydrogen to carbon dioxid is less than 1. Reaction alkaline.

Levulose.—A rapid fermentation takes place; more than one-half of the closed arm is filled with gas. The ratio of hydrogen to carbon dioxid is approximately 1 to 5; that is, less than 1. A slight formation of acid takes place at first, but the reaction rapidly becomes alkaline.

Maltose.—Formation of gas takes place with the result that at the end of 5 days approximately one-half of the tube is filled. The ratio of hydrogen to carbon dioxid will approximate that of 1 to 1. Reaction acid.

Mannite.—Gas is formed rapidly and abundantly; at the end of 5 days the closed arm is usually much more than half filled with the gas. The reaction is at first slightly acid, but soon becomes alkaline. The ratio of hydrogen to carbon dioxid is approximately 1 to 2; that is, less than 1.

Potato water.—Gas forms rapidly and fills half the closed arm. The ratio of hydrogen to carbon dioxid is as 1 to 2; that is, less than 1.

Agar slant.—A moderate, grayish-white, glistening, friable growth appears along the line of inoculation, which usually spreads to the sides of the tube.

Serum.—Moderate gray growth appears, which is confined quite closely to the line of inoculation. Liquefaction takes place slowly after 3 weeks.

Potato.—A moderate amount of gray fleshy growth covers the slope. The potato is slightly discolored.

Milk.—Coagulation takes place after 4 days' growth. Gas is formed.

Litmus milk.—A marked production of acid takes place, followed by firm coagulation.

Gelatin.—A heavy white growth takes place along the line of inoculation; the surface growth is flat, bluish-white, and spreads with an uneven margin. Slow infundibuliform liquefaction takes place after 2 weeks.

Acid agar.—A growth takes place.

Indol.—A trace is sometimes produced.

Nitrates.—Reduction to nitrites is positive.

B. coli communis.

Occurrence.—Found in the intestine of healthy honey bees.

Gelatin colonies.—The superficial colonies are blue, lobate-lobulate, and slightly spreading; when magnified they are brownish yellow in the center and more transparent toward the margin; the deep colonies are spherical to lenticular and brownish yellow, with well-defined borders.

Morphology.—The short rods with rounded ends measure 1.5μ to 2μ in length and 0.7μ to 0.8μ in thickness. They occur singly or in pairs, stain uniformly, and are motile by means of a few peritrichic flagella.

Motility.—The bacilli are actively motile from some cultures.

Spores.—No spores are formed.

Gram's stain.—The bacillus is decolorized by Gram's method.

Oxygen requirements.—It is a facultative anaërobe.

Bouillon.—The medium becomes uniformly clouded in 24 hours, with a slight acid reaction; the medium later becomes alkaline, with a gray and friable sediment. A feeble pellicle is formed and a growth of the organism often adheres to the glass at the surface of the liquid.

Glucose.—Both branches of the fermentation tube become clouded. The sugar splits by fermentation into gas and acid, one-half or more of the closed arm being filled. The ratio of hydrogen to carbon dioxid is 2 to 1.

Lactose.—Gas fills one-fourth of the closed tube. Reaction acid.

Saccharose.—Gas fills one-sixth of the closed tube. Reaction acid.

Levulose.—Gas fills one-half of the closed tube. The value of hydrogen to carbon dioxid is 2 to 1. Reaction acid.

Maltose.—One-sixth of the closed arm is filled with gas. Reaction acid.

Mannite.—One-half of the closed tube is filled with gas. Reaction acid.

Potato water.—Reaction acid.

Agar slant.—A moderate, gray, nonviscid, spreading growth takes place on the surface of the inclined agar.

Serum.—A gray, glistening, nonspreading growth is observed on the inclined serum. No liquefaction takes place.

Potato.—A moderate, fleshy, glistening growth spreads over the inoculated surface. Potato slightly discolored.

Milk.—Coagulation of the casein takes place in about 4 days. A small quantity of gas is produced.

Litmus milk.—Coagulation occurs. Reaction strongly acid.

Gelatin.—A moderate growth occurs along the line of inoculation; the growth is spreading with an irregular margin on the surface. No liquefication occurs.

Acid agar.—A moderate grayish growth occurs on surface.

Indol.—A trace was obtained in some cultures.

Nitrates.—Reduced to nitrites.

B. choleræ suis.

Occurrence.—Isolated from the intestine of healthy honey bees.

Gelatin colonies.—Colonies are translucent by transmitted light; bluish to gray by reflected, the border being uneven and well defined. When the colonies are magnified they appear brownish and finely granular.

Morphology.—The rods are short, with rounded ends, occurring singly and in pairs, and staining uniformly with carbol-fuchsin, 1 to 2.8μ in length, and 0.6μ to 0.8μ in thickness. A few peritrichic flagella are present.

Motility.—Usually only a few are motile at a time in the field, and these present a rapid whirling motion.

Spores.—No spores are formed.

Gram's stain.—The bacteria are decolorized by Gram's stain.

Oxygen requirements.—Facultatively anaërobic.

Bouillon.—A uniform, moderate cloudiness arises in this medium in 24 hours; later a grayish-white membrane is formed which, upon shaking the tube, sinks to the bottom, forming a gray sediment. The reaction is at first slightly acid, but later becomes alkaline.

Glucose.—The medium becomes clouded in both arms of the fermentation tube, with the production of a small amount of gas. Reaction acid.

Lactose.—Growth takes place in both arms of the tube, but the sugar is not split into either acid or gas.

Saccharose.—Growth occurs in both arms of the tube, neither acid nor gas being formed.

Levulose.—Growth takes place in both arms with the production of gas and acid; one-third of the closed arm is filled. The ratio of hydrogen to carbon dioxid is about 3 to 1—that is, greater than 1.

Maltose.—The medium in both arms of the tube becomes clouded. Fermentation results in the production of gas sufficient to fill about one-fifth of the tube. Only a small portion of the gas is absorbed by sodium hydroxid, leaving behind an explosive gas.

Mannite.—The medium in both branches of the tube becomes clouded; gas is not formed. Reaction alkaline.

Potato water.—About one-fifth of the closed arm is filled with gas. Reaction acid.

Agar slant.—A moderate, grayish-white, glistening, nonspreading growth is formed along the surface inoculated with the loop.

Serum.—A moderate, gray, glistening, nonspreading growth takes place on the inclined surface. No liquefaction occurs.

Potato.—A feeble, grayish growth is observed. The potato becomes slightly discolored.

Milk.—No coagulation occurs, and no gas is produced. Reaction alkaline.

Litmus milk.—The medium slowly becomes more and more alkaline.

Gelatin.—A moderate, white growth takes place along the line of inoculation. On the surface it spreads with irregular margin. No liquefaction occurs.

Acid agar.—A moderate growth appears.

Indol.—Indol is produced.

Nitrates.—Reduction to nitrites (?).

Bacillus E.

Occurrence.—Isolated from the intestine of healthy honey bees.

Gelatin colonies.—The colonies are lemon-yellow. Surface colonies are convex, smooth, with entire margin; when magnified they are finely granular. Deep colonies, when magnified, are lenticular, finely granular, and may appear dark green. Liquefaction takes place slowly.

Morphology.—The rods are short, with rounded ends, and usually occur singly. The bacilli are 1.5μ to 2μ in length and 0.7μ in thickness. This species possesses a few peritrichic flagella.

Motility.—The bacteria are actively motile.

Spores.—No spores are present.

Gram's stain.—They stain with Gram's stain.

Oxygen requirements.—Aërobic.

Bouillon.—The medium becomes uniformly clouded in 24 hours. Later a tough, yellowish-white membrane is formed, which sinks upon shaking. The medium is very viscid in old cultures. Reaction alkaline.

Glucose.—Growth is confined to the open bulb. No gas formation occurs. Reaction slightly acid.

Lactose.—There is a marked mucous-like appearance in the medium. Reaction alkaline.

Saccharose.—Reaction acid.

Levulose.—Reaction alkaline.

Maltose.—Reaction alkaline.

Mannite.—Reaction slightly acid.

Potato water.—Reaction alkaline.

Agar slant.—A moderate, yellowish-gray, nonviscid growth takes place on the surface.

Serum.—A strong growth takes place and the medium is liquefied.

Potato.—A yellowish-gray, nonviscid growth is observed over the entire inclined surface.

Milk.—Precipitation of casein takes place with very slight digestion (?).

Litmus milk.—Precipitation of the casein occurs. Reaction alkaline.

Gelatin.—A white growth forms along the line of inoculation, which becomes slowly liquefied from above.

Acid agar.—A moderate, slightly yellow growth is observed.

Indol.—None demonstrated.

Nitrates.—No reduction to nitrites occurs.

Bacillus subgastricus.

Occurrence.—Isolated from the intestine of a healthy honey bee.

Gelatin colony.—The colon-like, superficial colonies are thin, blue, spreading, and lobate-lobulate. When magnified they are finely granular, with brown center. Deep colonies are spherical and yellow.

Morphology.—Short rods, singly and in pairs, are from 1.5μ to 2.5μ long and from 0.6μ to 0.8μ thick. They stain uniformly with carbol-fuchsin.

Motility.—Marked whirling motion from gelatin cultures.

Spores.—No spores could be demonstrated.

Gram's stain.—The bacilli are decolorized with Gram's stain.

Oxygen requirements.—Facultatively anaërobic.

Bouillon.—This medium becomes clouded in 24 hours. A slight band of growth is formed on the glass at the surface of the liquid. Later a feeble pellicle is sometimes formed. Reaction at first slightly acid, later becomes alkaline.

Glucose.—The medium in both branches of the tube becomes clouded. Gas is readily formed until about one-fourth of the closed branch is filled. The ratio of hydrogen to carbon dioxid is 2 to 1—that is, greater than 1. Reaction strongly acid.

Lactose.—Gas formation occurs. About one-sixth of the tube is filled with gas, part of which is absorbed by sodium hydroxid and another part is explosive. Reaction acid.

Saccharose.—This sugar is fermented to the point of formation of acid, but no gas is formed.

Levulose.—This sugar splits in the process of fermentation to form acid and gas, the gas filling about one-sixth of the tube. A portion of the gas is absorbed by sodium hydroxid, the remainder being explosive.

Maltose.—Fermentation takes place with the formation of acid. No gas is produced.

Mannite.—One-fifth of the closed arm is filled with gas. A portion of the gas is absorbed by sodium hydroxid and a portion is explosive. Reaction acid.

Potato water.—Reaction alkaline.

Agar slant.—A moderate, translucent, gray, nonviscid and glistening growth spreads slowly from the surface inoculated with the loop.

Serum.—A moderate, glistening growth appears along the surface inoculated. No liquefaction occurs.

Potato.—A grayish growth takes place on the sloped surface.

Milk.—Firm coagulation of the milk takes place with the formation of a small amount of clear serum. A small amount of gas is produced.

Litmus milk.—Reaction strongly acid. Coagulation occurs in about six days.

Gelatin.—White, spherical colonies appear along the line of inoculation. The surface growth is grayish blue and spreading, with irregular margin. Slow liquefaction takes place, beginning usually in 2 weeks.

Acid agar.—A growth takes place.

Indol.—None could be demonstrated.

Nitrates.—No reduction to nitrites occurs.

Bacterium mycoides.

Occurrence.—Isolated from the intestine of a healthy honey bee.

Gelatin colonies.—A rapid growth of root-like colonies appears in 24 hours. In macroscopic appearance it somewhat resembles cotton fibers; when magnified these appear thick and somewhat felted in the center, while toward the margin they are beautifully filamentous. After a day or two the gelatin begins to liquefy.

Morphology.—The rods are large, scarcely rounded at the ends, and frequently in chains. They measure from 2.5μ to 5.5μ long and 1.5μ thick. No flagella have been demonstrated.

Motility.—No motility could be demonstrated.

Spores.—Spores are present.

Gram's stain.—The bacteria are not decolorized by Gram's stain.

Oxygen requirements.—Facultatively anaërobic.

Bouillon.—A decided fleecy growth with heavy, cotton-like sediment occurs.

Glucose.—No gas is formed. Reaction acid.

Lactose.—Reaction acid.

Saccharose.—Reaction acid.

Levulose.—Reaction acid.

Maltose.—Reaction acid.

Mannite.—Reaction acid.

Potato water.—Reaction alkaline.

Agar slant.—A luxuriant growth that appears root-like takes place on this medium. This growth tends to extend into the agar, which causes it to adhere to the medium.

Serum.—A luxuriant growth is formed, accompanied by liquefaction.

Potato.—A thick, gray, moist growth is found, the potato not being discolored.

Milk.—Coagulation occurs promptly, with formation of a clear serum.

Litmus milk.—The color is discharged in 48 hours.

Gelatin.—Hair-like outgrowths occur along the line of inoculation. Liquefaction begins at the surface and proceeds along the needle tract. In a few days the entire medium is liquefied.

Indol.—No indol is produced.

Nitrates.—Reduction to nitrites is positive.

Pseudomonas fluorescens liquefaciens.

Occurrence.—Isolated from the intestine of the healthy honey bee.

Gelatin colonies.—Before liquefaction, the superficial colonies, when magnified, are finely granular, with regular margin; deep colonies are spherical, brown, with regular margin. Liquefaction takes place rapidly. The surface of liquefied gelatin is covered by a friable membrane. Later the liquefied gelatin takes on a green fluorescence.

Morphology.—The bacteria are short rods, varying from 1μ to 2μ in length and from 0.5μ to 0.7μ in thickness. They stain uniformly with carbol-fuchsin and are motile by means of one or more polar flagella.

Spores.—No spores could be demonstrated.

Gram's stain.—The bacteria do not take Gram's stain.

Oxygen requirements.—Aërobic

Temperature requirements.—Culture must be grown at room temperature.

Bouillon.—The medium becomes clouded in 48 hours, forming a moderately tough pellicle. A greenish-yellow fluorescence begins at the surface, which gradually increases until the entire medium takes on that appearance. Reaction alkaline.

Glucose.—A cloudiness is formed in the open arm, but the closed arm is clear. Reaction alkaline.

Lactose.—Reaction alkaline.

Saccharose.—Reaction alkaline.

Levulose.—Reaction alkaline.

Maltose.—Reaction alkaline.

Mannite.—Reaction alkaline.

Agar slant.—At first a gray friable growth is formed confined to the surface inoculated, which later takes on a brown hue. Greenish-yellow fluorescence is observable in the medium.

Serum.—A slow liquefaction occurs.

Potato.—Very scanty growth occurs with slight discoloration.

Milk.—Rapid liquefaction of the casein takes place.

Litmus milk.—Rapid liquefaction of the casein takes place. Reaction alkaline.

Gelatin.—Infundibuliform liquefaction takes place rapidly.

Acid agar.—No growth occurs.

Indol.—No indol observed.

Nitrates.—No reduction to nitrites occurs.

SACCHAROMYCES AND FUNGI.

The first yeast plant described below is of very frequent occurrence in the intestine of the normal bee. *Saccharomyces roseus* can be isolated from the comb. A large number of common fungi were found in the flora of the intestines and in cultures from the pollen and combs.

In addition to the above the third Saccharomyces here described was found in two samples of brood apparently diseased, which could not be diagnosed as any disease commonly known.

Saccharomyces F.

Occurrence.—Very common in the intestine of healthy honey bees.

Gelatin colonies.—Colonies form slowly; the superficial colonies are white, glistening, convex, capitate, and about 1 to 2 millimeters in diameter. When magnified they are finely granular, brownish yellow, with entire margin. Deep colonies are finely granular, with uniform margin, spherical to lenticular, and brownish green.

Morphology.—The cells are oval and on agar in 24 hours attain their full size of 4.5μ in length and 3.5μ in thickness. They stain uniformly with carbol fuchsin.

Motility.—The yeast is not motile.

Gram's stain.—The cells take the Gram's stain.

Oxygen requirements.—Aërobic

Bouillon.—This medium remains clear, with the formation of a friable white sediment. Reaction neutral.

Glucose.—The closed arm remains clear. No gas is formed. Reaction acid.

Lactose.—Reaction neutral.

Saccharose.—Reaction neutral.

Levulose.—Reaction neutral.

Maltose.—Reaction neutral.

Mannite.—Reaction neutral.

Agar.—A white, nonspreading growth occurs.

Serum.—White, moderate, nonviscid, nonspreading growth occurs along the surface inoculated. No liquefaction takes place.

Potato water.—Reaction neutral.

Potato.—Gray, luxuriant, fleshy growth occurs.

Milk.—No change occurs.

Litmus milk.—No change occurs.

Gelatin.—A moderate growth is formed, accompanied by no liquefaction.

Acid agar.—Moderate growth takes place.

Indol.—Negative.

Nitrates.—Reduced to nitrites.

Saccharomyces roseus.

Occurrence.—Isolated from comb of healthy hive.

Gelatin colonies.—Superficial colonies are pink, convex, capitate, with lobate-lobulate margin; when magnified, the deep colonies are irregular, brownish-yellow, and finely granular.

Morphology.—This cell is oval, attaining about 6.5μ in length and 3.5μ in thickness. The cells stain uniformly.

Motility.—No motility occurs.

Gram's stain.—The cells are not decolorized by Gram's stain.

Oxygen requirements.—Aërobic.

Bouillon.—This medium remains clear, forming a pink, friable sediment. A pink band forms at the surface of the medium and adheres to the glass.

Glucose.—The closed arm remains clear. No gas is formed. Reaction acid.

Lactose.—Reaction neutral.

Saccharose.—Reaction neutral.

Levulose.—Reaction slightly acid.

Maltose.—Reaction slightly acid.

Mannite.—Reaction neutral.

Potato water.—Reaction acid.

Glucose agar.—Luxuriant, red growth forms on the surface.

Serum.—A pink, fleshy, nonspreading growth is formed, accompanied by no liquefaction.

Potato.—A thick, nonspreading, red growth occurs.

Milk.—No apparent change takes place. The milk coagulates on boiling.

Litmus milk.—Reaction alkaline.

Gelatin.—Moderate pink growth is formed, accompanied by no liquefaction.

Acid agar.—Slow growth occurs.

Indol.—Negative.

Nitrates.—Reduction to nitrites is positive.

Saccharomyces G.

Occurrence.—Found in the dead larvæ of diseased adult bees.

Morphology.—They appear in hanging-drop preparation in large clusters,

stain uniformly with carbol-fuchsin and are oval, nearly spherical, attaining the length of 4.5μ and thickness of 3.5μ.

Gram's stain.—The cells are not decolorized by Gram's stain.

Oxygen requirements.—Aërobic.

Bouillon.—A slight, friable, white sediment is formed, with a clear medium above. Reaction slightly acid.

Glucose.—The medium in the closed arm remains practically clear and about one-fifth of the closed arm is filled with gas. Reaction acid.

Lactose.—Reaction neutral.

Saccharose.—Reaction neutral.

Levulose.—Reaction slightly acid.

Maltose.—Reaction slightly acid.

Mannite.—Reaction neutral.

Potato water.—Reaction acid.

Agar.—A moderate, white growth is formed.

Serum.—Very feeble growth occurs, accompanied by no liquefaction.

Potato.—A luxuriant, moist, white growth occurs.

Milk.—No appreciable change takes place.

Litmus milk.—No appreciable change takes place.

Gelatin.—A moderate, white growth occurs along needle tract and on the surface. No liquefaction results.

Acid agar.—A feeble white growth occurs.

Indol.—None could be demonstrated.

Nitrates.—No reduction to nitrites occurs.

Glucose agar.—A thick, white, fleshy growth occurs.

TABULATION OF MICRO-ORGANISMS NORMALLY PRESENT IN THE APIARY.

The following table will serve to summarize the descriptions of the micro-organisms considered in the foregoing pages:

Species.	Biology. Morphology. Dimensions in microns. Length.	Thickness.	Motility.	Position in rod.	Spores. Germination.	Stain by grams.	Growth at room temperature.	Growth at 37° C.	Oxygen requirements.	Physiology. Cultural features. Gelatin colonies. Colon-like.	Entire.	Curled.	Bouillon. Membrane.	Cloudy.	Agar.	Serum.	Potato. Degree.	Discolored.	Gelatin.	Biochemic properties. Growth on acid agar.	Milk. Coagulation.	Lit. discharged.	Chromogenesis. Potato.	Agar.	Fluorescence. Agar.	Gelatin.	Liquefaction. Casein.	Serum.	Gas production. Glucose.	Lactose.	Saccharose.	Levulose.	Maltose.	Mannite.	Potato.	Acid production. Milk.	Glucose.	Lactose.	Saccharose.	Levulose.	Maltose.	Mannite.	Potato.	Milk.	Milk alkaline.	Nitrate to nitrite.	Indol.	Remarks.
Bacillus A	3 –4	0.9–1.2	+	–	1	–	+	+	2	+	.	.	+	+	+	+	3	1	+	+	1	.	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	B. mesentericus (?).
Bact. acidiformans	1.6	.8	–	–	–	–	+	+	2	+	.	.	+	+	+	+	2	–	+	+	3	.	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	
Bacillus B	1 –2	.....	+	–	–	–	+	+	2	+	.	.	+	+	+	+	1	+	+	+	3	.	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	
Bact. cyaneus	.8–1.7	.7– .8	–	–	–	–	+	+	1	+	.	.	+	+	+	+	2	.	+	+	1	+	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	Indol trace.
Micrococcus C	.8	.8	–	–	–	–	+	+	1	+	.	.	+	+	+	+	2	.	+	+	2	+	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	Spores (?).
Bacterium D	.7–1.5	.5– .7	–	–	–	–	+	+	3	+	.	.	+	+	+	+	2	.	+	+	1	+	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	Indol (?).
B. cloacæ	1 –2	.7– .9	+	–	–	–	+	+	2	+	.	.	+	+	+	+	2	.	+	+	1	+	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	Indol trace.
B. coli communis	1.5–2	.7– .8	+	–	–	–	+	+	2	+	.	.	+	+	+	+	2	.	+	+	1	+	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	
B. coleræ suis	1 –2.8	.6– .8	+	–	–	–	+	+	2	+	.	.	+	+	+	+	2	.	+	+	1	+	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	
Bacillus E	1.5–2	.7	+	–	–	–	+	+	1	.	.	.	+	+	+	+	2	.	+	+	1	.	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	
B. subgastricus	1.5–2.5	.6– .8	+	–	–	–	+	+	2	+	.	.	+	+	+	+	2	.	+	+	2	+	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	
Bact. mycoides	2.5–5.5	1.5	?	+	.	.	+	+	2	.	.	.	+	+	+	+	3	.	+	+	2	+	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	
Ps. fluor. lique	1 –2	.5– .7	+	–	–	–	+	+	1	.	.	.	+	+	+	+	1	1	+	+	1	.	+	.	.	+	+	+	+	.	.	.	.	.	.	–	–	–	–	–	–	–	–	+	+	–	
Saccharomyces F	4.5	3.5	–	.	.	.	+	+	1	.	.	.	–	–	+	+	3	.	+	+	3	.	+	.	.	+	+	+	+	.	.	.	.	.	.	+	+	+	+	+	+	+	+	–	–	–	
Saccharomyces roseus	6.5	3.5	–	.	.	.	+	+	1	.	.	.	–	–	+	+	3	.	+	+	3	.	+	.	.	+	+	+	+	.	.	.	.	.	.	+	+	+	+	+	+	+	+	–	–	–	
Saccharomyces G	4.5	3.5	–	.	.	.	+	+	1	.	.	.	–	–	+	+	3	.	+	+	3	.	+	.	.	+	+	+	+	.	.	.	.	.	.	+	+	+	+	+	+	+	+	–	–	–	

SUMMARY TO PART I.

The results of the study of the bacteria found normally in the apiary may be briefly summarized as follows:

(1) The temperature of the hive approximates that of warm-blooded animals.

(2) Upon adult bees and upon the comb there occurs quite constantly a species of bacteria which we refer to in this paper as *Bacillus A*, and which, it is believed, is the organism that some workers have confused with *Bacillus alvei*, the cause of European foul brood (p. 33).

(3) There occurs very constantly in the pollen and intestine of adult bees a species here referred to as *Bacillus B*.

(4) From the combs *Bacterium cyaneus*, *Saccharomyces roseus*, and a Micrococcus referred to here as *Micrococcus C*, have been isolated and studied.

(5) Honey from a healthy hive is, as a rule, sterile.

(6) The normal larvæ are, as a rule, sterile.

(7) There is an anaërobe found quite constantly in the intestine of the healthy honey bee. It is referred to in this paper as *Bacterium D*.

(8) From the intestine there have been isolated and studied the following micro-organisms: *Bacillus cloacæ*, *Bacillus coli communis*, *Bacillus choleræ suis*, *Bacillus subgastricus*, *Bacterium mycoides*, *Pseudomonas fluorescens liquefaciens*, and two referred to as *Bacillus E*, and *Saccharomyces F*. Others less frequently present have been isolated, but not studied.

(9) In two samples of brood with unknown disease there was found a species of yeast plant here referred to as *Saccharomyces G*.

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PART II.—THE DISEASES OF BEES.

The bee industry in this country, and other countries as well, is suffering large losses from various diseases among bees. Those which are most destructive attack the brood and weaken the colony by killing off large numbers of the young larvæ which would otherwise mature. There are other diseases which attack the adults and so decrease the strength of the colony in that way.

In order to combat a disease to the best advantage it is clear that its cause must be known, as well as the means by which the infection is transmitted and the environmental conditions which are favorable for the breaking out of an epidemic. The brood diseases among bees are on the increase. The custom of selling and shipping the honey, which is now carried on more extensively than formerly, the manner in which the products of the apiary are handled, and the absence of a general knowledge by the mass of bee keepers of the nature of the diseases are conditions which must be met before the spread of these diseases can be checked. When a colony is diseased, very little or no profit is realized from it; consequently the wealth and comfort of a very large number of people are greatly endangered by the existence of bee diseases. This suggests the importance, from an economic standpoint, of a thoro knowledge of these disorders.

BRIEF HISTORY.

The attention of investigators has been attracted by these diseased conditions, not only from the economic interests attached thereto, but from the scientific point of view as well. The writings of Aristotle (12) contain an account of certain disorders which were then prevalent among bees; at that time it was thought that the blight of flowers bore a relation to bee diseases. In 1769 Schirach (13) gave the name foul brood to a diseased condition of the brood of bees; he attributed the cause to (*a*) unwholesome food, and (*b*) the placing of the larvæ with head inward in the cell. Leuckhart (14) thought the cause to be a fungus, related to the cause (*Panhistophyton ovatum*) of the disease of the silkworm. Muhlfeld (15), in 1868, thought the trouble to be of two kinds—infectious and noninfectious—and that the cause of the infectious one is the larva of a parasitic fly (*Ichneumon apium mellificarium*) feeding upon the larvæ of the bee. In 1868 Preuss (16) exprest the view that the cause of

foul brood is a fermenting fungus belonging to the genus Cryptococcus. Geilen (17), in 1868, thought that when bees alight on the remains of animal bodies the putrefying matter thus carried with them may cause foul brood. The fermentation of bee bread was thought by Lambrecht (18) to be a sufficient cause of the disease; while Hallier (19) thought that various fungi could produce the disorder. On the contrary, Cornallia (20), in 1870, exprest the opinion that a fungus (*Cryptococcus alvearis*) is the specific cause of the trouble. Fischer (21), in 1871, supposed that a predisposing factor of foul brood is to be found in insufficient nourishment. In 1874 Cohn and Eidem received from Schonfeld samples of foul brood and, upon examination, they found spores and rods. In 1885 Cheshire and Cheyne (22) determined the cause and named the germ *Bacillus alvei*. Dickel (23) claimed that a number of different species might be the cause of foul brood. In 1900 Harrison (24) writes on foul brood and *Bacillus alvei*, its cause. Doctor Lambotte (25), in 1902, made some interesting studies concerning the relation of *Bacillus alvei* and *Bacillus mesentericus vulgatus*.

Since so many conflicting views have been held as to the cause of foul brood, one might conclude that the term " foul brood " has been applied incorrectly to a number of different disorders. In the light of more recent work this supposition is strengthened.

In June, 1902, the author, under the direction of Dr. Veranus A. Moore, began an investigation of bee diseases, especially as they existed in New York State. There were recognized at that time by bee inspectors of that State a number of distinct diseases which attacked the brood. Those which caused the greatest loss to the apiarists were known to the bee experts as " black brood," " foul brood," and " pickle brood." The results of the investigations of 1902 (26), 1903 (27), and 1904 (28) on these disorders, and on palsy or paralysis, are embodied in the following pages.

THE TERM " FOUL BROOD " AS HITHERTO APPLIED.

In the discussion of foul brood of bees it must be remembered that until recent years the name has been applied to what is now known to be two distinct diseases.

Schirach, in 1769, gave the name foul brood to a diseased condition in the brood of bees, but it is impossible to know to which of the two he referred. It may be that both diseases existed then as now and that he did not observe the fact that the two were different. We have reason to think that there are, at the present time in Europe, two distinct diseases to which the name foul brood is being applied. It is definitely known that such is the case in America.

It becomes necessary, then, to have two names to designate these

two diseased conditions in the brood of bees. For reasons given by Dr. E. F. Phillips, in the preface to this paper, it has been considered advisable to retain the name foul brood and to use a qualifying word to distinguish the two diseases. "European foul brood" and "American foul brood" are the names by which these two diseased conditions are to be designated.

In 1885 Cheyne (22) in England (Europe) found present in the decayed larvæ suffering from a diseased condition known as "foul brood" a new bacillus, which he named *Bacillus alvei* and to which he ascribed the cause of the disease. The diseased condition which contains *Bacillus alvei* is to be called "European foul brood," because this fact was first observed by an investigator working in Europe (England). In 1903 (27) the author observed that there was constantly present in the other diseased condition known as "foul brood" another bacillus which was new, and to which the name *Bacillus larvæ* is given. In view of the fact that *Bacillus larvæ* was constantly found to be present in the larvæ suffering from this disorder in the brood of bees, by investigations carried on in New York State (America) (27) (28), this diseased condition is to be called "American foul brood." From a scientific standpoint this choice of names for two distinct diseases might be easily criticized, but from the standpoint of the apiarist the selection of these names as the common ones for these two distinct disorders seemed almost necessary, or at least advisable.

EUROPEAN FOUL BROOD (FOUL BROOD OF CHEYNE).

The first scientific investigation of this disease bacteriologically was performed by Cheyne in 1885 (22). At this time he isolated a new bacillus from the dead larvæ. It was described by him and given the name *Bacillus alvei* (literally, hive bacillus). This afforded, then, a means for a positive diagnosis of this diseased condition.

Symptoms.

The symptoms of European foul brood, as given by Dr. E. F. Phillips in Circular No. 79, Bureau of Entomology, are as follows:

Adult bees in infected colonies are not very active, but do succeed in cleaning out some of the dried scales. This disease attacks larvæ earlier than does American foul brood, and a comparatively small percentage of the diseased brood is ever capped; the diseased larvæ which are capped over have sunken and perforated cappings. The larvæ when first attacked show a small yellow spot on the body near the head and move uneasily in the cell; when death occurs they turn yellow, then brown, and finally almost black. Decaying larvæ which have died of this disease do not usually stretch out in a long thread when a small stick is inserted and slowly removed; occasionally there is a very slight "ropiness," but this is never very marked. The thoroly dried larvæ form irregular scales which are not strongly adherent to the lower side wall of the

cell. There is very little odor from decaying larvæ which have died from this disease, and when an odor is noticeable it is not the "glue pot" odor of American foul brood, but more nearly resembles that of soured dead brood. This disease attacks drone and queen larvæ very soon after the colony is infected. It is, as a rule, much more infectious than American foul brood and spreads more rapidly. On the other hand, it sometimes happens that the disease will disappear of its own accord, a thing which the author never knew to occur in a genuine case of American foul brood. European foul brood is most destructive during the spring and early summer, often almost disappearing in late summer and autumn.

Confusion Regarding Foul Brood in America.

Prof. J. J. Mackenzie in 1882 made what seems to have been a short study of a bee disease as it appeared in Ontario, Canada, which was known to the apiarists of that Province as foul brood. He says very little of the character of the species of bacteria with which he was working, but he supposed that they were *Bacillus alvei* of Cheyne. The author has examined samples of brood from Ontario which have what, in the opinion of bee experts, is the most prevalent disease, and has not found *Bacillus alvei* present in any one. The bacteriological findings and the experience of bee-disease experts show that American foul brood is the prevalent disease in that Province. As the bee experts see the disease in the light of recent studies, there is no authentic report of which we are aware that European foul brood exists in Ontario. We can safely say, then, that *Bacillus alvei* can not be isolated from larvæ taken from the prevalent disease in the above-named Province. No difficulty is exprest on the part of Professor Mackenzie in the isolation of *Bacillus alvei* from any sample. The author is inclined to think, therefore, that this investigator was in error as to the identity of his culture, and therefore his conclusion can have little weight.

The foul brood of bees received some attention also from Prof. F. C. Harrison, of Ontario. In a paper of some length he gives a description of a species of bacteria which he identified as *Bacillus alvei*. The description which he gives and the accompanying photomicrographs (another plate which was given being after Cheyne and correct for *Bacillus alvei*) might easily be that of a member of a group represented by and described as *Bacillus* "*A*" in Part I of this paper. He also says that he has isolated *Bacillus alvei* from diseased larvæ from 13 States of the Union, ranging from New York to California and from Michigan to Florida. European foul brood has had a very limited geographical distribution, spreading only recently from New York to adjoining States. In Professor Harrison's work, too, there seems to have been no difficulty in isolating *Bacillus alvei* from diseased brood diagnosed by bee inspectors

as foul brood thruout the United States and Canada. In the experience of the author it has not been possible to obtain *Bacillus alvei* from diseased brood which the inspectors in most of the States and in Canada have been calling foul brood. For the above reasons the author believes that Harrison, too, has made a serious error in the identity of his culture and therefore was not working with *Bacillus alvei* at all. The author considers himself unfortunate in that he was unable to obtain a culture of *Bacillus alvei* for study and identification from Professor Harrison.

Dr. William R. Howard, of Fort Worth, Tex., also studied foul brood somewhat, and gave a description of *Bacillus alvei* as he found it. From his description and from the fact that he, too, worked with a diseased condition which does not contain *Bacillus alvei*, and exprest no difficulty in obtaining his cultures from any samples, the author believes that this investigator made an error in the identification of the culture with which he was working.

Some writers—Cowan, Bertrand, and others—have attempted the positive diagnosis of foul brood with the microscope alone from a preparation made direct from the dead larvæ. If the reader will remember that with the microscope alone it would be impossible to distinguish between *Bacillus larvæ* and *Bacillus alvei*, the verdict of these men can have no weight. As shown later in this paper under black brood (pp. 43–44), the Doctor Howard, of Fort Worth, Tex., referred to above, made an error in supposing that the European foul brood was a new disease and naming it "New York bee disease" or "black brood."

It is very unfortunate for the apiarist that these men should have fallen into error as to the identity of their culture with *Bacillus alvei*, as it has caused great confusion in the names of bee diseases. This confusion in the identity of cultures may be excused to a certain extent by the fact that European foul brood did not appear in this country, or at least did not attract much attention, until after Mackenzie, Harrison, and William R. Howard had done their work on foul brood.

The Present Investigation.

When the author's investigations were begun in 1902 there were two especially troublesome diseases in this country, which were then known to the bee experts as "black brood" and "foul brood."

The following summary and table shows the results of the examination of a number of samples of diseased brood from different apiaries, sent by the New York State bee inspectors during the summer of the year 1902:

Table showing the results of examinations of European foul brood. (The samples were called "black brood" by the apiarists at that time.)

Brood sent by—	Date.	Bacteriological findings.
W. D. Wright	June 4	*Bacillus alvei.*
W. D. Wright	June 12	*Bacillus alvei.*
N. D. West	June 12	*Bacillus alvei.*
N. D. West	June 12	*Bacillus alvei.*
N. D. West	June 12	*Bacillus alvei.*
N. D. West	June 12	*Bacillus alvei.*
N. D. West	June 12	*Bacillus alvei.*
N. D. West	June 12	*Bacillus alvei.*
N. D. West	Aug. 5	*Bacillus alvei.*
W. D. Wright	Oct. 8	*Bacillus alvei.*

It can be seen clearly from the above table that the diseased condition which the apiarists were calling "black brood" is really the disease "foul brood" of Cheshire and Cheyne, because of the constant presence of *Bacillus alvei.*

The work upon European foul brood was continued during the year 1903. The following table gives the results of the examination of specimens received during that year. The samples were taken from different apiaries.

Table giving a summary of the examination of specimens of European foul brood ("black brood").

Brood sent by—	Date.	Sources of brood in New York.	Bacteriological findings.
W. D. Wright	May 1	Columbia County	*Bacillus alvei.*
W. D. Wright	May 1	Albany County	*Bacillus alvei.*
N. D. West	June 25	Schoharie County	*Bacillus alvei.*
N. D. West	June 29	Schoharie County	*Bacillus alvei.*
N. D. West	June 29	Schoharie County	*Bacillus alvei.*
N. D. West	June 29	Schoharie County	*Bacillus alvei.*
N. D. West	June 29	Schoharie County	*Bacillus alvei.*
N. D. West	July 6	Schoharie County	*Bacillus alvei.*
N. D. West	July 6	Schoharie County	*Bacillus alvei.*
N. D. West	July 6	Schoharie County	*Bacillus alvei.*
N. D. West	July 10	Montgomery County	*Bacillus alvei.*
N. D. West	July 10	Schoharie County	*Bacillus alvei.*
N. D. West	July 10	Schoharie County	*Bacillus alvei.*
N. D. West	July 10	Schoharie County	*Bacillus alvei.*
N. D. West	July 15	Schoharie County	*Bacillus alvei.*
N. D. West	July 15	Montgomery County	*Bacillus alvei.*
N. D. West	July 22	Schoharie County	*Bacillus alvei.*
N. D. West	July 22	Schoharie County	*Bacillus alvei.*
N. D. West	July 22	Schoharie County	*Bacillus alvei.*
N. D. West	July 30	Schoharie County	*Bacillus alvei.*
N. D. West	July 30	Schoharie County	*Bacillus alvei.*
N. D. West	July 30	Greene County	*Bacillus alvei.*
N. D. West	July 30	Albany County	*Bacillus alvei.*
N. D. West	July 30	Greene County	*Bacillus alvei.*
N. D. West	Aug. 20	Greene County	*Bacillus alvei.*
N. D. West	Aug. 20	Greene County	*Bacillus alvei.*

The above table shows that *Bacillus alvei* was present in each specimen of European foul brood received. Frequently pure cultures of this species were obtained from dead larvæ, but with it sometimes were associated other rod-shaped bacteria of different species.

In 1904 the work upon bee diseases was confined principally to the diagnosis of the diseased brood sent in and a further study of the organisms found. *Bacillus alvei* was found in a large number of

samples received from New York State and in some received from Pennsylvania.

Bacillus alvei.

Occurrence.—This bacillus was found in all samples of European foul brood examined.

Morphology.—The bacillus is a motile, rod-shaped organism, occurring singly and in pairs, and varying when taken from the surface of agar from 1.2μ to 3.9μ in length, and from 0.5μ to 0.7μ in width. Involution forms are sometimes present. Spores are produced and occupy an intermediate position in the organism. They are oval and vary from 1.5μ to 2μ in length and from 0.7μ to 1μ in breadth; they exhibit polar germination. The few flagella are arranged peritrichic.

Oxygen requirements.—This bacillus is a facultative anaërobe which grows at room temperature, but better at 37° C.

Bouillon.—The medium becomes uniformly clouded in 24 hours; later it shows a tendency to clear by a settling of the organisms. A somewhat viscid sediment is thus formed in the bottom of the tube. In older cultures a slightly gray band of growth adheres to the glass at the surface of the medium. The acidity is at first slightly increased, and a pellicle is sometimes formed.

Glucose.—The medium in both branches of the fermentation tube becomes uniformly clouded. Gas is not formed. Reaction acid.

Lactose.—The medium becomes uniformly clouded in both branches of the fermentation tube, but the cloudiness is not so marked as when glucose is used. The acidity is slightly increased, as shown by phenolphthalein. No gas is formed.

Saccharose.—The bouillon in this case also becomes clouded in both arms. A heavier growth is observed than when lactose is used, but less than when glucose is used. Acidity is slightly increased. Gas is not formed.

Agar plates.—Small, grayish, circular colonies form in 24 hours. When many are on the plate, they do not exceed 2 millimeters in diameter. Under low magnification they appear granular, with no definite margin. When fewer colonies are on the plate, the granular center of the colony is surrounded by numerous smaller but similar growths. The organism has a tendency to grow into the medium rather than upon the surface. Sometimes, however, when there are but a few colonies on the plate a thin, transparent growth spreads rapidly over the surface. Later it takes on a brown tint.

Agar slant.—A gray layer spreads over the surface in 24 hours, which later takes on a slightly brown color. A strong, slightly viscid growth occurs in the condensation water.

Acid agar.—Growth takes place with the reactions varying from neutral to +3.5 to phenolphthalein.

Serum.—A slightly raised growth which is confined quite closely to the line of inoculation appears on the surface of solidified serum.

Potato.—On this medium the bacillus grows rather slowly at first, but after 3 or 4 days a milky growth is observed, which increases until a luxuriant growth is formed, which varies from a lemon-yellow to a gray color, and which later becomes tinted with brown.

Milk.—Acidity is increased after inoculation. Coagulation usually takes place after the third day.

Litmus milk.—Much of the blue color is discharged, leaving the coagulated milk of a light brown.

Gelatin colonies.—Gelatin is a medium in which it develops slowly. The colony becomes very irregular in outline, owing to thread-like outgrowths which take place in curves from its border. Growth is better when 5 per cent glycerin is added. From the small, white, spherical colonies which form along the line of puncture gray, thread-like growths shoot out thru the medium. In about 2 months the gelatin is changed to a thick liquid, holding gray flocculent masses of organisms which gradually settle, forming a strong, slightly viscid sediment.

Indol.—In old cultures a decided indol reaction is obtained.

Power to resist disinfectants.—Preliminary observations give the following results: The spore form resists drying for a considerable time. Spores which have been drying for 1 year germinate promptly when introduced into bouillon. The vegetative form: One per cent carbolic acid kills in 10 minutes; 3 per cent carbolic acid kills in 2 minutes; mercuric chlorid solution, 1 to 1,000. kills in 1 minute; mercuric chlorid solution, 1 to 2,000, kills in 2 minutes.

Spore form.—Mercuric chlorid, 1 to 1,000, kills in 30 minutes.

Pathogenesis in vertebrates.—Inoculations into guinea pigs and frogs have not proven this organism to be pathogenic to these animals.

Inoculation Experiments.

That part of the investigation which involves the producing of the disease experimentally by inoculating with pure cultures of the organism is usually the most difficult one. Very rarely indeed is one able to produce the disease with symptoms closely simulating those found in nature. The experimental production of a disease involves many variable factors, such as attenuation of the organism, methods of inoculation, resistance of the host, and the immediate environment.

On August 4, 1902, we inoculated a hive containing nothing but healthy brood, free from bacteria, by feeding with sirup (sugar and water in equal parts) to which was added the growth from the surface of the plate cultures containing spores and bouillon cultures of *Bacillus alvei.* Similar feedings were given to these bees from one to three times a week until September 28, but symptoms of foul brood did not develop. On August 6 cultures were made from a few of the hive larvæ. They were found to contain the bacilli.

Inoculation experiments were again made in 1903. Because of a failure to produce a diseased condition with cultures of *Bacillus alvei* in the experiment of 1902, the variable factors above mentioned were carefully considered in the experiment of this year. The inoculations were made when climatic conditions were such as seemed to favor the ravages of the disease in the apiaries; namely, low temperature, dampness, and cloudiness. A colony of black bees was used, as they were almost universally considered more susceptible. Cultures of *Bacillus alvei* were freshly isolated from foul-brood specimens and kept in stock on bee-larvæ agar (described under American foul brood, pp. 41–42). All cultures were incubated at 34° C., which temperature is observed to be slightly below that of the hive. The spore form of *Bacillus alvei* was used.

Inoculations were made in different ways. A diseased condition

appeared in the hive when the following method was used: The agar from plates on which the culture was grown was finely crusht and mixt with sterile sirup. A jelly glass, in the lid of which holes had been punctured, was filled and inverted on strips of wood inside the hive. In this way the bees take up the culture with the sirup as rapidly as it flows out of the glasses. A colony having brood free from *Bacillus alvei* was fed in the above manner on August 8, with repeated feedings on the 9th, 10th, 12th, 13th, 15th, and 17th. On the 12th *Bacillus alvei* was found in the living larvæ and on the 17th many larvæ were dead under cappings and some were dead which were not capped; all were soft and of a dull color. Many of the capped cells containing dead larvæ had their capping freshly punctured. *Bacillus alvei* was usually obtained from these larvæ in pure cultures. In no cell examined where the cell capping was punctured did we find gas-producing organisms; this fact would suggest the conclusion that these punctures which are found in the capping in foul brood are made by the bees and not by gas-producing organisms. During this series of inoculations the days were quite cool and sometimes cloudy and damp. On the 20th of August the temperature was much higher, the bees were more active, and much of the dead brood had been cleaned out by the bees. On the 22d no dead brood was noticed by casually looking over the brood nest. On the 24th of the same month a careful search was made by uncapping all the cells of one brood frame, and 12 decaying larvæ of a brown color were found. At this time the larvæ were not viscid. All the remaining dead brood had evidently been cleaned out by the bees. A condition similar to this, where only a few scattered about in the brood nests contain dead larvæ, occurs sometimes in affected apiaries. Two other colonies which were near by but not inoculated gave no signs of disease.

Mr. N. D. West reports that the climatic conditions seem to have something to do with the extent of the ravages of European foul brood, since the disease is much more destructive in cool, damp weather. This seems to be a very plausible idea. The larvæ at such times may receive more infected food than when fresh is being rapidly gathered; the resistance of the body of the larvæ to the growth of *Bacillus alvei* is at such times much lessened; and the adult bees being less active, the dead larvæ are not cleaned out of the combs so rapidly. The results of the experimental work seem to confirm this theory.

Distribution of Bacillus alvei in Infected Hives.

In order to combat this disease it is well to know where these pathogenic bacteria may be found. The following is a summary of the results of the investigation along this line:

1. The greatest number of infecting germs are found in the bodies of dead larvæ.

2. The pollen stored in the cells of the foul-brood combs contains many of these infecting organisms.

3. The honey stored in brood combs infected with this disease has been found to contain *Bacillus alvei* in small numbers.

4. The surface of the combs, frames, and hives may be contaminated.

5. The wings, legs, head, thorax, abdomen, and intestinal contents of adult bees are found to be contaminated with *Bacillus alvei.*

6. Cheshire (29), Mackenzie (30), and others have found *Bacillus alvei* in the ovary of the queen. This has suggested a means of infection. From a bacteriological examination of queens from three badly infected hives we were able to isolate *Bacillus alvei* in small numbers in two cases. Since a very large number of this species of bacteria may be found in the intestinal tract and upon all parts of the body, it is very probable that such findings are the results of contamination in making cultures and have no special significance.

Experiments with Formaldehyde Gas.

Within the last few years several articles have appeared in the bee journals entertaining great hopes that a cure for foul brood has been found in the use of formaldehyde gas. The methods described for its use have been tested by the apiarists and bee experts in New York State, with the result that the disease sometimes breaks out anew in colonies so treated.

In order to test the value of formaldehyde gas as a disinfectant when used in foul-brood combs a number of experiments were made in the laboratory. A common frame hive was first used, in which were placed specimens of foul brood. The hive was charged with gas by heating formalin in a closed vessel which was in communication with the hive; 15 c. c. was used each time and evaporated to dryness. The charging of the hive with gas was repeated in this way at the end of 2, 4, 6, and 20 hours. Before each charging and at the end of 24 hours after the first application of gas, cultures were made. Of all the tubes inoculated 90 per cent showed *Bacillus alvei* to be present. There was no decrease in the number of tubes in which *Bacillus alvei* appeared following the several applications of formaldehyde gas.

The examination of specimens of foul brood which had been treated with the gas by an apiarist gave the following results:

Thirty tubes which were inoculated from larvæ, capped and uncapped, showed the presence of *Bacillus alvei* in 21.

Thirty tubes which were inoculated with pollen in cells gave *Bacillus alvei* in 28.

Four series of agar plates showed apparently no diminution in the number of bacteria present.

Further experiments were made by using Novy's anaërobic jar (a very tight chamber) as a chamber in which to put the diseased brood combs and cultures. This vessel will retain the gas much more perfectly than the devices made for practical use in the apiary. Treatment of brood in this jar by recharging with the gas resulted usually in complete disinfection after 2 days. Agar plates containing spores and cheese cloth on which cultures were spread and dried were disinfected after a short length of time by the application of formaldehyde gas.

From the experiments made the conclusion can be drawn that formaldehyde gas is a good disinfectant, but that it penetrates very slowly and that 24 hours' application of the gas to the combs, as usually applied, is not sufficient to kill all the spores in the decayed larvæ (27).

AMERICAN FOUL BROOD.

The diseased condition which we shall call American foul brood and the micro-organism found constantly present in the diseased and dead larvæ, which we shall call *Bacillus larvæ*, were, for convenience, referred to, respectively, as "X Brood" and *Bacillus* "*X*" in a former report (27). This disease has been called "foul brood" by many bee keepers in this country and in other countries as well. It is the diseased condition with which Mackenzie, Harrison, and William R. Howard were working largely, if not altogether, in their investigations of foul brood. The disorder is, as a rule, dreaded less than European foul brood by the apiarist, yet in the aggregate the bee industry suffers enormous losses from the trouble. The general character of the diseased brood is so much like that of foul brood that the two may be easily confused by those unfamiliar with the variety of appearances which one finds in each disease and a few characters which are differential. Therefore it is not strange that the mistaken diagnosis should be made from the symptoms manifested by these two diseases. When, however, European foul brood and American foul brood are subjected to a bacteriological examination, the diagnosis is easy. Experts when comparing specimens of the two diseased conditions are able to see a difference in the gross appearance.

Symptoms.

The symptoms are given by Dr. E. F. Phillips in Circular No. 79, Bureau of Entomology, as follows:

The adult bees of an infected colony are usually rather inactive and do little toward cleaning out infected material. When the larvæ are first affected they turn to a light chocolate color, and in the advanced stages of decay they become

darker, resembling roasted coffee in color. Usually the larvæ are attacked at about the time of capping, and most of the cells containing infected larvæ are capped. As decay proceeds these cappings become sunken and perforated, and, as the healthy brood emerges, the comb shows the scattered cells containing larvæ which have died of disease still capped. The most noticeable characteristic of this infection is the fact that when a small stick is inserted in a larva which has died of the disease, and slowly removed, the broken-down tissues adhere to it and will often stretch out for several inches before breaking. When the larva dries it forms a tightly adhering scale of very dark brown color, which can best be observed when the comb is held so that a bright light strikes the lower side wall. Decaying larvæ which have died of this disease have a very characteristic odor, which resembles a poor quality of glue. This disease seldom attacks drone or queen larvæ. It appears to be much more virulent in the western part of the United States than in the East.

A microscopic preparation from the diseased, but not dead larvæ, or from larvæ recently dead, at first shows a few comparatively long slender rods; later these increase rapidly in number, and spores also are seen. In the later stages of decay in the ropy mass and the dried scales spores only are found; these occur in very large numbers. When this investigation was begun, in 1902, it was observed (26) that in the dried dead larvæ there are very large numbers of spores, but these, when inoculated into the media commonly used in the laboratory, fail to grow. The cultures were sterile, except for an occasional contamination.

The Present Investigation.

The following samples from different sources were examined in 1902:

Results of examination of specimens of American foul brood diagnosed by the experts at that time simply as "foul brood."

Brood sent by—	Date.	Source.	Bacteriological findings.
Charles Stewart	June 12	New York	No growth.
W. D. Wright	Sept. 19	Wisconsin	2 unidentified bacilli.
W. D. Wright	Oct. 19	Canada	No growth.
W. D. Wright	Nov. 11	Wisconsin	No growth; 4 samples.

Inasmuch as *Bacillus alvei* was absent, it is evident that this condition is not European foul brood (26).

In 1903 the investigations were continued. Several media were devised in which it was hoped that it would be possible to obtain a germination of the spores which were observed the year before and which failed to grow on our ordinary media. The one which proved successful was prepared as follows: Larvæ are picked from the brood combs of a number of frames of healthy brood and a bouillon (bee-larvæ bouillon) is made from them following the same directions as when bouillon is made from meat. Our first growth from these

spores was secured in an agar (bee-larvæ agar) made from this special bouillon when Liborius's method for cultivating anaërobes was used.

The technique for making cultures successfully from the diseased material is not difficult if the following method is used: Place a loopful of the decayed tissue of the larvæ into a tube of bouillon; heat to 65° C. for 10 minutes to kill any vegetative forms which might be present; incubate for 12 hours, and heat again to 65° C. for 10 minutes. This is usually sufficient, but it may be necessary to repeat the same process. Liquefied bee-larvæ agar in a test tube is then inoculated and incubated. The successive heating will destroy the vegetative stage of any spore-producing species which is common about the apiary, *e. g.*, members of the group represented by *Bacillus A*, as described on pp. 13–14 of this paper. Agar slant and bouillon, when inoculated from this source, remain sterile; but when bee-larvæ agar is used a slow but abundant growth takes place. Under certain conditions the growth appears very near or at the surface when cultures are made in the above manner. A surface growth can be obtained after a few generations by reinoculating slant agar of this same medium.

The above method was used successfully in diagnosing the following samples from different apiaries:

Results of examination of specimens of American foul brood, formerly called simply "foul brood."

Brood sent by—	Date.	Source.	Bacteriological findings.
W. D. Wright	Oct. 19, 1902	Canada	*Bacillus larvæ.*
W. D. Wright	Nov. 11, 1902	Wisconsin	*Bacillus larvæ.*
W. D. Wright	Nov. 11, 1902	Wisconsin	*Bacillus larvæ.*
C. H. W. Weber	July 24, 1903	Ohio	*Bacillus larvæ.*
N. D. West	Aug. 3, 1903	Broome County, N. Y.	*Bacillus larvæ.*
N. D. West	Aug. 3, 1903	Broome County, N. Y.	*Bacillus larvæ.*
N. D. West	Aug. 3, 1903	Chenango County, N. Y.	*Bacillus larvæ.*

The results of these examinations show that *Bacillus larvæ* was present in all the specimens examined, which suggests that it very probably figures as an etiological factor in this disease. Other bacteria of different species are occasionally found associated with this bacillus.

Bacillus larvæ.

Occurrence.—Constantly present in diseased brood from colonies affected with American foul brood.

Gelatin.—There is no growth.

Morphology.—It is a slender rod, having a tendency to form in chains. This is especially true when grown in bee-larvæ bouillon.

Motility.—The bacillus is rather sluggishly motile.

Spores.—Spore formation takes place. This can be observed best in the different stages of the disease and decay of the larvæ.

Oxygen requirements.—When Liborius's method is used, the best growth usually appears near to but not on the surface. After a few generations a surface growth may be obtained.

Bouillon.—There is no growth.

Glucose bouillon.—There is no growth.

Lactose.—There is no growth.

Saccharose.—There is no growth.

Agar plate.—There is no growth.

Bee-larvæ agar.—The inoculations must be made with the medium liquefied. The growth takes place near to but rarely on the surface. Cultures must pass thru a few generations before a satisfactory surface growth can be secured.

Bee-larvæ agar slant.—On the surface of this medium a thin, gray, nonviscid growth takes place.

Glucose agar.—Slight growth has been observed in the medium. No gas is produced.

Potato.—There is no growth.

Milk.—There is no growth.

Litmus milk.—There is no growth.

Fermentation.—In bee-larvæ bouillon no gas is produced.

Indol.—There is no growth in sugar-free bouillon.

THE SO-CALLED "PICKLE BROOD."

The name "pickle brood" was given by Dr. William R. Howard, of Fort Worth, Tex., to a disorder found in the brood of bees. He stated that the cause of the disease was a specific fungus which he called *Aspergillus pollinis*. His results have not been confirmed by other investigators.

The bee keepers are sustaining a loss from a diseased condition in their apiaries which they are diagnosing as "pickle brood." The larvæ usually die late in the larval stage. Most of them are found on end in the cell, the head frequently blackened and the body of a watery, granular consistency.

The following table gives a summary of the results of an examination of specimens received labeled "pickle brood:"

Results of examination of specimens of so-called "pickle brood."

Brood sent by—	Date.	Bacteriological findings.
W. D. Wright	June 17, 1902	Two unidentified micrococci.
W. D. Wright	July 31, 1902	No growth.
W. D. Wright	Aug. 4, 1902	No growth.
M. Stevens	Aug. 20, 1902	Unidentified bacilli.
W. D. Wright	Sept. 2, 1902	Unidentified bacilli.
W. D. Wright	June 24, 1903	Unidentified bacilli and yeast.
N. D. West	Aug. 5, 1903	No growth.
M. Stevens	Aug. 20, 1903	No growth.

The results of the examinations show that *Aspergillus pollinis* was not found. Further investigations must be made before any conclusion can be drawn as to the real cause of this trouble.

THE SO-CALLED "BLACK BROOD."

In 1890 some specimens of diseased brood were sent from New York State to Dr. William R. Howard, of Fort Worth, Tex., and unfortunately, after a short and inadequate study of the disease, he

reported it to be a new disease and called it "New York bee disease" or "black brood." He described as its cause a species of bacteria which he called *Bacillus millii* (31).

In our investigations of this diseased condition, which have covered five years, we have not found an organism corresponding to *Bacillus millii* in any of the specimens that we have received; but we have found *Bacillus alvei*, the supposed cause of foul brood, to be present constantly in samples of brood which the bee experts of New York State say are samples of the same diseased condition as that received by Howard.

From this we conclude that the diseased brood that has received the name of "New York bee disease" or "black brood" is really genuine European foul brood.

PALSY OR PARALYSIS.

The disease known to the apiarists as palsy or paralysis attacks the adult bees. The name is suggestive of the symptoms manifested by the diseased bees. A number of bees affected were received from Messrs. W. D. Wright and Charles Stewart, taken from apiaries in New York State. In 1903 bacteriological examinations were made of a number of bees so affected. Several species of bacteria were isolated and some experimental inoculations made, but no conclusions could be drawn from the results obtained as to the cause of the disorder.

From a study of the normal flora of the bee it was soon found that we had here quite a number of species of bacteria present. This fact stimulated a study of the normal flora, the results of which are recorded in Part I. From this point the work can be carried on with the hope that, if the disease has a bacterium as an etiological factor, it may be found. It is believed by some bee keepers that *Bacillus gaytoni* of Cheshire is the cause of paralysis, but this is not claimed by Cheshire, and the belief is not grounded on bacteriological findings.

SUMMARY TO PART II.

Following is a brief summary of the results of the present investigation of bee diseases:

(1) There are a number of diseased conditions which affect the apiary.

(2) The disease which seems to cause the most rapid loss to the apiarist is European foul brood, in which is found *Bacillus alvei*—first isolated, studied, and named by Cheshire and Cheyne in 1885.

(3) The distribution of *Bacillus alvei* in the infected hive is as follows:

(*a*) The greatest number of infecting germs are found in the bodies of dead larvæ.

(*b*) The pollen stored in the cells of the foul-brood combs contains many of these infecting organisms.

(*c*) The honey stored in brood combs infected with this disease has been found to contain a few bacilli of this species.

(*d*) The surface of combs, frames, and hives may be contaminated.

(*e*) The wings, head, legs, thorax, abdomen, and intestinal contents of adult bees were found to be contaminated with *Bacillus alvei.*

(*f*) *Bacillus alvei* may appear in cultures made from the ovary of queens from European foul-brood colonies, but the presence of this species suggests contamination from the body of the queen while the cultures are being made and has no special significance.

(4) The disease which seems to be most widespread in the United States we have called American foul brood, and the organism which has been found constantly present in the disease we have called *Bacillus larvæ.* This disorder was thought by many in this country and other countries as well to be the foul brood described by Cheshire and Cheyne, but such is not the case.

(5) From the nature of American foul brood it is thought that the organism has a similar distribution to that of *Bacillus alvei.*

(6) It appears that European foul brood was erroneously called "New York bee disease" or "black brood" by Dr. Wm. R. Howard in 1900.

(7) There is a diseased condition affecting the brood of bees which is being called by the bee keepers "pickle brood." No conclusion can be drawn from the investigation so far as to the cause of the disease.

(8) *Aspergillus pollinis*, ascribed by Dr. William R. Howard as the cause of pickle brood, has not been found in this investigation and is not believed by the author to have any etiological relation to the so-called "pickle brood."

(9) Palsy or paralysis is a diseased condition of the adult bees. No conclusion can yet be drawn as to its cause.

(10) Formaldehyde gas as ordinarily used in the apiaries is insufficient to insure complete disinfection.

CONCLUSIONS.

In a paragraph the author wishes, if possible, to present the status of the bee diseases in this country. It should be remembered, firstly, that "black brood" can now be dropt from our vocabulary, and probably does not exist; secondly, that the term "foul brood" was being applied to two distinct diseases. One of these diseases we now refer to as European foul brood, because it first received a scientific study from a European investigator. We refer to the other disease as American foul brood, because it was first studied scientifically in America. There is one more disorder in the brood of bees which has attracted considerable attention—the so-called "pickle brood." There are, then, these three principal diseases: European foul brood, American foul brood, and the so-called "pickle brood."

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INTRODUCTION

European foulbrood is an infectious disease of the brood of bees caused by *Bacillus pluton*. It is characterized by the death of brood during its uncapped stage and by the absence of any marked odor. The disorder has a wide distribution and is fairly well known to beekeepers. The losses sustained by the infected apiary vary from a slight weakening of the colonies in some instances to the destruction of all of them in others.

Practical apiarists have determined much concerning the disorder while pursuing their profession. The writer in an earlier paper (15)[1] referred to the nature and extent of the progress that had been made in the study of the disease from the laboratory point of view. The present paper deals with results which have been obtained from a continuation of the work. Among the problems considered are: The re-

[1] Figures in parentheses refer to "Literature cited," p. 34.

sistance of *Bacillus pluton* to heat, drying, sunlight, fermentation, and disinfectants; the effect of the disease on the colony and on the apiary; and the transmission, diagnosis, and prognosis of the disease. Work directly on the treatment of the disease has not been attempted by the writer. Naturally, however, any treatment that is devised, if it is to be efficient and at the same time economical, must be based upon results obtained from the solution of such problems as those which have received attention in these studies.

Results obtained from a study of the disease in the laboratory and in the experimental apiary form the basis of the discussions contained in the present paper. Since the disease encountered in nature is very similar to the one produced by artificial inoculation, the importance of the studies is at once evident.

The paper [1] will be of interest, it is believed, not only to the apiarist who may wish to apply the facts here determined in the pursuit of his profession, but also to the investigator whose desire primarily is a further study of the disease.

NAME OF THE DISEASE

The term "foulbrood" was quite generally used in the past, as it still frequently is, for the two infectious diseases now known in America as European foulbrood and American foulbrood. In 1885 when Cheshire and Cheyne (4) in England made their studies on foulbrood and described *Bacillus alvei*, evidently they were not convinced that there were two distinct diseases that were being called by the one name foulbrood. The disease studied by them is the one which is the subject of discussion in the present paper. In the names for the two diseases it will be observed that the word "foulbrood" is retained in both instances. To this "European" is added for the disease on which early laboratory studies were made by these Europeans (Cheshire and Cheyne).

Dr. William R. Howard (6), of Texas, in 1900, worked for a brief period with this disease, reached the conclusion that it was a new one, and referred to it by the names "New York bee disease," or "black brood." Work by Moore and White (11) in 1902 showed that the disease was not new, but was the foulbrood studied by Cheshire and Cheyne (4). The names "New York bee disease," or "black brood," therefore, were superfluous, and as their use would have added to the confusion that already existed they were discarded. Beekeepers, entomologists, and pathologists, as a rule, are more or less familiar with the terms "foulbrood" and "*Bacillus alvei*." Usually, however, the ropy foulbrood—American foulbrood—is the one that is thought of,

[1] The present studies are similar to those made by the writer on sacbrood (17), Nosema-disease (18), and American foulbrood (19). A reference to these papers may be found helpful where the discussions in the present one are especially brief. The investigations were completed in September, 1916, and the paper was submitted for publication in October, 1918.

and the one that frequently has been associated in the literature with *Bacillus alvei*. This is unfortunate. While *B. alvei* is not the cause of any bee disease, it occurs very frequently with European foulbrood and is found only seldom in the ropy disease. In using the names European foulbrood and American foulbrood it is possible, however, to avoid confusion by bearing well in mind the history of the disease.

HEALTHY LARVÆ OF THE AGE AT WHICH THEY DIE OF EUROPEAN FOULBROOD

Bees dying of European foulbrood do so during the larval stage.[1] Death may take place at any time from the fourth day of larval life to pupation. For convenience of description the brood of the age at which death from European foulbrood occurs is placed here in three groups. Groups 1 and 2 include the uncapped and group 3 the capped larvæ.

GROUP 1

The youngest larva (Pl. II, D, G) that dies of European foulbrood practically covers the bottom of the cell. It lies either on its right or its left side, with its dorsal portion extending to the lateral walls of the cell. Its form is **C** shaped with the anterior and posterior extremities almost together. Its color is bluish white with a glistening surface, presenting a pearly appearance. The body is more or less opaque, due largely to the adipose tissue. Folds and furrows divide the surface into segments. In health these are quite prominent and the entire larva is turgid in appearance.

With the unaided eye spiracles and tracheæ can be seen with difficulty, but by slight magnification they are readily observed. Most of the tracheæ, appearing as white lines, extend either dorsally or ventrally on the lateral side of the larva, but a distinct chain connecting them will be observed to extend at right angles to these.

GROUP 2

Healthy larvæ (Pl. III, D, G) slightly older than those described in Group 1 constitute Group 2. The larva now completely fills the bottom of the cell. The dorsal side pressing against the lateral side walls of the cell causes the contour of the body to be in general hexagonal. The tracheæ are seen less easily than in younger larvæ, while the color, glistening appearance, prominence of segments, and turgidity are similar to those of the younger larvæ described in Group 1.

By turning the larva so that its dorsal surface may be brought into view (Pl. III, A) there is observed a more or less transparent narrow

[1] The term larvæ as used in the present paper applies to the prepupæ as well as to earlier stages of the brood.

area along the dorsal median line extending nearly the length of the body. The contents of the stomach may be seen through this area. The color of the mass is due chiefly to the presence of pollen. It is usually some shade of yellow. The median area presents in its appearance a sharp contrast to the bluish-white, opaque portions on either side of it. Similar appearances are to be noted in the larvæ of Group 1.

The larva removed from the cell performs only slight movements, lies partly coiled, and is more or less turgid. The segments are prominent. When the body wall is torn there flows from the ruptured wall the clear larval blood, in which are suspended often fat and other tissue cells which give to it a somewhat milky appearance. The stomach, a transparent tube easily torn into segments, contains a mass of partially digested food, pollen constituting usually a conspicuous portion of it.

GROUP 3

Group 3 consists of capped larvæ. These are, therefore, larger than those described in Groups 1 and 2. In the group are included the larvæ which have spun a cocoon as well as those which have not. An endwise position in the cell may or may not have been assumed. The larvæ are seen in various positions. Not infrequently some portion of the dorsal surface is turned toward the observer, the narrow, median, transparent area being in evidence as in younger larvæ. Healthy larvæ occupying an endwise position are described in papers on sacbrood and American foulbrood (17, 19) and will not be referred to further at this time.

SYMPTOMS

In European foulbrood, as in other brood diseases, the colony as a whole and not the individual bee should be considered as the unit in the discussion of the symptoms of the disease. The description of the symptoms recorded in the present paper is based chiefly upon observations made on the disease produced through artificial inoculations. In making the studies in the experimental apiary observations made by beekeepers have been duplicated and new facts determined. It has been possible also to locate errors which have been made in discussions of symptoms of the disease.

GENERAL SYMPTOMS FROM A CASUAL EXAMINATION

Death of brood during the feeding stage, in uncapped cells, is a characteristic of European foulbrood. The brood nest in the disease usually presents an irregular appearance, capped cells and uncapped ones being found scattered irregularly over the brood frames, giving to them the "pepper box" appearance (Pl. I) often referred to by

beekeepers, a condition noticeable when the disease is fairly well advanced in the colony.

The dead larvæ lose their pearly whiteness and assume a yellowish color, later becoming brownish. This deepens often to a dark brown. The decaying remains are not characteristically ropy, as in American foulbrood. Marked viscidity is usually absent. When it is present the decaying mass can be drawn into threads but to a less extent than in the ropy disease. In advanced cases the disease may be accompanied by an odor, but in the writer's experience this never has been marked and never offensive.

As the disease in the colony advances, weakness becomes a symptom. In severe cases queenlessness may result from the infection. This, however, is by no means the rule.

SYMPTOMS MANIFESTED BY INDIVIDUAL LARVÆ SICK OR DEAD OF EUROPEAN FOULBROOD

Evidences of European foulbrood in the individual larvæ appear before and after death. The colony symptoms used most frequently in the diagnosis of the disease are largely post-mortem appearances of larvæ. Of much interest and frequently of considerable diagnostic value are the symptoms manifest by larvæ sick but not dead of the disease. For convenience in the description of the appearances of the sick or dead larvæ, the grouping used in describing the healthy larvæ (p. 3) is followed. The appearances of affected larvæ both living and dead are, of course, changing constantly. A description which is correct for one day or hour, it should be realized, is not likely to be entirely correct for the next.

GROUP 1

The youngest larvæ manifesting symptoms of European foulbrood are approximately 4 days old (Pl. II, A, B, C, E, F, H, I). In many cases at this stage of the disease a peristalsis-like movement of the body is marked and is readily observed by the unaided eye, but in others no such bodily movements are observed. The diseased larvæ at the time may be more transparent (Pl. II, B, H) than healthy ones of the same size. In such larvæ the tracheæ are quite prominent and more readily seen than in healthy ones. Occasionally numerous minute opaque areas are observed in these more transparent larvæ, giving to them a punctate appearance. Very often, however, this sign is not present. In many instances, indeed, no distinct symptom is observed until the larva approaches death. (Pl. II, A).

Larvæ (Pl. II, A, B, C, E, H, I) of this group dying or just dead of the disease lose their marked glistening appearance; their pearly whiteness gives way to a yellowish tint; the turgidity seen in healthy larvæ is diminished in the sick; and the folds and furrows indicat-

ing the segments of the body become less prominent. As the process of decay advances the yellowish hue changes, the color assuming a brownish tone. The segmental markings are less prominent, while the tracheæ often become quite distinct, appearing as white lines contrasted with the darker color of the larval remains (Pl. II, B). Not infrequently at this time there will be seen a chitinous envelope containing a watery-looking fluid in which is the larva proper (Pl. II, C; Pl. IV, A). The decay proceeds and the drying becomes evident. The larval mass settling upon the concave bottom of the cell causes the upper surface of the mass to be depressed about the center. At this stage the tracheæ not infrequently are seen distinctly in the drying mass. When the larval remains become dry they are known as the scale (Pl. II, F). The scales do not adhere closely to the cell and when removed are found to be thin and more or less circular in outline. They are convex and smooth on the side which was in contact with the bottom of the cell while the opposite surface—the one which, while in the cell, was toward the observer—is slightly roughened and concave.

GROUP 2

Larvæ (Pl. III) showing symptoms of European foulbrood and classed in this group have reached a sufficient size to fill the deepest third or more of the cell. The yellowish tint appears in contrast to the bluish white of the healthy larvæ (Pl. III, D, G). Increased movement may or may not be observed. Before and after death the remains may assume one of a number of positions in the cell. Not infrequently a portion of the dorsal surface is turned toward the observer (Pl. III, B). Usually through the transparent area along the median dorsal line a whitish or yellowish-white mass is to be observed. This mass is within the stomach of the larva and contains a large amount of bacterial growth (Pl. VIII, a, b, c) consisting very largely of *Bacillus pluton*. Often before death this mass is seen to move within the stomach in response to the peristalsis-like movements of the body of the larva.

At the time of death the larva usually occupies some unnatural position, being more or less curled up and lying upon the floor of the cell (Pl. III, C, E, F, H, I). Lessened turgidity, a relative dullness of the surface appearance, and a yellowish tint are present. Not infrequently the two ends of the larva are directed more or less toward the bottom of the cell and some portion of the dorsal surface is toward the opening of it (Pl. III, E, H, I). Among the dead larvæ will be found some with one end directed toward the bottom, and the other toward the mouth of the cell, the body occupying a more or less spiral position against the side walls and floor of the cell (Pl. III, F).

Later the dead larval remains assume a brownish tint which deepens to varying shades as decay continues and drying takes place. During the early part of the decay, the firmness of the body wall permits the removal of the larva intact from the cell. Later, however, it offers but little resistance and is easily ruptured. The decaying mass before drying often attains a certain amount of viscidity. Sometimes it is of a doughy consistency, at other times it is purulent or sputum-like, while at times it assumes a viscidity that will permit of its being drawn out to the extent of an inch or more. When the larval mass becomes dry it forms an irregular scale, usually brown in color, lying on the floor or side wall of the cell or both, but not adhering closely to them.

GROUP 3

A larva dying of European foulbrood after being capped may be found occupying one of many positions within the cell (Pl. IV, C, D, E; Pl. V, D, E, F, G, H). Dying before the two-day quiescent period that precedes pupation, the remains during decay and as a scale resemble in many respects those of larvæ described in group 2. The dry scales occupy usually an irregular position on the floor of the cell (Pl. IV, F, G). Dying during the two-day quiescent period, however, the scales (Pl. V, F, I) resemble very much those of larvæ dying at the same age of American foulbrood. The larval mass assumes the brownish hue which deepens as the decay advances, reaching a dark brown. Viscidity is present in the decaying larval mass, but the extent to which the decaying material may be drawn out is less than in American foulbrood. The scale is less brittle and more rubberlike.

At no time has the writer observed pupæ dead of European foulbrood. If they die of the disease it is a rare occurrence.

The removal of larvæ sick or dead of the disease is accomplished to a greater or less degree by adult workers. The larvæ are either partially or entirely removed. This is usually done piecemeal. In an infected colony will be found, therefore, the remains of larvæ of different ages (Pl. IV, B) and (Pl. V, A) in varying numbers.

ETIOLOGY

PREDISPOSING CAUSES

Age.—Infection in European foulbrood takes place during the feeding stage and at some time after the first day of larval life, the larvæ being more often 2 days of age, or older. Death takes place somewhat more than 2 days from the time of infection. As a rule, therefore, a larva has passed its fourth day of larval life before death from European foulbrood occurs. From this age to pupation larvæ may die of the disease. The writer has not encoun-

tered death among brood which has reached the pupal stage. Adult bees are not susceptible to infection.

Sex.—Worker, drone, and queen larvæ are all susceptible to infection with *Bacillus pluton* and any of these may die of European foulbrood.

Race.—Complete immunity from European foulbrood has not been found among the races of bees studied. Experimental work recorded in the present paper involved the use of at least five colonies of "tested Italians," two of "tested Carniolans," and two of "tested Caucasians." For the most part the bees used were "untested Italians," but among the colonies were a few common blacks. In all these strains the disease was readily produced through experimental inoculation. The examination of numerous samples of diseased brood received from beekeepers throughout the United States suggests that all races commonly kept by American beekepeers are susceptible to European foulbrood. The relative immunity of the different races has not been demonstrated by the studies. These facts, however, do not dispute the observation by practical beekeepers that some strains of bees show a greater colony resistance than others.

Climate.—From reports of studies made in Austria by Muck (12), in Denmark by Bahr (1), in England by Cheshire and Cheyne (4), in Germany by Zander (20), and in Switzerland by Burri (3), it is clearly evident that the disease discussed in the present paper occurs in these different countries. It has been encountered also in many sections of the United States and Canada. This distribution shows that the infection can exist under a variety of climatic conditions. The practical import of the fact is that the presence of European foulbrood in any locality can not be attributed entirely to the climate of the region.

Season.—Beekeepers have observed that European foulbrood occurs with greatest severity before midsummer rather than later in the season. The disease, it has been shown experimentally, can be produced, however, at any season of the year at which brood is being reared. Its severity at any given season is to be attributed, therefore, to environmental conditions rather than to the difference in the susceptibility of larvæ during the different seasons.

Food.—As in American foulbrood it is found that the cause of the disease in the colony is governed very little if at all by the quality of food gathered by bees. Indirectly, however, the quantity present in the hive or obtainable often does influence its course materially.

EXCITING CAUSE

That *Bacillus alvei* may be present in large numbers in brood dead of foulbrood was demonstrated by Cheshire and Cheyne (4) in 1885.

For a decade and a half following the observation the belief was quite general that this bacterium was the exciting cause of a bee disease. The view was then seriously challenged. In 1906 the only positive conclusion in regard to the relation between European foulbrood and *Bacillus alvei* that could be drawn by the writer (13) was that this species occurs in brood dead of the disease.

William R. Howard (6), of Texas, after a brief study of the disease reported in 1900 the presence of an organism which he called *Bacillus millii*. He cultivated the species apparently with ease. In 1904 Bahr (1) in Denmark found a small oval bacterium in a brood disease in which larvæ dying in uncapped cells are yellowish in color and not ropy in consistency. Burri (3) in 1906 encountered in his studies on the brood diseases a small bacterium which he referred to as *guntheri*-forms. The species was cultured and compared with *Bacterium guntheri* and found to be somewhat different. In 1907 Maassen (7) obtained from brood material cultures of a species which he named *Streptococcus apis*. White (14) in 1908 reported the presence of a small organism in European foulbrood which had refused to grow on artificial media. The species was not the one, therefore, with which the investigators just referred to had worked. That this organism might be the exciting cause of the disease was noted. Pending more information regarding it, the species was not given a name but was referred to as bacillus "Y." That this species bears a direct etiological relation to the disease was demonstrated in 1912 by the writer (15) and the name *Bacillus pluton* was then given to it.

As the cultivation of *Bacillus pluton* on artificial media had not been accomplished the conclusion that it is the exciting cause of European foulbrood was arrived at by eliminating all other possible agencies. The observations furnishing the proof appear in an earlier paper (15). By demonstrating *Bacillus pluton* to be the cause of the disease, *Bacillus alvei*, *Streptococcus apis*, *Bacterium eurydice*, and *Bacillus orpheus*, and still other species occasionally encountered, were thereby proven to be secondary invaders.

To eliminate the possibility of a filterable virus in European foulbrood 10 colonies were inoculated with filtrates obtained from aqueous suspensions of brood sick and dead of the disease. In six instances the Berkefeld N filter was used and in four the Pasteur-Chamberland F was employed. In no case was the disease produced. Studies recorded in the present paper on the resistance of *Bacillus pluton* to heating, drying, fermentation, and disinfectants show that when the virus of the disease is not destroyed this species is still alive. This fact is further evidence in support of the conclusion that the species *Bacillus pluton* is the virus of the disease.

BACILLUS PLUTON

An artificial medium for the cultivation of *Bacillus pluton* has not yet been devised. To accomplish this may or may not be a particularly difficult task. The media ordinarily used in the laboratory are not suitable. Bee-larvæ agar, brood-filtrate media, egg-yolk-suspension agar (19), and combinations of these have not thus far proved sufficient for the purpose. The species is an unusual one. The generic classification has not been determined definitely and this may not be possible until the proper condition for the artificial cultivation of the species has been supplied.

The morphology of *Bacillus pluton* is somewhat variable. In very early infection its form is that of a short rod in pairs or in chains, or possibly of a coccus with the individuals similarly arranged (fig. 1; Pl. VII, B). The length is then equal to or somewhat greater than the breadth. In slightly later stages of infection the predominating form is that of a lancet-shaped coccus (fig. 1; Pl. VII, A), and in late stages this form is present almost exclusively. The lancet form occurs singly, varying greatly in size and having a length which approximates twice the width. The length is more often less than 1 μ than greater. The organism colors uniformly with the aniline stains, stains with iron hematoxylin, and is gram-positive. It does not form spores. This is evidenced by the microscopic appearance and also by the thermal death point of the species. Its resistance to drying, disinfectants, and other environments is discussed later in the present paper.

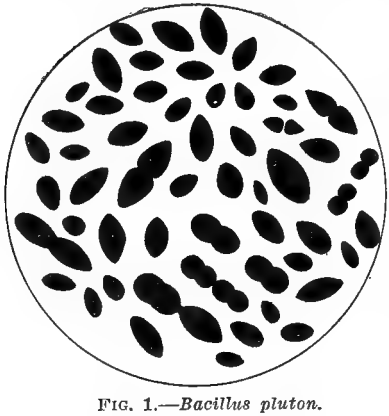

FIG. 1.—*Bacillus pluton.*

Seven rabbits inoculated, six subcutaneously and one intraperitoneally, with a suspension of larvæ dead of European foulbrood proved to be refractory. Only a slight rise of temperature followed the inoculations and the weight was not materially affected. Six guinea pigs inoculated subcutaneously with similar material proved not to be susceptible to infection with the species. Four pigeons inoculated in the pectoral muscles and two white rats inoculated subcutaneously also proved refractory. In none of these inoculated animals were there any lesions of particular note produced.

Growth of *Bacillus pluton* in the infected larva begins close to the surface of the peritrophic membrane (Pl. VII, I) in contact with the food of the larva. As growth continues the bacterial mass extends toward the center of the lumen of the peritrophic sac (Pl. VII, K),

finally filling it more or less completely (Pl. VII, J). The growth does not always take place uniformly along the peritrophic membrane (Pl. VII, J), nor does it extend beyond it (Pl. VII, I, J, K), but is inclosed within the sac, the tissues of the larvæ not being reached. The multiplication of the organism after the death of the host, if, indeed, it takes place at all, is limited.

Secondary invaders, chiefly *Bacillus alvei*, *Bacterium eurydice*, *Streptococcus apis*, and occasionally *Bacillus orpheus*, and a few others, are encountered at various stages of the disease and during the decay of the larva. During the life of the larva these species also remain within the peritrophic sac.

BACILLUS ALVEI

Bacillus alvei (fig. 2; Pl. VII, D, F) is present very frequently and in very large numbers in larvæ dead of European foulbrood. The species was well described by Cheyne (4). Descriptions may be found elsewhere also (11, 13). It is readily recognized and may be differentiated easily from other spore-producing species occasionally encountered in the diseased brood.

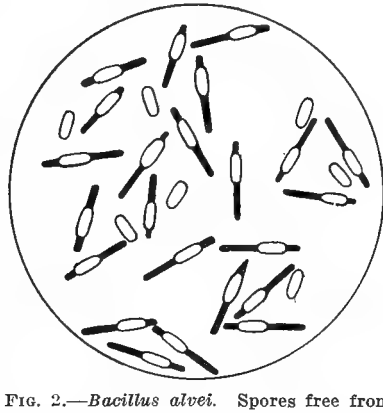

FIG. 2.—*Bacillus alvei*. Spores free from and others within rods.

Bacillus alvei is not the active cause of any bee disease. It seems probable, however, that it plays a rôle in European foulbrood, but the extent is not fully known. The species is present usually, if not invariably, in large numbers in the rubberlike scales (Pl. V, F, I), which resemble so much those of American foulbrood. The decayed larval mass, which forms the scale, before becoming dry is ropy in consistency similar to that of American foulbrood but to a less degree. It seems probable that this ropiness is due more or less directly to *Bacillus alvei*. On account of this viscidity the decaying mass, as well as the scales, are removed with greater difficulty than are most larvæ dead of European foulbrood. The result, as often observed, is that these brown viscid decaying larvæ or the rubberlike scales resulting from them are the only evidence that European foulbrood is present in the colony.

While *Bacillus pluton* in such larval masses and scales is often difficult to detect microscopically, its presence can be demonstrated through the experimental inoculation of healthy larvæ. Inasmuch as *Bacillus pluton* will live for a considerable period in the scales, it

seems quite probable that the disease might in some cases be carried over for months or even over winter through the medium of these rubberlike scales.

It is of interest to know that the amount of disease resulting immediately from inoculations in which scale material is used is much less than when larvæ recently dead of the disease are used. This is true also of dead larvæ stored in Petri dishes compared with smears allowed to dry immediately from larvæ recently dead of the disease. These facts indicate a possible deleterious effect on *Bacillus pluton* of the secondary invaders multiplying in the decaying larvæ.

STREPTOCOCCUS APIS

It is most probable that *Streptococcus apis* is the species that was isolated from diseased brood by Burri (3) and referred to by him in 1906 as "*güntheri*-forms." Maassen described it in 1908 (8). The organism grows well at incubator, room, and refrigerator temperatures in most of the media ordinarily used in the laboratory. Its cultural characteristics suggest the micrococci rather than the streptococci. Confusion in some of the earlier investigations was due evidently to the resemblance of *Streptococcus apis* and *Bacillus pluton* morphologically. To this fact is due the chief interest in the species *Streptococcus apis*. When encountered in larvæ dead of European foulbrood it can be identified readily by culturing. The generic position of this species should be considered as being not altogether certain.

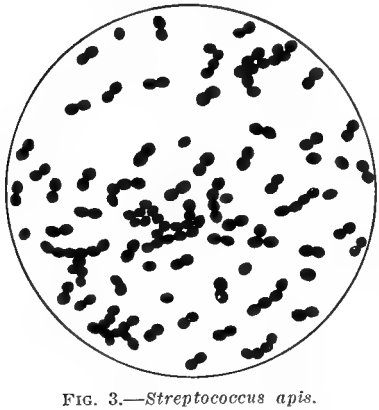

FIG. 3.—*Streptococcus apis.*

Occurrence.—*Streptococcus apis* is occasionally encountered in larvæ dead of European foulbrood and often is present in large numbers.

Morphology.—It is more or less spherical (fig. 3; Pl. VII, E), occurring singly and in pairs with occasionally a chain of 2 or more pairs when grown in liquid media. In larval remains not infrequently the ends may be somewhat pointed.

Staining properties.—It colors uniformly and readily with the common stains, and retains the stain after Gram's method.

Glucose agar plate.—Within a day growth is visible. Colonies never become large. Surface colonies are usually less than 2 mm. They are circular with uniform outline and a well-defined border, are grayish by reflected and bluish by transmitted light, are smooth and convex, are moist and glistening in appearance, and are friable in consistency. When magnified the surface colonies appear light brown in color, and granular in structure, the density decreasing from the center to the periphery. Deep colonies appear dense, dark brown, and coarsely granular. They are in general lenticular to oval but are sometimes almost spherical in form.

Glucose gelatin plate.—At refrigerator temperature and within 3 days, the surface colonies begin to liquefy the gelatin, each liquefied area appearing somewhat as a minute drop of water.

Agar slant.—In one day numerous gray colonies cover the inoculated surface.

Bouillon.—Within a day the medium is uniformly and moderately clouded.

Fermentation.—In glucose, lactose, saccharose, levulose, maltose, and mannite bouillons, a uniform clouding of the media occurs. The growth takes place in both arms of the tube, but is heavier in the open one. Considerable acidity, but no gas, is produced.

Milk.—Milk is rapidly coagulated. Digestion of the coagulum follows. In from 3 to 5 days more than one-half has been changed. Within 24 hours the color is discharged in litmus milk, except at the top of the medium. In other respects it is like the plain milk.

Potato.—No visible growth. That growth in the potato water takes place is confirmed by microscopic examination.

Gelatin stab.—Liquefaction along the line of puncture is appreciable after one day. In four days a cylinder of liquefied gelatin 1 cm. in diameter surrounds the original line of puncture and soon extends to the walls of the tube.

Pathogenesis.—No disease results when the brood of bees is fed cultures of *Streptococcus apis* either by the direct or indirect method. A rabbit and two guinea pigs inoculated with a pure culture of *Streptococcus apis* were not susceptible to infection with the species.

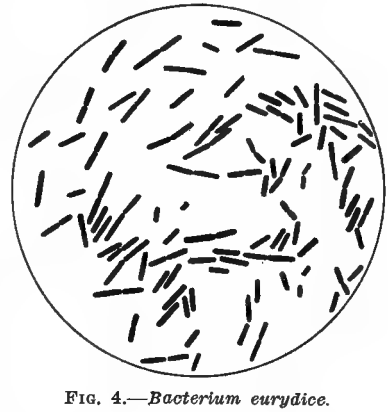

FIG. 4.—*Bacterium eurydice.*

BACTERIUM EURYDICE

The presence of this species in European foulbrood was pointed out by the writer in an earlier publication (15). Among the secondary invaders in larvæ infected with *Bacillus pluton*, *Bacterium eurydice* is one of the earliest to be found. It is often present in considerable numbers. In plating for the species the stomach contents from larvæ sick, but not dead, of the disease should be used. In studying this species cultures were isolated which in some respects differed from it. Whether these are different species or belong to a group of which *Bacterium eurydice* is a representative has not been definitely determined.

To isolate *Bacterium eurydice* the plating has been done with glucose agar. Incubation must be carried out at room temperature. Growth of the species is always slow and never luxuriant. Under favorable conditions colonies are visible after one day. To preserve cultures they must be renewed frequently.

Occurrence.—*Bacterium eurydice* is frequently present in larvæ sick or recently dead of European foulbrood.

Glucose agar plate.—To the naked eye the surface colonies are slightly convex, smooth, and glistening. They are from 1 to 2 mm. in diameter, cir-

cular and uniform in outline. The color is bluish by transmitted and grayish by reflected light. Under a two-thirds objective they are a light brown, and are finely granular near the periphery, but more coarsely granular near the center.

Morphology.—The rod (fig. 4; Pl. VII, C) is small and slender with slightly rounded ends, occurring usually in pairs or singly. It is nonmotile and no spores are produced.

Staining properties.—It is stained easily and uniformly with the ordinary aniline stains and is Gram-negative.

Oxygen requirements—Growth is better in the presence of air than in anaerobic conditions.

Bouillon.—Growth takes place slowly, producing a uniform cloudiness with no pellicle. After a week or more a somewhat viscid sediment is present.

Sugars.—Growth in the sugar media is slow, variable, and never luxuriant. Both arms may be clouded. Glucose or levulose when added improves a medium. Fermentation with gas does not take place in any of the sugars. A noticeable amount of acid is formed when glucose and levulose are used, the other sugars being less affected. A 1 per cent honey solution supports a moderate growth. Brood filtrate as a rule improves media.

Milk.—In plain and litmus milk no changes are visible.

Potato.—Growth on potato is slow. When present, the culture is for the most part grayish in color.

Gelatin stab.—A bluish gray growth appears slowly along the line of inoculation. No liquefaction follows.

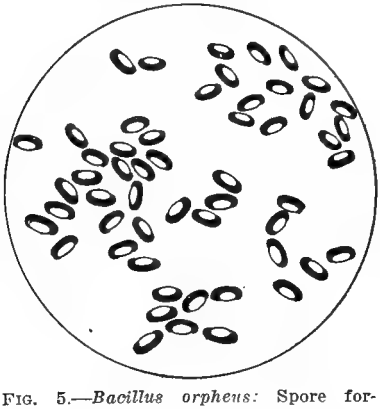

FIG. 5.—*Bacillus orpheus:* Spore formation.

Pathogenesis.—No ill results are observed when cultures of *Bacterium eurydice* are fed to healthy colonies of bees. A rabbit inoculated subcutaneously with a pure culture proved to be refractory.

BACILLUS ORPHEUS

The name *Bacillus orpheus* was given to an interesting species occasionally encountered in European foulbrood (15). In one instance the species was found very widely distributed in an apiary in which heavy losses were being sustained from the disease. In this case the dead larvæ when dry were stonelike in character, the petrified remains breaking like so much marble. Usually the species is met with in a less number of the affected larvæ. It can be readily identified from its morphology and cultural characteristics. A description of the species has been made by McCray (9). An organism similar to *B. orpheus* in many respects has been described by Laubach (5) and named *Bacillus laterosporus.*

The organism is a motile spore-bearing rod with a few peritrichic flagella. Spore formation begins in a few hours on the surface of the agar at incubator temperature, the rod swelling toward the center and becoming fusiform. Soon, as determined from stained preparations, the spore is seen occupying one side

of the rod with the protoplasm distributed along the opposite side and the two ends (fig. 5; Pl. VII, H). The rod, together with the spore within it, measures about 2.4μ in length and 1.2μ in width. This relation of spore and rod persists in cultures on a solid medium for a long period, especially at room temperature. Good growth, no gas, and only slight changes in reaction occur in the sugar media. A slight coagulum forms in the milk which is slowly digested. Gelatin is rapidly fluidified.

Bacillus orpheus is not pathogenic for the brood of bees when inoculated by feeding either by the direct or indirect method. Silkworm larvæ succumb following inoculation by feeding and also by puncture.

TECHNIQUE[1]

Artificial conditions for the successful cultivation of *Bacillus pluton* have not yet been obtained. That this can be achieved by further study is not at all improbable. Without having accomplished this, it has been possible, however, to make the studies on the biology of the parasite that were most desired. This was done through experimental work, using the larvæ of bees. The inoculations were made by feeding a suspension of the organism in sugar sirup.

Two methods were employed in making the feedings, which will be referred to here as (*a*) the indirect method, in which the colony is inoculated, and (*b*) the direct method, in which only a few larvæ are inoculated. Cane sugar and water were used in preparing the sirup in the proportion approximately of 3 to 2. This solution was then brought to the boiling point.

From 5 to 10 diseased larvæ furnish sufficient infective material when the indirect method is followed. These, after being picked from the brood frame, are thoroughly crushed, added to about 300 c. c. of the cooled sirup, and fed to a colony.[2] When the suspension contains the living virus, symptoms of European foulbrood appear in 3 days following the inoculation. The earliest evidence of disease is manifested by sick rather than dead larvæ (p. 5). Often fragments of larvæ (Pl. IV, B) are found upon examination of the brood nest.

In the direct method *Bacillus pluton* is taken from the stomachs of infected bees. Sick rather than dead larvæ are preferred for obtaining the virus free from the body tissues. By the use of dissecting needles and with a little care the stomach contents (Pl. VIII) can be pulled out of the blind end of the organ (15). The virus-containing material thus obtained is triturated with water and the aqueous suspension is added to sirup. The suspension of *Bacillus pluton* in a thin sirup is used in making the inoculation. Larvæ about 2 days old are especially desirable for the direct method. The inoculation is made by adding a small amount of the suspension to

[1] The technique in general which was found to be satisfactory for bee-disease studies is detailed to some extent in the sacbrood paper (17).

[2] The experimental colony is described in earlier papers (17, 18).

the food of the larvæ by means of a capillary pipette made from glass tubing of small bore. Care must be exercised in thus feeding the larvæ. Too much of the suspension will often float the larva. There is danger also that it will be changed in position mechanically by means of the feeding pipette. In either event the chances are that such larvæ will be removed subsequently by the bees. Considerable larval food already in the cell is advantageous. This method has proved to be especially useful in much of the experimental work recorded in the present paper. It has the advantage of being both economical as to the number of colonies needed, and definite. During the third day following the hour of inoculation symptoms of European foulbrood will be observed if infection is produced. By the fourth day frequently all of the infected larvæ will have been removed by the bees. Symptoms of European foulbrood infection manifested by larvæ sick rather than dead have proved to be especially useful for experimental purposes in these studies.

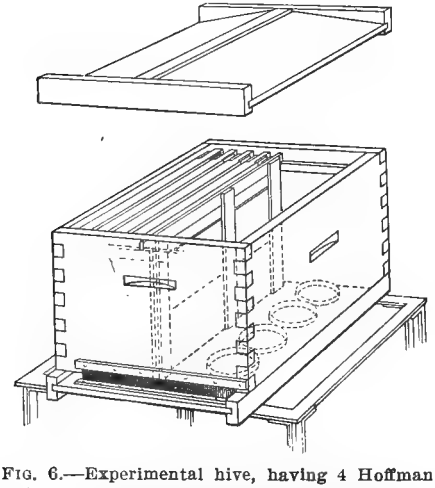

FIG. 6.—Experimental hive, having 4 Hoffman frames, a division board, Petri dishes as feeders, the entrance nearly closed with wire cloth, and the opening on the side of the hive body occupied by the frames. (Author's illustration.)

During most of the time that experimental studies are being made it is necessary to have fresh diseased material at hand. A supply can be maintained by using one or more colonies for this purpose. Repeated inoculations of the colony usually must be made at intervals of a few days or after longer periods, depending on its condition and the need for the virus. The indirect method is especially indicated in inoculating these colonies.

Frequently colonies which have been employed in European foulbrood experiments can be used again for further experiments on the disease. This must be done with some care, however. The condition of the brood always should be noted before an inoculation is made. European foulbrood colonies serve very well the purpose of experimental colonies for the other brood diseases and for Nosema-disease. In fact, not infrequently during these studies experiments on two or more of the diseases were in progress in a colony at the same time.

The apiary (Pl. VI) used in the experimental work with European foulbrood was the same as the one employed in the study of sacbrood (17), Nosema-disease (18), and American foulbrood (19). The hive (fig. 6) and the experimental colonies, where they were not the

same, were similar to those used in the other studies. The method of making the inoculations was also similar. The colonies were, therefore, in the open and the bees had free access of flight. The same precautions taken to minimize robbing, swarming, absconding, and drifting of bees were observed in the experiments with this disease as with the other diseases. All hives which had housed European foulbrood colonies were flamed before they were used again to be certain that there would be no infection from such a source. Whether the queens used had been in diseased colonies need not give one any concern. Further reference to the technique followed in the present studies will be made as the experiments are discussed.

THERMAL DEATH POINT OF BACILLUS PLUTON

The result of the experiments recorded by the writer in an earlier paper (16) shows that when suspended in water the thermal death point of *Bacillus pluton* is approximately 63° C., the period of application being 10 minutes. Further experiments have been conducted in which the organism was suspended in honey and heated. After being heated, healthy larvæ are inoculated by feeding, using the direct or pipette method. Table I summarizes the experiments made:

TABLE I.—*Resistance to heat of Bacillus pluton suspended in honey*

Date of inoculation.	Temperature.		Period of heating.	Results of inoculation.
1915.	°C.	°F.	*Minutes.*	
June 15	67	153	10	European foulbrood produced.
Do	70	158	10	Do.
Do	75	167	10	Do.
June 22	76	169	10	Do.
Do	78	172	10	Do.
Sept. 22	79	174	10	No disease produced.
June 15	80	176	10	Do.
June 18	80	176	10	Do.
Sept. 27	81	178	10	Do.
June 19	85	185	10	Do.
Do	90	194	10	Do.

The results given in the foregoing table show that the thermal death point of *Bacillus pluton* suspended in honey is approximately 79° C., maintained for 10 minutes.

RESISTANCE OF BACILLUS PLUTON TO DRYING

In conducting experiments relative to the effect of drying on *Bacillus pluton* the stomach contents (Pl. VIII) of larvæ sick or recently dead of European foulbrood are spread in a thin layer in Petri dishes or on slides. From time to time after the films are made healthy larvæ are inoculated by feeding a suspension of the drying larval material suspended in a weak sirup solution. When no in-

fection results the germ is considered as having been destroyed. Observations have been made on the virus kept at incubator, room, outdoor, and refrigerator temperatures and shielded from the light in each instance. The experiments conducted with *Bacillus pluton* in these environments are summarized in Tables II, III, IV, and V which follow:

TABLE II.—*Resistance of Bacillus pluton to drying at incubator temperature*

Date of inoculation.	Period of exposure.		Results of inoculation.
1914.	*Months.*	*Days.*	
July 3		10	European foulbrood produced.
July 10		17	Do.
July 17		24	Do.
July 25	1	2	Do.
Aug. 3	1	7	Do.
Aug. 15	1	17	Do.
Sept. 1	2	0	Do.
Sept. 16	2	11	Do.
Sept. 29	3	0	Do.
1915.			
June 29	9	10	Do.
July 13	12	2	Do.
July 9	12	0	No disease produced.
Sept. 20	14	18	Do.
Sept. 17	14	20	Do.
1916.			
Sept. 8	24	0	Do.

TABLE III.—*Resistance of Bacillus pluton to drying at room temperature*

Date of inoculation.	Period of drying.		Results of inoculation.
	Months.	*Days.*	
July 25, 1914	1	1	European foulbrood produced.
Sept. 16, 1914	1	21	Do.
Sept. 1, 1914	2	8	Do.
Sept. 28, 1914	2	21	Do.
Sept. 29, 1914	3	0	Do.
Oct. 6, 1914	3	14	Do.
Jan. 29, 1915	9	10	Do.
July 9, 1915	12	6	Do.
Aug. 3, 1914	11	13	No disease produced.
June 22, 1915	11	15	Do.
June 25, 1915	11	18	Do.
Aug. 9, 1915	12	2	Do.
Sept. 17, 1915	14	10	Do.
Sept. 20, 1915	14	18	Do.
Sept. 8, 1916	24	0	Do.
Do	36	0	Do.

TABLE IV.—*Resistance of Bacillus pluton to drying at outdoor temperature*

Date of inoculation.	Period of drying.		Results of inoculation.
	Months.	*Days.*	
Sept. 2, 1914		33	European foulbrood produced.
Sept. 16, 1914		47	Do.
Oct. 13, 1914		74	Do.
May 26, 1915	9	21	Do.
June 19, 1915	10	18	Do.
May 17, 1915	13	17	Do.
Aug. 3, 1915	12	3	No disease produced.
Aug. 17, 1915	12	17	Do.
June 23, 1916	23		Do.

TABLE V.—*Resistance of Bacillus pluton to drying at refrigerator temperature*

Date of inoculation.	Period of drying.		Results of inoculation.
	Months.	*Days.*	
Oct. 17, 1915		26	European foulbrood produced.
Sept. 18, 1916	3	28	Do.
May 3, 1916	6	12	Do.
June 23, 1916	8	0	Do.
May 26, 1916	8	2	Do.
June 23, 1916	9	0	Do.
July 31, 1916	10	7	Do.
Sept. 18, 1916	10	18	Do.

From Table II it will be observed that *Bacillus pluton* in a dry film made from the contents of infected larvæ resisted drying at incubator temperature for approximately one year. Table III shows that at room temperature, other conditions being similar, the resistance is approximately equal to that at incubator temperature. At outdoor temperature, as shown by Table IV, the resistance is again approximately the same. At refrigerator temperature, Table V, the experiments do not include the period at which *Bacillus pluton* is destroyed. In 10 months the organism was still viable and the results of the inoculations indicate from the character of the infection produced after such a period that at refrigerator temperature *Bacillus pluton* will remain alive for a longer period than at the other temperatures studied.

RESISTANCE OF BACILLUS PLUTON WHEN DRY TO DIRECT SUNLIGHT

In experiments relative to the resistance of *Bacillus pluton*, when dry, to the direct rays of the sun, smears are made of the contents of stomachs of European foulbrood larvæ in Petri dishes or on slides, and after becoming dry are exposed to the direct rays of the sun. After intervals reckoned in hours inoculations are made by feeding, using the direct method. Infection resulting from such inoculations shows that the drying has not killed the organism. In Table VI experiments performed in this connection are summarized:

TABLE VI.—*Results of inoculation with Bacillus pluton in a dry film exposed to direct sunlight*

Date of inoculations.	Period of exposure.	Results of inoculations.
	Hours.	
July 21, 1914	3	European foulbrood produced.
Sept. 18, 1913	6	Do.
July 31, 1914	7	Do.
Sept. 22, 1915	8	Do.
July 21, 1914	10	Do.
Sept. 27, 1915	14	Do.
Aug. 7, 1914	15	Do.
July 21, 1914	16	Do.
Aug. 7, 1914	20	Do.
Sept. 10, 1915	20	Do.
Sept. 25, 1915	21	Do.
July 22, 1914	23	Do.
Sept. 8, 1915	27	Do.

TABLE VI.—*Results of inoculation with Bacillus pluton, etc.*—Continued

Date of inoculations.	Period of exposure.	Results of inoculation.
	Hours.	
Sept. 20, 1915	21	No disease produced.
Sept. 24, 1915	23	Do.
Sept. 14, 1915	24	Do.
Aug. 16, 1915	26	Do.
Sept. 14, 1915	26	Do.
Sept. 13, 1915	31	Do.
Sept. 20, 1915	38	Do.
Aug. 3, 1915	40	Do.
Sept. 14, 1915	44	Do.
Aug. 16, 1915	46	Do.
Aug. 23, 1915	63	Do.
Sept. 14, 1915	95	Do.

Observations recorded in Table VI show that *Bacillus pluton* in a dry film made from the contents of the stomachs of larvæ sick or recently dead of European foulbrood resists the direct rays of the sun for from 21 to 31 hours.

RESISTANCE OF BACILLUS PLUTON IN WATER TO DIRECT SUNLIGHT

In performing the experiments relative to the effect of direct sunlight on *Bacillus pluton* suspended in water, an aqueous suspension of the contents of stomachs of infected larvæ is exposed, in a Petri dish with the top removed, to the direct rays of the sun. After intervals reckoned in hours inoculations of healthy larvæ are made to determine whether the organism is viable. The direct method is used. Experiments made in this connection are summarized in Table VII:

TABLE VII.—*Resistance of Bacillus pluton suspended in water exposed to the direct rays of the sun*

Date of inoculation.	Period of exposure.	Results of inoculation.
1915.	*Hours.*	
Aug. 24	1	European foulbrood produced.
Do	2	Do.
Aug. 16	2	Do.
Aug. 8	3	Do.
Aug. 9	3	Do.
Aug. 16	3	Do.
Aug. 24	3	Do.
Sept. 13	4	Do.
Aug. 18	5	Do.
Aug. 16	5	No disease produced.
Sept. 14	5	Do.
Aug. 17	6	Do.
Aug. 20	7	Do.
Sept. 14	7	Do.
Do	8	Do.
Do	9	Do.
July 28	10	Do.
Aug. 20	18	Do.

Table VII shows that *Bacillus pluton*, when suspended in water and exposed to the direct rays of the sun, was destroyed in from 5 to 6 hours.

RESISTANCE OF BACILLUS PLUTON IN HONEY TO DIRECT SUNLIGHT

Experiments were made to determine the resistance of *Bacillus pluton* when suspended in honey to the direct rays of the sun. In these experiments a honey suspension of the organism obtained from the stomachs of infected bees is exposed to the sun in a Petri dish with the top removed. After intervals, reckoned in hours, inoculation tests are made using healthy larvæ and the direct method.* Table VIII contains a summary of the experiments performed:

TABLE VIII.—*Resistance of Bacillus pluton suspended in honey and exposed to direct sunlight*

Date of inoculation.	Period of exposure.	Results of inoculation.
1915.	*Hours.*	
Aug. 24	1	European foulbrood produced.
Do	2	Do.
Do	3	Do.
Aug. 3	3	No disease produced.
Aug. 20	3	Do.
Sept. 13	3	Do.
Sept. 19	4	Do.
Aug. 20	5	Do.
Sept. 14	5	Do.
Do	6	Do.
Sept. 11	7	Do.
Sept. 14	8	Do.
Do	9	Do.
Sept. 28	10	Do.

It is shown by the experiments recorded in Table VIII that in direct sunlight *Bacillus pluton* was destroyed in from 3 to 4 hours.

The results obtained by the experiments summarized in the last three tables above, it will be noted, show that *Bacillus pluton* is susceptible to the destructive effects of the direct rays of the sun; that the resistance of the organism suspended in honey is about equal to its resistance when suspended in water; and when dry the resistance is considerably greater than when suspended in either water or honey. It is to be expected that the period required for the destruction of the organism by the rays of the sun will vary with the intensity of the rays at the time of the exposure. In the foregoing experiments clear days were chosen and preference was given to the middle of the day for the exposures.

RESISTANCE OF BACILLUS PLUTON TO FERMENTATION

In obtaining data relative to the resistance of *Bacillus pluton* to fermentation, the stomach contents of larvæ sick or recently dead of European foulbrood were suspended in a 10 per cent sugar (saccha-

rose) solution. A bit of soil was added to inoculate it further. Records were made on suspensions fermenting at incubator and room temperatures, respectively. Tables IX and X which follow summarize experiments made:

TABLE IX.—*Bacillus pluton in a 10 per cent sugar solution fermenting at incubator temperature*

Date of inoculation.	Period of fermentation.	Results of inoculation.
	Days.	
Aug. 12, 1916	3	European foulbrood produced.
June 26, 1916	5	No disease produced.
Sept. 2, 1916	7	Do.
Sept. 7, 1915	8	Do.
Aug. 9, 1915	10	Do.
June 30, 1916	15	Do.
July 5, 1915	15	Do.
Aug. 24, 1915	24	Do.

TABLE X.—*Bacillus pluton in a 10 per cent sugar solution fermenting at room temperature*

Date of inoculation.	Period of fermentation.	Results of inoculation.
	Days.	
June 30, 1916	9	European foulbrood produced.
July 17, 1915	10	Do.
Sept. 8, 1915	10	Do.
July 21, 1915	14	Do.
Aug. 25, 1916	16	Do.
July 8, 1916	17	Do.
Sept. 10, 1916	11	No disease produced.
July 5, 1916	14	Do.
Aug. 26, 1916	21	Do.
Aug. 3, 1915	27	Do.
Aug. 9, 1915	32	Do.
Aug. 25, 1915	49	Do.

The experimental results contained in Tables IX and X show that *Bacillus pluton* is destroyed in a fermenting solution. At incubator temperature the virus was destroyed in from 3 to 5 days, and at room temperature it was killed in from 11 to 21 days.

Similar experiments were made in which suspensions in 20 per cent honey solutions were allowed to ferment at outdoor temperature. The records obtained show that *Bacillus pluton* in this environment was still alive and virulent after one month.

RESISTANCE OF BACILLUS PLUTON TO PUTREFACTION

Suspensions of the contents of stomachs from larvæ sick or dead of European foulbrood were made in a 1 per cent peptone solution. Soil was added to inoculate it further. Putrefactive changes were allowed to take place at incubator and room temperatures, respec-

tively. In Tables XI and XII, which follow, are summarized the experiments performed:

TABLE XI.—*Bacillus pluton in the presence of putrefactive processes at incubator temperature*

Date of inoculation.	Period of putrefaction.	Results of inoculation.
	Days.	
June 30, 1916	9	European foulbrood produced.
Sept. 2, 1916	7	No disease produced.
Aug. 15, 1916	8	Do.
Sept. 7, 1915	13	Do.
July 5, 1916	15	Do.
Sept. 10, 1915	16	Do.
July 8, 1916	18	Do.
Aug. 23, 1915	19	Do.
Aug. 30, 1915	26	Do.

TABLE XII.—*Bacillus pluton in the presence of putrefactive processes at room temperature*

Date of inoculation.	Period of putrefaction.	Results of inoculation.
	Days.	
Aug. 4, 1914	8	European foulbrood produced.
July 17, 1915	10	Do.
July 5, 1916	14	Do.
July 23, 1915	16	Do.
Aug. 14, 1914	18	Do.
Sept. 17, 1915	18	Do.
Aug. 25, 1916	18	Do.
Sept. 2, 1916	26	Do.
Aug. 3, 1915	27	Do.
Aug. 28, 1916	21	No disease produced.
Sept. 1, 1914	35	Do.
Sept. 16, 1914	51	Do.
Aug. 12, 1916	52	Do.

As shown by Tables XI and XII *Bacillus pluton* is destroyed in the presence of putrefactive processes. At incubator temperature it resisted the effects of these processes for from 7 to 13 days and at room temperature for from 21 to 35 days.

During August and September, 1916, preliminary experiments were made testing the resistance of *Bacillus pluton* to putrefaction at outdoor temperature. The parasite was alive and virulent after 40 days. The maximum period during which it will remain so has not been determined.

VIABILITY OF BACILLUS PLUTON IN HONEY

Honey suspensions of *Bacillus pluton* from the stomach contents of larvæ sick or recently dead of European foulbrood were made and distributed in flasks each containing about 300 c. c. These were allowed to stand at room temperature shielded from the light. At intervals thereafter colonies free from the disease were inoculated

each with the contents of a single flask. A summary of the experiments is contained in Table XIII:

TABLE XIII.—*Resistance of Bacillus pluton in honey at room temperature*

Date of inoculation.	Period in honey.		Results of inoculation.
	Months.	*Days.*	
May 22, 1915		4	European foulbrood produced.
June 12, 1915		25	Do.
July 23, 1915	1	6	Do.
June 25, 1915	1	11	Do.
Aug. 23, 1915	1	15	Do.
Aug. 3, 1915	1	17	Do.
July 12, 1915	1	25	Do.
Aug. 23, 1915	2	7	Do.
Sept. 10, 1915	2	25	Do.
Aug. 16, 1916	3	0	Do.
May 19, 1916	7	0	No disease produced.
May 4, 1915	7	17	Do.
June 7, 1913	8	0	Do.
June 13, 1913	8	0	Do.
May 13, 1915	8	25	Do.
May 14, 1915	8	26	Do.
May 22, 1915	9	5	Do.
May 24, 1915	9	5	Do.
July 31, 1916	9	11	Do.
May 15, 1916	10	6	Do.

Experimental evidence recorded in Table XIII shows that the virus of European foulbrood when suspended in honey at room temperature ceased to be virulent in from 3 to 7 months.

VIABILITY OF BACILLUS PLUTON IN POLLEN

Preliminary experiments were made to determine the viability of *Bacillus pluton* in pollen. Pollen is removed from brood-comb, and an aqueous suspension of the organism obtained from the stomachs of larvæ sick or recently dead of the disease is added to it until a moderately thick, pastelike mass is obtained. This is distributed in Petri dishes and allowed to stand at room and refrigerator temperatures, respectively. After different intervals of time the contents of a single dish, after being suspended in water, are added to about 300 c. c. of sirup and the suspension is fed to a colony, using the indirect method. The results show that *Bacillus pluton* was virulent after 7 months at room temperature and for more than 10 months in the refrigerator. The maximum period during which the organism will remain alive in these two environments has not been determined.

RESISTANCE OF BACILLUS PLUTON TO CARBOLIC ACID

Preliminary experiments were made to determine the effect of carbolic acid on the virus of European foulbrood. An aqueous suspension of the contents of the stomachs of larvæ sick or dead of the disease is first made. A measured quantity of this suspension is added to an equal quantity of an aqueous suspension of carbolic acid

of a strength twice that desired in the experiment. After shaking, it is allowed to stand at room temperature. At intervals brood free from the disease is fed a bit of this suspension, using the direct method. Table XIV summarizes the experiments performed:

TABLE XIV.—*Effect of carbolic acid on Bacillus pluton*

Date of inoculation.	Strength of solution.	Period of suspension.	Results of inoculation.
	Per cent.	*Days.*	
Aug. 22, 1914	¼	¾	European foulbrood produced.
Aug. 14, 1914	⅓	1	Do.
July 3, 1915	½	4	Do.
Aug. 21, 1914	½	8	Do.
Sept. 4, 1914	½	18	No disease produced.
June 29, 1915	1	[1]5	European foulbrood produced.
July 3, 1915	1	4	No disease produced.
June 29, 1915	2	[1]5	Do.
Aug. 22, 1914	2	[1]18	Do.
Aug. 14, 1914	2	1	Do.
Aug. 17, 1914	2	4	Do.
Aug. 25, 1914	2	4	Do.
Aug. 22, 1914	2	9	Do.
June 29, 1915	4	[1]5½	Do.

[1] Hours.

The experiments outlined in Table XIV show that *Bacillus pluton* withstood a one-half per cent solution of carbolic acid for 8 days but not for 18 days; that it withstood 1 per cent for 5 hours but not for 4 days; and that it was destroyed by 2 and 4 per cent solutions, respectively, in less than 6 hours. Probably it is destroyed by these latter strengths in considerably less time than this.

It is seen by these preliminary experiments that *Bacillus pluton* is destroyed easily by carbolic acid as a disinfectant. As a drug, however, less can be expected of it, inasmuch as a strength twice that which the bees will accept in honey (Table XV) requires days to destroy the germ. While the fact does not furnish conclusive proof of the value of carbolic acid as a drug, it indicates what might be expected of it in the treatment of the disease.

In using the results recorded on the foregoing pages for the purpose of destroying the virus of European foulbrood and controlling the disease in practical apiculture, it must be borne in mind, as has been urged in the discussions on the other bee diseases, that due allowance must be made by the beekeeper for variations which always occur. These, however, are relatively slight and can be met readily. In the destruction of the virus through heating, for example, the temperature can be raised a few degrees above that which is found to be the minimum required, or the time can be extended somewhat. Similarly for the other destructive agencies the effectiveness of the process can be increased.

EFFECT OF DRUGS ON EUROPEAN FOULBROOD

Preliminary experiments have been made to obtain data relative to the effect of drugs on *Bacillus pluton*. In conducting the experiments a suspension of the stomach contents of larvæ sick or recently dead of European foulbrood is made in an aqueous solution of the drug. This is added to diluted honey and healthy brood is fed this suspension. In some instances the direct and in others the indirect method was followed. In Table XV are summarized the experiments which were performed:

TABLE XV.—*The effect of drugs on European foulbrood*

Date of experiment.	Drugs.	Strength.	Results of inoculation.
1915.			
July 11	Betanaphthol	1:2000	European foulbrood produced.
May 31	do	1:1000	
June 7	do	2:1000	
July 11	Carbolic acid	1:2000	
June 21	do	1:1000	
Do	do	2:1000	
May 31	Oil of eucalyptus	4:1000	
July 11	do	4:1000	
June 7	Formic acid	1:1000	
July 11	do	3:1000	
Do	Salicylic acid	1:2000	
May 31	do	1:1000	
June 7	do	2:1000	
July 11	Salol	1:2000	
May 31	do	1:1000	
June 7	do	2:1000	
July 11	Quinin	2:1000	
May 31	do	4:1000	
June 7	do	10:1000	

It will be observed from Table XV that European foulbrood was produced in all cases in which larvæ were fed a suspension of *Bacillus pluton* in sirup medicated with betanaphthol, carbolic acid, eucalyptus, formic acid, salicylic acid, salol, and quinin (bisulphate of quinin), respectively, in the proportions noted.

The strongest solutions of the drugs used in the experiments are in most instances approximately the maximum proportion of the chemical in honey that will be taken by the bees. These preliminary results indicate that drugs should not be depended upon, for the present at least, in the treatment of European foulbrood, and emphasize the fact that beekeepers should make sure that the value of a drug has been demonstrated fully before it is used.

TRANSMISSION OF EUROPEAN FOULBROOD

While there is yet much to be learned concerning the transmission of European foulbrood, the data at hand relative to this important phase in the study of the disease justify certain statements in regard to it. The disease can be produced experimentally by feeding a healthy colony the crushed larvæ sick or dead of the disease, suggesting that infection takes place by way of the alimentary tract.

Through the study of microtome sections of such larvæ, it has been conclusively proved that infection takes place in this way. The fact is naturally one of special moment in the solution of the transmission of the disease. There is a tendency on the part of adult bees to remove sick and dead larvæ from the brood comb. This is done largely at least in a piecemeal manner. Were the fate of the fragments removed known definitely the solution of the problem naturally would be aided greatly.

If infective material thus removed were fed to susceptible healthy larvae, disease would result. On the other hand should the fragments of diseased larvæ be stored with the honey of the hive or with the pollen, or consumed by the adult bees, or by larvæ later in the feeding stage, the chances that such material would ever reach susceptible larvæ to cause infection are very much reduced. Stored in honey the virus remains virulent only a few months (p. 24); in pollen, however, it remains virulent much longer (p. 24). Drying within the hive *Bacillus pluton* would probably remain alive more than a year (p. 19).

The chances that any portion of the infectious material of any given fragment, if it is removed entirely from the hive by the bees of the colony, and released from them, will be taken up by other bees and carried to healthy brood and cause infection are comparatively slight. If thus removed and exposed to the direct rays of the sun, the virus will be destroyed within a few hours (p. 19); or if subjected to fermentative or putrefactive processes it will be destroyed in a few weeks (p. 23). If *Bacillus pluton* is present in honey extracted from diseased colonies it will be destroyed within a few months while in storage (p. 24). It is seen, therefore, that in nature there are many means that destroy the virus of European foulbrood and thus limit the spread of the disease.

All of the colonies of the experimental apiary used in making the inoculations cited in the present paper had free access to the fields and there was no evidence at any time of the transmission of the disease from infected to healthy colonies. This fact supports the conclusion that the disease is not spread by way of flowers visited by bees from healthy colonies which had been visited previously by bees from diseased ones. The fact further indicates that if the disease is transmitted at all by way of the water supply of the bees, it takes place to a limited extent only. The fact still further indicates that if drones or straying or drifting workers transmit European foulbrood they do so to a slight extent only. If these observations are at variance with the experience of the practical beekeepers, as the writer has been informed that they are, they will probably be of particular interest.

Observations made during the present studies indicate that queens from European foulbrood colonies are not likely to transmit the disease when introduced into healthy colonies. The experiences further show, and the facts in general regarding the disease support the conclusions, that the infection will not be transmitted by the hands or clothing of the beekeeper, or by visitors to the apiary when the manipulations ordinarily practiced are followed. Tools and equipment used about the apiary are not to be feared unless they supply a source for robbing. Hives which have housed infected colonies are not likely to be a medium for the spread of the disease.

Robbing of infected colonies is the most fruitful source of infection. A colony weakened by disease (p. 5) becomes a prey for other bees. Infectious material is carried to other colonies, thereby transmitting the infection. Manipulations in the apiary, whereby brood combs from diseased colonies are placed in healthy ones, are another fruitful source for the transmission of the disease. Preliminary work[1] indicates that stored brood combs from European foulbrood colonies may transmit the disease after a considerable period.

The disease, it would seem, might be spread through the medium of honey from infected colonies. The danger from this source, however, probably has been overestimated at times (p. 23). That pollen stored in the comb would serve as a protection to *Bacillus pluton*, if the parasite were lodged with it, has been determined (p. 24).

DIAGNOSIS

The diagnosis of European foulbrood offers more difficulty than does that of either American foulbrood or sacbrood. It can usually be made, however, from the symptoms alone. Inasmuch as these symptoms (p. 4) are rather varied, much care should be exercised in diagnosing the disease.

The appearance of the adult bees does not aid in the diagnosis. A weak colony should arouse suspicion. Increased suspicion is justified when no other readily discernible cause for the weakness is to be observed. The disease may be present, however, in a strong colony. Such a case may be one of recent infection or one which late in the recovery from the disease has gained in strength. It may be, however, a colony which has suffered only a slight attack of the disease.

The following outstanding gross characters are often sufficient for a diagnosis: The dying of the brood before the time for capping (Pls.

[1] Brood combs were removed from European foulbrood colonies in October, 1914, and stored in the laboratory. In May, 1915, one frame of brood comb was placed in each of two colonies with the result that European foulbrood was produced in both instances. When a frame of the comb was placed in the colony in May, 1916, no disease resulted. After 6 months the combs were still able to transmit the disease; after 18 months they did not. These experiments are not sufficient to justify definite conclusions but are suggestive.

II, III, IV), the yellow hue of the larvæ more recently dead, and the brown shade of those longer dead, the irregularity of the brood (Pl. I), and the absence of a disagreeable odor.

Not infrequently, however, the diagnosis is not so simple. During recovery from the disease scales (Pl. V, F, I) of larvæ dying in capped cells may be the only remains of diseased brood to be found, all of the younger larvæ having been removed by the bees. These scales[1] are, as a rule, comparatively few in number and resemble somwhat those of American foulbrood, but would rarely be mistaken for those of sacbrood. In these cases a diagnosis can be made frequently by a microscopic examination alone. Cultures, however, are needed in some instances.

Special attention is needed in cases of early infection and in other instances where only a small amount of diseased brood in uncapped cells is present (Pl. I, A). The symptoms manifested by larvæ sick or only recently dead of the disease furnish often the readiest and most conclusive evidence of the presence of the disease. Larvæ of the age at which they comfortably fill the bottom of the cell exhibiting increased peristalsis-like movements of the body suggest European foulbrood. Increased transparency of larvæ of this age (Pl. II, B) is also suggestive. The presence of a white or yellowish-white mass within the stomach (midgut) as seen through the dorsal median line of the body is strong evidence of the presence of the disease. If on puncturing the body of larvæ nearly dead or only recently dead the contents of the stomach flows out as a fluid and more or less finely granular mass, the fact furnishes further evidence of European foulbrood.

A symptom which is pathognomonic of the disease is to be seen in larvæ that have been infected somewhat more than two days, but wherein the disease has not reached an advanced stage. The test (15) involves the removal of the stomach contents, which consist of a bacterial mass, together with a small amount of larval food and a clear envelope (Pl. VIII, a, b, c). The slight tension necessary to remove the contents stretches the envelope and breaks the whitish bacterial mass into a number of fragments.

[1] The number of larvæ that die of European foulbrood in capped cells after assuming the endwise position represents a very small percentage of the brood that dies of the disease. These remains may be found in practically all colonies in which the disease has been present for a sufficiently long period and in which a considerable amount of dead brood has resulted. Before becoming dry they are somewhat viscid and are less easily removed than are those of larvæ dying at an earlier age. These and the scales resulting from them are used in diagnosis principally (1) when the younger larvæ sick or dead of the disease have been removed, (2) when a demonstration of the presence of *Bacillus alvei* is desired, and (3) when both European foulbrood and American foulbrood infection is suspected. Such a double infection has been encountered in the writer's experience very rarely. In making diagnoses, therefore, after European foulbrood has been found in the sample American foulbrood is seldom looked for.

By one or more of these colony symptoms manifested by larvæ sick or only recently dead of the disease the experienced can diagnose European foulbrood definitely without a microscopic examination. The methods not only give definite results, but are also easy of application. They have been indispensable in much of the writer's experimental work and it is believed that the beekeeper will find them to be valuable in practical apiculture where other gross methods fail.

BACTERIOLOGICAL EXAMINATION

The findings from microscopic examinations and from cultures have been set forth in an earlier publication (10). These are always adequate for a definite diagnosis when a suitable sample is at hand. *Bacillus alvei* (p. 11) (fig. 2; Pl. VII, D, F) frequently overshadows all other species. In larvæ sick of the disease *Bacillus pluton* (Pl. VII, A, B) overshadows all others. With experience one learns to recognize this species in stained preparations. The individuals are seen frequently in groups. They are more or less lancet shaped, and a variation in size is often sufficient to be noticeable (fig. 1).[1] In larvæ nearly dead and in those only recently dead *Bacterium eurydice* (p. 13) (fig. 4; Pl. VII, C) is frequently encountered. *Streptococcus apis* (p. 12) (fig. 3; Pl. VII, E) occurs in a small number of cases. *Bacillus orpheus* (p. 14) (fig. 5; Pl. VII, H), *B. vulgatus*, and *B. mesentericus* are occasionally encountered. While *B. pluton* is present in all cases of European foulbrood, not infrequently in routine examinations it is so masked by the secondary invaders that the microscopic examination fails to reveal it. In many cases *B. alvei* and *B. orpheus* are recognized microscopically. Cultures are necessary for the differentiation of *B. vulgatus* and *B. mesentericus*. In many cases cultures are needed to differentiate *Strep. apis* and *B. pluton*. *Strep. apis* grows on the ordinary media, *B. pluton* does not.

DIFFERENTIAL DIAGNOSIS

AMERICAN FOULBROOD

American foulbrood is recognized by the death of larvæ in capped cells and of pupæ soon after transformation, the viscidity of the decaying remains of the brood, and the "foulbrood" odor which is frequently present. The presence of the spores of *Bacillus larvae* in large numbers and the absence of other species is conclusive proof of American foulbrood.

[1] Smears made from larvæ sick of European foulbrood and quite early in the course of the disease were selected in making a study of the morphology of *B. pluton*. These were stained with iron hematoxylin. In smears made from dead larvæ and stained with carbol fuchsin, as is usually done, the pointed ends and the more or less rod-shaped forms are less prominent than illustrated in figure 1.

SACBROOD

Sacbrood is recognized by the death of larvæ after capping, by the saclike appearance, the watery granular consistency of the larval remains, and the absence of viscidity. The absence of microorganisms characterizes the microscopic picture in sacbrood.

OTHER CONDITIONS

Conditions referred to as chilled brood, overheated brood, and starved brood must be differentiated from European foulbrood. This can usually be done with little difficulty by a comparison of the symptoms present with those of European foulbrood. The history of the case is of much value. Brood dying after being removed from the hive and before examination is made shows often an interesting similarity to European foulbrood. *B. alvei* and *B. pluton* are not found in these conditions. The absence of bacteria, or their presence in small numbers only, and a lack of uniformity of the species when present, characterize the bacteriological findings in these cases.

PROGNOSIS

There is no uniformity in the prognosis in European foulbrood. The diseased colony may recover completely from the infection, suffering only a slight loss in strength as a result of it; the colony may recover but sustain considerable loss; or it may die out entirely, as a result of the disease. The infection may spread only slightly to other colonies of the apiary or the entire apiary may become infected. The losses sustained vary from slight to total. The tendency for European foulbrood to disappear is greater after midsummer than before.

Whether a larva once infected ever recovers from this disease is not known, but the evidence at hand indicates that it may. This seems to be especially probable when the infection takes place during the latter part of the feeding period of the larva. Queen larvæ are susceptible to infection, but sufficient data are wanting from which to estimate the extent to which queenlessness may result from the disease. In experimental colonies queens have been reared in the presence of a considerable amount of European foulbrood infection.

The prognosis for the colony in the case of European foulbrood may be said, therefore, to vary from very good to very grave, many recovering entirely from the infection without treatment and without appreciable losses, while others rapidly decline and finally die out.

SUMMARY AND CONCLUSIONS

The following is a brief summary of facts regarding European foulbrood, together with some conclusions based upon them:

1. European foulbrood is an infectious brood disease of bees caused by *Bacillus pluton.*

2. All larvæ—worker, drone, and queen—are susceptible to the disease; adult bees are not.
3. Man evidently is not susceptible to infection with *Bacillus pluton* nor are the experimental animals.
4. As far as is known insects other than bees are not susceptible.
5. Brood can be infected by feeding the colony a suspension of crushed larvæ sick or dead of the disease. This is described in the present paper as the indirect method.
6. The virus contained in a single larva recently dead of European foulbrood will produce a considerable amount of disease when fed to a colony.
7. The larvæ can be infected also by a more direct method. A fraction of a drop of a suspension of the stomach contents of a larva sick of the disease added with a capillary pipette directly to the food surrounding the larva to be inoculated will result in infection.
8. *Bacillus pluton* gains entrance to the larva by way of the mouth. The growth and multiplication of the parasite take place within the stomach (mid-intestine) of the larva and do not, during the life of the larva, get beyond the peritrophic membrane. The tissues, therefore, are not invaded by it.
9. The secondary invaders in European foulbrood, *Bacillus alvei*, *Streptococcus apis*, *Bacterium eurydice*, and *Bacillus orpheus*, rarely, if ever, invade the tissues until the larva is dead or nearly so. In a few instances in microtome sections rod forms have been encountered in the act of invading the tissues of living larvæ. The species, however, was not determined definitely.
10. The period of incubation is slightly less than 3 days.
11. Brood is susceptible to infection at all seasons of the year.
12. More brood die of the disease during the first half of the brood-rearing season than during the second half.
13. The writer has examined samples of the disease from Canada and the United States. From written reports it seems quite certain that it occurs also at least in Denmark, England, Germany, France, and Switzerland.
14. Occurring as it does in this somewhat wide range of climatic conditions, the presence of the disease in any particular locality can not be attributed entirely to the prevailing climatic conditions.
15. The quality of food obtained by the bees does not affect greatly, if at all, the course of the disease in the colony, although the quantity may affect it to a variable extent.

16. Experimental colonies may be inoculated and kept in the apiary without transmitting the disease to others. This fact is of special importance, not only in connection with the technique of making studies on the disease, but also in the control of the malady.
17. The thermal death point of *Bacillus pluton* suspended in water is approximately 63° C. maintained for 10 minutes.
18. When suspended in honey *Bacillus pluton* is destroyed in 10 minutes at approximately 79° C.
19. Drying at room or incubator temperature *Bacillus pluton* remains alive and virulent for approximately one year.
20. When dry, *Bacillus pluton* resisted the direct rays of the sun for from 21 to 31 hours.
21. When suspended in water *Bacillus pluton* was destroyed by the direct rays of the sun in from 5 to 6 hours.
22. When suspended in honey and exposed to the direct rays of the sun *Bacillus pluton* was destroyed in from 3 to 4 hours.
23. In the presence of fermentative processes in a 10 per cent sugar solution *Bacillus pluton* was destroyed in from 3 to 5 days at incubator temperature and in from 11 to 21 days at room temperature.
24. In a fermenting honey solution outdoors *Bacillus pluton* was still alive and virulent after one month.
25. In the presence of putrefactive processes at incubator temperature *Bacillus pluton* was destroyed in from 7 to 13 days and at room temperature in from 21 to 35 days.
26. In a putrefying medium at outdoor temperature *Bacillus pluton* remained alive and virulent for more than 40 days. The maximum period has not been determined.
27. In honey at room temperature *Bacillus pluton* ceased to be virulent in from 3 to 7 months.
28. Mixed with pollen, *Bacillus pluton* remained alive and virulent for more than 7 months at room temperature and more than 10 months at refrigerator temperature, the maximum time not being determined.
29. In one-half per cent carbolic acid solution *Bacillus pluton* was destroyed in from 8 to 18 days; in 1 per cent it was destroyed in from 5 hours to 4 days, and in 2 and 4 per cent in less than 6 hours. The probability is that at these higher strengths of the solution minutes rather than hours are sufficient for the destruction of the virus.
30. Experimental evidence indicates that at the present time drugs should not be depended upon in the treatment of European foulbrood.

31. Robbing from diseased colonies of the apiary or from neighboring apiaries is the most likely manner in which European foulbrood is transmitted in nature.
32. Brood-combs containing diseased brood, if given to a healthy colony, serve as a medium for the transmission of the disease.
33. European foulbrood is not likely to be transmitted by queens or drones. Whether they ever do so has not been demonstrated.
34. As a rule a hive which has housed a European foulbrood colony should not be considered as a fruitful source of infection. The facts indicate that often such hives could be used with impunity for housing colonies without treatment. Flaming them inside certainly removes all danger.
35. The transmission of European foulbrood by way of flowers, visited by bees from diseased colonies and subsequently by those from healthy ones, is not to be considered as a likely source of infection. Whether the water supply is ever a source of danger is not known. It is evidently not a fruitful source.
36. The disease is not likely to be transmitted through the medium of the clothing or hands of the apiarist.
37. Tools and bee supplies in general do not serve as means for the transmission of the disease in the absence of robbing from such sources.
38. It is usually possible to diagnose European foulbrood from the symptoms alone. A definite diagnosis can be made from suitable samples by bacteriological methods.
39. The prognosis in European foulbrood varies from very good to exceedingly grave. The tendency for a colony to recover entirely from the disease is much greater than in American foulbrood.
40. Considered from the technical point of view, much is yet to be learned concerning European foulbrood. For practical purposes, however, it can be said that sufficient knowledge has been gained to make it possible for the beekeeper to devise a treatment which will be logical, efficient, and at the same time economical.

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EXPLANATION OF PLATES

Plate I

Brood-combs containing larvæ that are sick and others that are dead of European foulbrood, showing the irregular appearance of the brood. About one-half natural size.

A.—The dead larvæ have all been removed. Some of the remaining larvæ are sick, others are not infected. The disease was produced by experimental inoculation.

B.—Many of the dead larvæ have not been removed. The comb had been out of the colony for a considerable period. The larvæ that are quite young showing abnormal position and appearance are not sick or dead of European foulbrood, but are so as a result of the comb being away from the colony. Disease was produced by experimental inoculation.

C.—The comb was taken from a colony in which the disease had appeared in nature and not as the result of artificial inoculation. Before being photographed the brood-comb had been out of the hive for a few days. Aside from the larvæ which are dead of European foulbrood, other larvæ present are dead from lack of attention by adult bees—starvation, exposure, and other causes.

Plate II

A.—Live larva showing first symptoms of European foulbrood. The turgidity is slightly less than in a healthy larva (D).

B.—Live larva showing early symptoms of European foulbrood. The body is more transparent than that of a healthy larva (D). Small opaque areas give it a punctate appearance.

C.—Larva dead of European foulbrood contained within a chitinous envelope filled with a watery-appearing fluid.

D.—Healthy larva of the earliest age at which larvæ die of European foulbrood. Turgidity marked.

E.—European foulbrood larva which may or may not be dead. Surface less glistening than in healthy larvæ. Marked turgidity lost. Prominence of tracheæ not increased.

F.—Scale formed by drying of larvæ dead at early age. Prominence of tracheæ marked.

G.—View of healthy larva in normal position with roof of cell removed. Larva turgid. Surface glistening.

H.—Larva sick with European foulbrood. Lack of turgidity and increased prominence of tracheæ observed.

I.—European foulbrood larva which may or may not be dead. Less turgidity, a relative dullness in the surface appearance, and punctate condition present. Similar to E.

Plate III

A.—Healthy larva immediately preceding the age at which the capping of the cell is done. Dorsal surface turned toward the observer. The narrow transparent area along the dorsal median line is prominent.

B.—Larva dead of European foulbrood of the same age as A. The turgidity, glistening surface, and transparent area are less marked.

C.—Larva dead of European foulbrood partly coiled and partly endwise in cell.

D.—Healthy larva near the age at which capping takes place.

E.—Dorsolateral view of a larva dead of European foulbrood. The ends are directed toward the bottom of the cell.

F.—Larva dead of European foulbrood. The body occupies a spiral position in the cell.

G.—Healthy larva approaching the age at which capping takes place.

H.—Lateral view of larva dead of European foulbrood seen with the roof of the cell removed. The ends are directed toward the bottom and the dorsal surface toward the mouth of the cell.

I.—Dead larva similar to H but having been dead somewhat longer.

PLATE IV

A.—Young larva dead of European foulbrood. The chitinous capsule and tracheæ are prominent.

B.—Fragments of young larva dead of European foulbrood, a portion having been removed by adult bees after its death.

C.—Lateral view of larva dead of European foulbrood, the roof of the cell having been removed. The ends in this instance are directed more or less toward the mouth of the cell.

D.—Lateroventral view of larva dead of European foulbrood. The body lies with the dorsal portion against the floor of the cell.

E.—Larva dead of European foulbrood lying on the floor of the cell in somewhat lengthwise position.

F.—Scale of European foulbrood larva which had occupied a somewhat spiral position in the cell.

G.—Scale of a European foulbrood larva which had occupied a position somewhat as shown in D. This scale and the one shown in F can be removed intact rather easily and without tearing the wall of the cell.

PLATE V

Larvæ (prepupæ) of bees dead of European foulbrood which had already assumed before death a lengthwise position in the cell.

A.—Fragment of European foulbrood soon after death. A portion of the larva has been removed by the adult bee.

B.—Entire cap of cell containing larva dead of European foulbrood.

C.—Punctured cap of cell containing the remains of a larva dead of European foulbrood.

D.—End view of larva dead of European foulbrood.

E.—End view of larva dead of European foulbrood, lying with its dorsal surface against the floor of the cell. Considerable drying of the remains has taken place.

F.—End view of scale of European foulbrood larva which had reached before death the age at which the endwise position in the cell is assumed.

G.—Ventral view of European foulbrood larva. Stage similar to D. Turgidity is lost to a large extent and the segmented markings are less distinct than in healthy larvæ.

H.—Larva which has been dead of European foulbrood for a longer period than illustrated in G. The ridge and furrows indicating the segments of the body are not marked.

I.—Scale of European foulbrood similar to F. The larva before death had reached the endwise position in the cell. These scales resemble very much those of American foulbrood. They are more easily removed, however, do not adhere so closely to the floor of the cell, and are more rubberlike in consistency, breaking less readily than those of American foulbrood.

PLATE VI

A view of the experimental apiary of 54 colonies in which the inoculation experiments made during the summer of 1915 were conducted.

PLATE VII

Photomicrographs illustrating the more commonly encountered bacteria in European foulbrood.

A.—*Bacillus pluton:* A smear from the stomach of a larva sick with European foulbrood. Note the paired forms and short chains. These forms are numerous in a recent infection, suggesting the organism in the process of multiplication. The lancet-shaped form is by far the predominant one in all later stages of the disease. × 1000.

B.—*Bacillus pluton:* A smear from a larva quite recently infected. The multiplying paired forms are at this stage present almost exclusively. × 1000.

C.—*Bacterium eurydice:* Stained preparation from a pure culture on the surface of agar. × 1000.

D.—*Bacillus alvei:* Stained preparation showing spores and spore formation. × 800.

E.—*Streptococcus apis:* Stained preparation from a pure culture. × 800.

F.—*Bacillus alvei:* The peculiar arrangement of the spores as sometimes seen. From a pure culture, the smear having been made by suspending the culture on the slide in normal salt solution. × 1000.

G.—*Bacillus orpheus:* Stained preparation made from a pure culture only a few hours old. Grown on the surface of agar. × 1000.

H.—*Bacillus orpheus:* Stained preparation showing spore formation. Note the stained portion along one side and about both ends of the spore. The stage is soon reached in a culture at incubator temperature. At room temperature it remains in this stage for a considerable period. × 800.

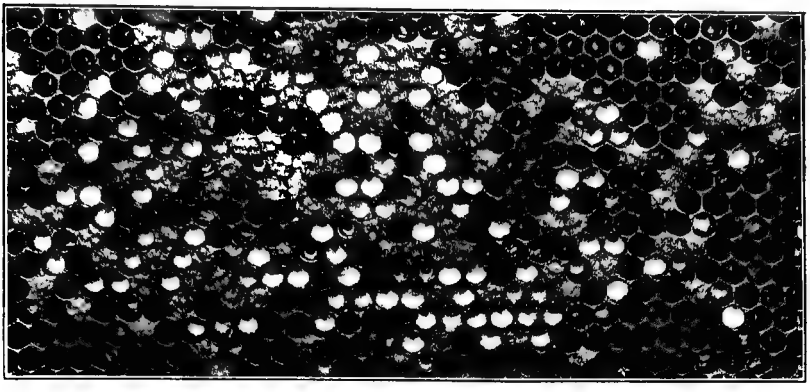
I.—Longisection of a young larva showing early infection in European foulbrood. The bacterial growth is seen as a narrow black area just within the peritrophic membrane on one side of the food mass.

J.—Longisection of larva sick of European foulbrood, showing a later stage of infection than that present in I. The dark area in the food mass shows the bacterial growth. Note that the growth mass does not extend beyond the peritrophic membrane and that it does not extend uniformly along this membrane and throughout the food mass.

K.—Transverse section of larva about the time of its death from European foulbrood infection. Note the bacterial mass along the peritrophic membrane and extending from the membrane into the food mass. As seen within the living larva this bacterial mass in the sick larva is practically white, but is more or less yellowish white when present with larval food material. The gelatinous-like envelope outside the peritrophic membrane and inside the stomach epithelium in healthy larvæ thins out as the disease advances.

PLATE VIII

The stomach contents of larvæ sick of European foulbrood removed from the organ. The anterior end of the larva is shown. Fairly early stage of infection (a) showing the white bacterial mass broken into fragments as a result of the tension produced in removing the stomach contents from the organ. A somewhat later stage (b) in the course of the disease, showing the bacterial growth contained in the stomach fragmented, also the mucous or gelatinous envelope surrounding the petritrophic membrane. The stomach contents removed from a European foulbrood larva (c) about the time of its death. The bacterial growth at this time is surrounded by very little other than the peritrophic membrane. When this membrane is ruptured the contents flow out as a thin yellowish-white mass.



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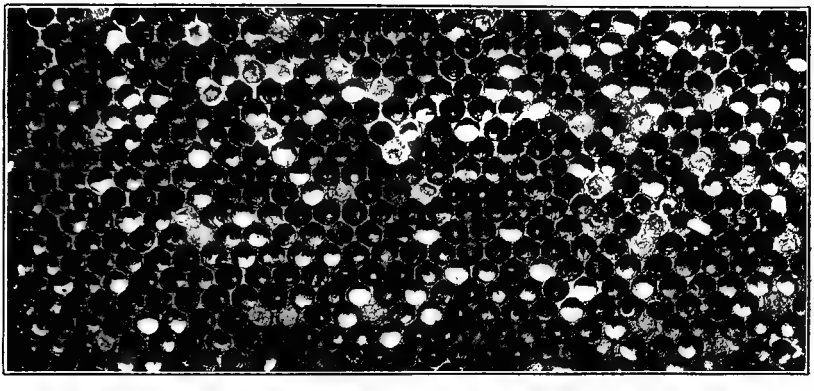

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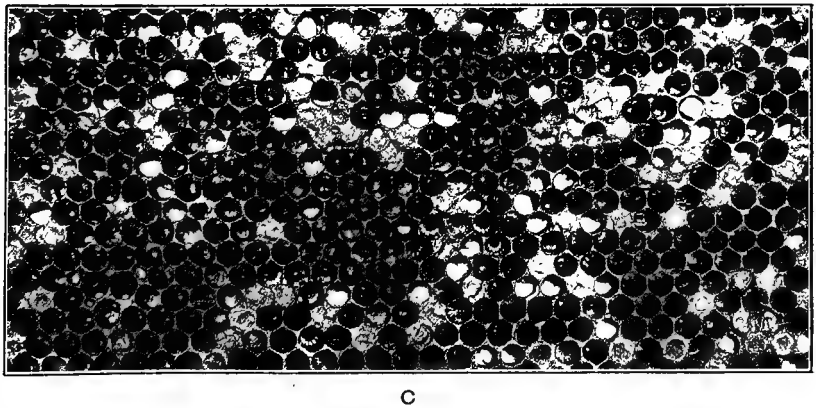
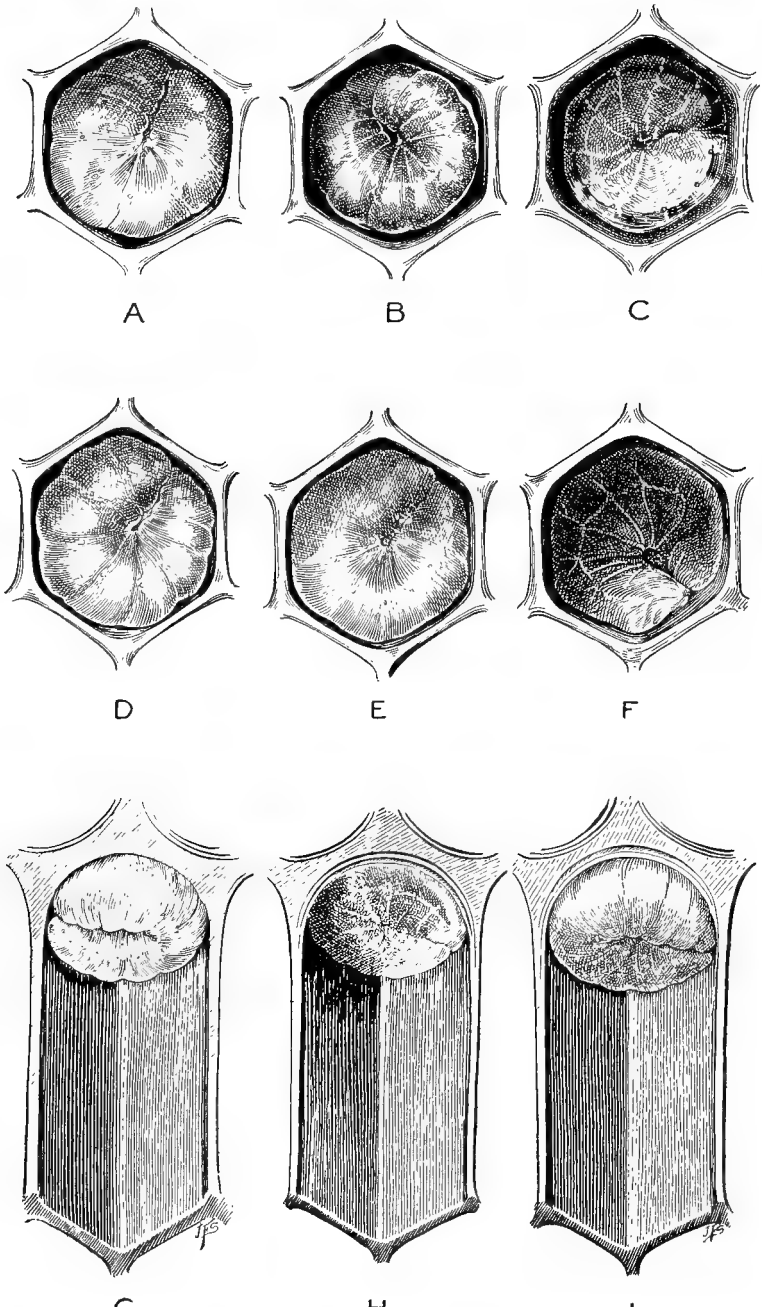


C

EUROPEAN FOULBROOD.



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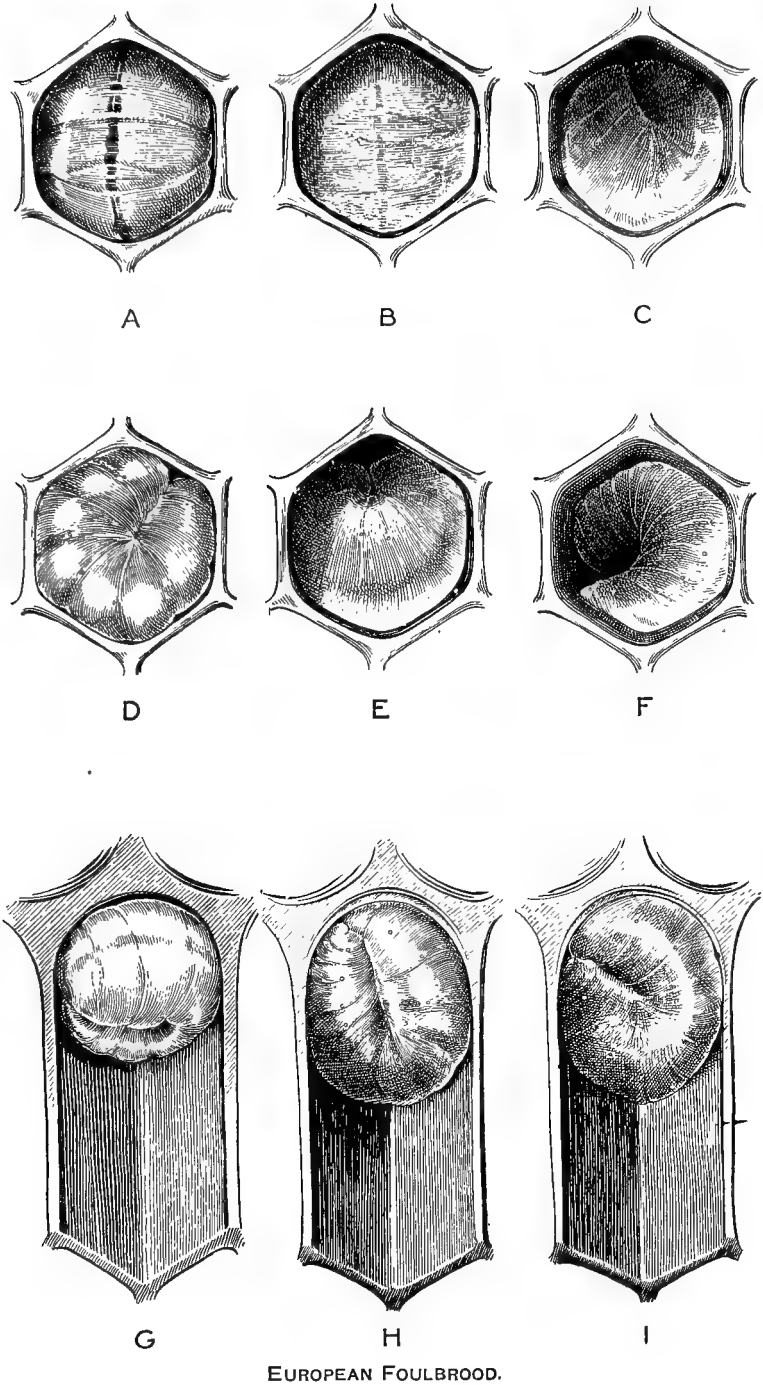

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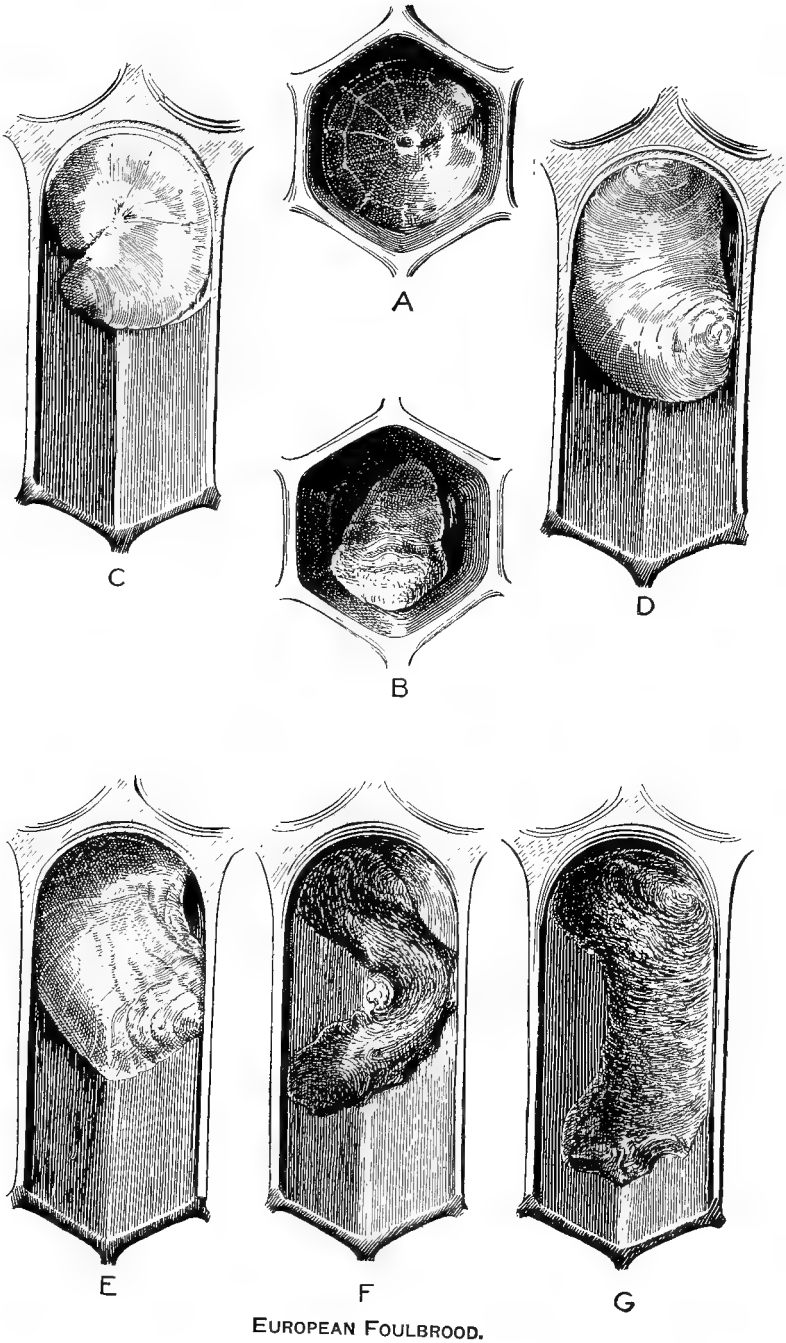

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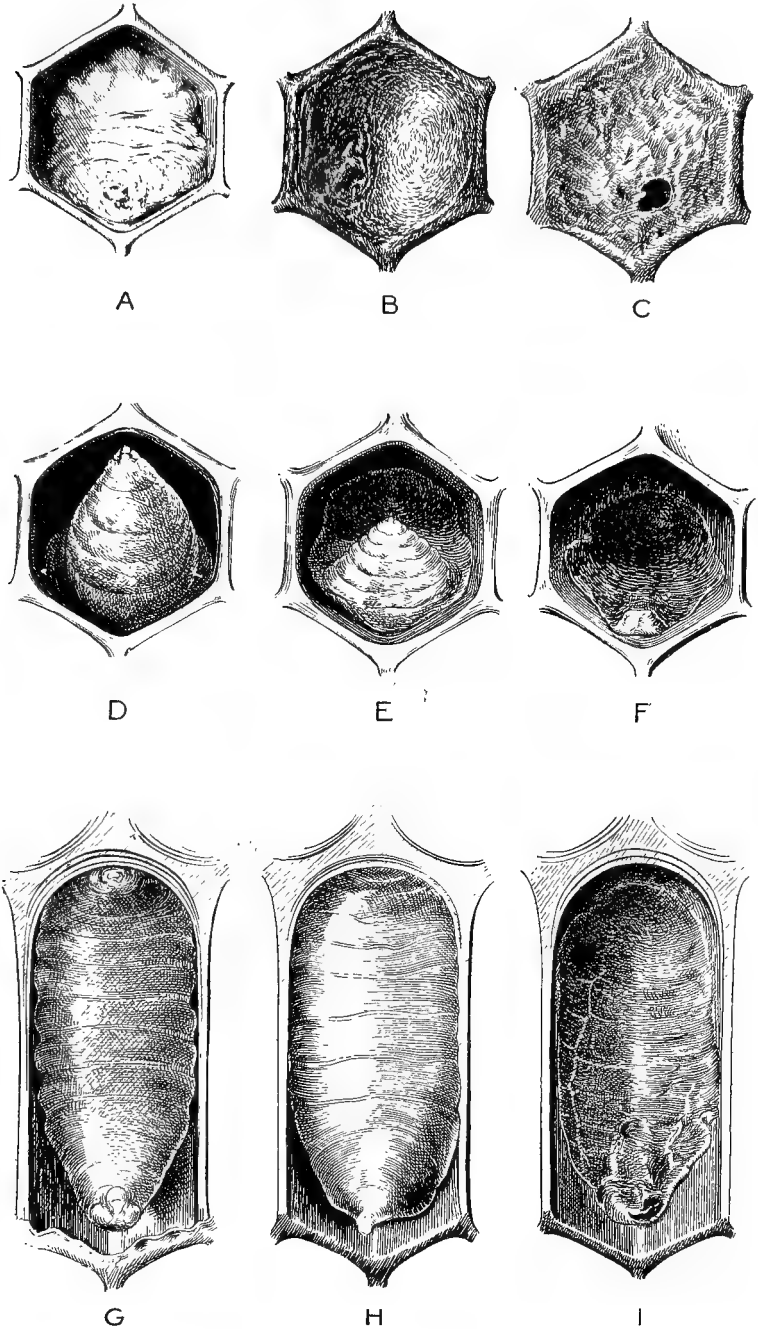

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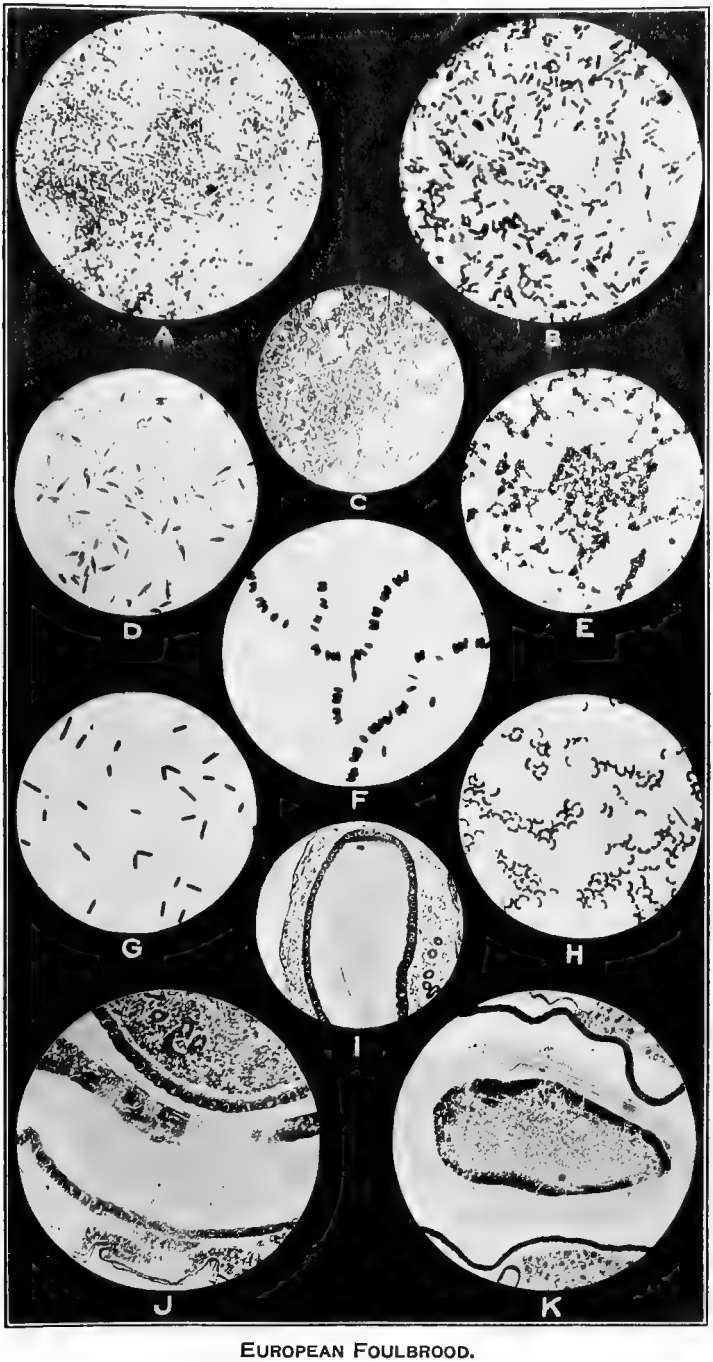

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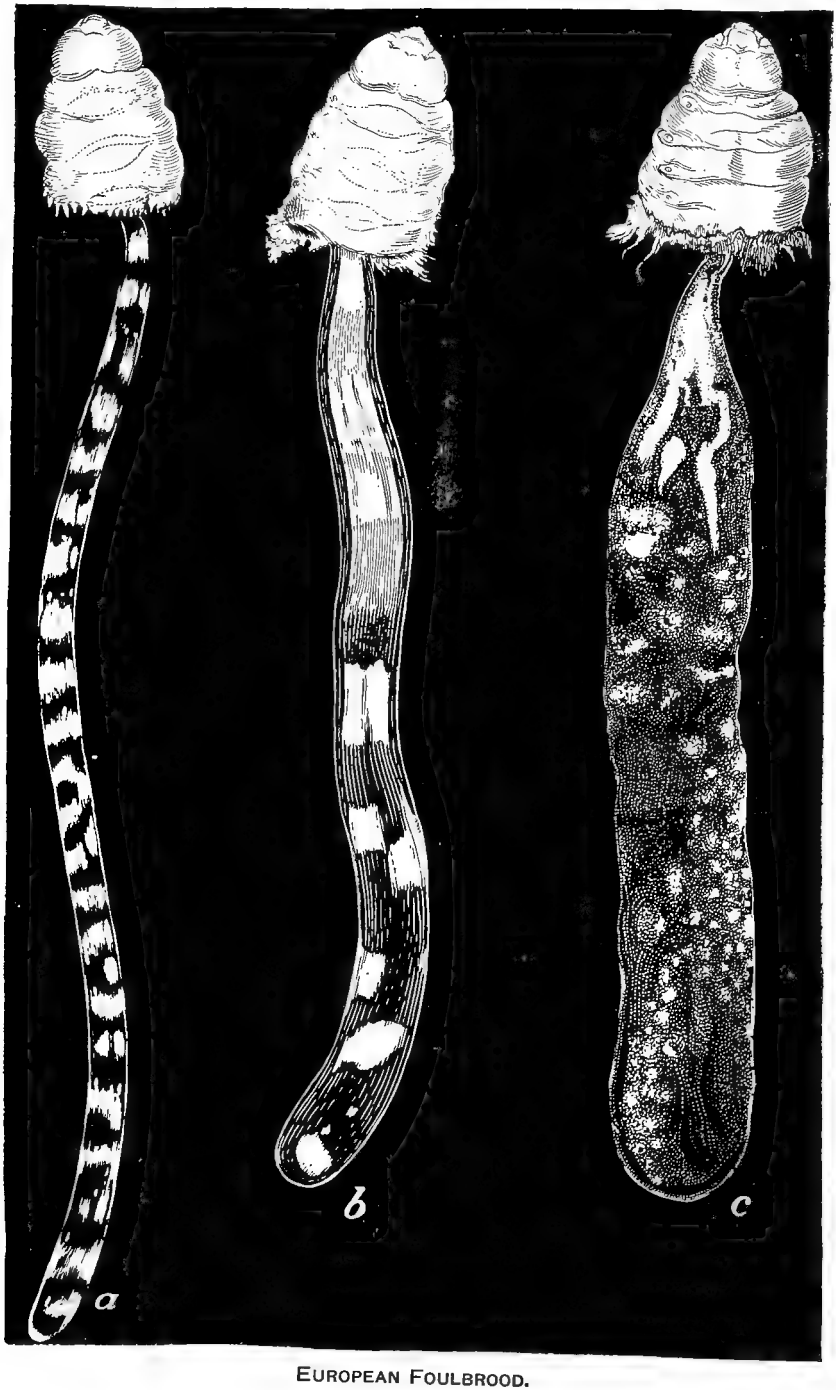

EUROPEAN FOULBROOD.

UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 431

Contribution from the Bureau of Entomology
L. O. HOWARD, Chief

Washington, D. C. PROFESSIONAL PAPER February 9, 1917

SACBROOD

By

G. F. WHITE
Expert, Engaged in the Investigation of Bee Diseases

CONTENTS

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CONTENTS.

INTRODUCTION.

Sacbrood is an infectious disease of the brood of bees. It is frequently encountered and has often been the cause of fear on the part of beekeepers through a suspicion that one of the more serious maladies—the foulbroods—was present.

The disease is more benign than malignant. It is insidious in its nature and somewhat transient in its character. The number of colonies that die as a direct result of sacbrood is comparatively small; the loss of individual bees from it, however, in the aggregate is enormous. The loss tends naturally to weaken the colony in which the disease is present, a fact which makes the disease one of great economic importance.

Until recently no laboratory study has been made of this disease. Circular No. 169, Bureau of Entomology, is a preliminary report on recent studies made by the writer. The present bulletin represents the results obtained from a continuation of these studies. In it are included only such results as it is believed can be applied by the beekeeper directly to his needs or as will be otherwise of particular interest to him.

HISTORICAL ACCOUNT.

There are a number of references in beekeeping literature to a disorder of the brood of bees which had been recognized by the presence of dead brood that was different from that dead of "foulbrood." It will be profitable to cite here a few of these articles:

Langstroth (1857) writes as follows:

> There are two kinds of foul-brood, one of which the Germans call the *dry* and the other the *moist* or *fœtid*. The dry appears to be only partial in its effects and not contagious, the brood simply dying and drying up in certain parts of the combs. The moist differs from the dry in this that the brood dies and speedily rots and softens, diffusing a noisome stench through the hive.

In this statement it will be seen that beekeepers had already recognized differences in the brood diseases which caused Langstroth to write that there were two kinds of "foulbrood." The kind referred to as "dry" foulbrood might easily have been sacbrood.

Doolittle (1881), following a description of "foulbrood," writes:

> We have been thus particular in describing the disease [foulbrood] so none can mistake it; and also because there is another disease similar, *called* foul brood, which is not foul brood. With this last-named, the caps to the cells have very much the same appearance as in the genuine, but the dead larva is of a grayish color, and instead of being stretched out at full length in the cell, it is drawn up in a more compact shape. After a time it so dries up that the bees remove it, and no harm seems to arise from it, only as there are a few larvæ that die here and there through the combs at different periods; sometimes never to appear again, and sometimes appearing with the next season; * * *.

Doolittle, therefore, as early as 1881, had also observed a brood disease which he says is similar to foulbrood and called foulbrood, but which is different from the genuine foulbrood. From his description one can readily believe that the disease which he says was not foulbrood was sacbrood.

Jones (1883), of Beeton, Ontario, Canada, writes the following:

> There is also another disease of the larvæ which is sometimes found both in Europe and America, which is more like foul brood than any of the above [chilled, starved, or neglected brood] and which frequently deceives those who we might claim should be good judges, but which, however, is not the genuine article. It is a dying of the brood both before and after it has been capped over. The appearance of this and the genuine is much the same during the earlier stages of their existence, but the former is usually removed by the bees and no further trouble ensues.

It will be noted that Jones also recognized that there was a disease that resembled somewhat the genuine foul brood, but was different from it, and that it was also different from chilled, starved, or neglected brood. Most likely the disorder referred to in his article was sacbrood.

Simmins (1887), writing from Rottingdean, England, points out the difference between "deadbrood" and foulbrood:

> That foul brood is often confused with simple dead brood I am well aware. * * * But that every bee keeper may decide for himself without the aid of a microscope, which is the genuine foulbrood and which is not, I will show how I have always been able to detect the difference. With simple deadbrood, while some may appear like the foul disease, much of the older brood *dries up to a white cinder*, in many cases retaining its original form, which I have never found to occur when genuine foulbrood is present. Chilled brood can be distinguished from the more serious malady in like manner.

In addition to emphasizing the difference between "deadbrood" and "foulbrood," Simmins says that these two diseases are in turn to be differentiated from chilled brood. He adds the additional fact also that Cheshire had examined this "deadbrood" and failed to find any microscopic evidence of disease.

Cook (1904), under the heading "New Bee Disease," writes as follows:

> In California and some other sections the brood dies without losing its form. We use the pin-head, and we draw forth a larva much discolored, often black, but not at all like the salvy mass that we see in foulbrood.

From his description, and from the fact that the disease is quite prevalent in California, it is very probable that the disorder mentioned by Cook is sacbrood.

A study of this "dead brood" recognized by the beekeepers as being different from foulbrood was begun by the writer in New York State in 1902, under the direction of Dr. V. A. Moore. In a brief report on the work (1904) the following is found:

> The beekeepers are sustaining a loss from a diseased condition in their apiaries which they are diagnosing as "pickled brood." The larvæ usually die late in the larval stage. The most of them are found on end in the cell, the head frequently blackened and the body of a watery granular consistency. * * *
>
> The results of the examinations showed that *Aspergillus pollinis* was not found. Further investigations must be made before any conclusion can be drawn as to the real cause of this trouble.

It will be observed from this quotation that the so-called pickled brood did not conform to the description of pickled brood and could not therefore be the condition which had called forth the description of and the name, "pickled brood" (see p. 4).

Burri (1906), of Switzerland, writes:

> Dead brood, said to have been black brood, I have occasionally met with in my investigations. It occurred in the older larvæ, and showed a gray to blackish colora-

tion, partially drying the larvæ until mummified. These larvæ of the black-brood type gave a negative result both in microscopic examination and in the usual bacteriological culture experiments. Bacteria seem to take no part in this disease, and so far as I have come in contact with black brood, I have been able to reach no certain opinion as to its cause. [Translation.]

It is very probable that the disorder encountered by Burri, which was free from bacteria, was sacbrood. Out of 25 samples examined between 1903 and 1905, he found four samples containing this disease alone, while in a few of the samples the disorder was accompanied by one of the other brood diseases.

Kursteiner (1910), of Switzerland, gives a summary of all samples examined by Burri and himself from 1903 to 1909. Out of 360 samples of suspected disease examined, 94 were diagnosed as "dead brood free from bacteria." These were probably samples of sacbrood. As shown by his later reports, Kursteiner has continued to find this disease in the examination of suspected samples.

The foregoing references to the literature show that beekeepers in different countries had been observing dead brood in their apiaries which was unlike brood dead of "foulbrood." On this point all of the observers practically agreed. No name had been given to the disorder.

NAME OF THE DISEASE.

Before 1912, very little definite information concerning this somewhat mysterious disorder of the brood had been obtained. After discovering its cause and determining its true nature, the writer (1913) used the name "sacbrood" to designate it. The name was coined to suggest the saclike appearance of the dead larvæ in this disease at the time they are most frequently seen by the beekeeper.

The fact should here be emphasized that sacbrood is not a new disease. It is only the knowledge concerning the disease and its name that is of recent origin. It is far better, and in all probability much more accurate, to think of sacbrood as a disease which has affected bees longer than history records the keeping of bees by man. The disease, therefore, has been collecting its toll of death for centuries, often unawares to the beekeeper. Simply knowing that there is such a disease should not be the cause of any additional anxiety concerning its losses. On the other hand, less fear should be experienced, since by knowing of it hope may be entertained that the losses resulting from it may be reduced.

PICKLED BROOD.

The term "pickled brood" was introduced into beekeeping literature 20 years ago (1896), by William R. Howard of Texas. The condition which he described under this term he declared was caused

by a fungus to which he gave the name *Aspergillus pollini*. In a second article (1898) he writes that pupæ and adult bees, as well as the larvæ, are attacked by the disease, stating his belief that the disease in adult bees had been diagnosed as paralysis. Technically, therefore, the term "pickled brood" refers to an infectious disorder of bees affecting both the brood and adult bees and caused by a specific fungus, *Aspergillus pollini*.

It was particularly unfortunate that these articles on pickled brood should have appeared at the time they did, as through them some beekeepers have been led to the mistaken belief that the brood disease, which they had so long observed as being similar to "foulbrood," but differing from it, had been described in his articles as pickled brood.

Whether such a disease (pickled brood) does exist, can not be definitely stated. It may be said, however, that it probably does not. The writer has not encountered such a disorder during his study on the bee diseases. He believes that if the condition is present it certainly has not attracted the attention of beekeepers to any great extent. It can safely be advised, therefore, that all fear of losses from such a possible condition should be dispelled, at least until the disease is met with again.

It would seem that the name "pickled brood" is being used among beekeepers at present in a very general sense. Root (1913) writes:

> The name pickled brood has been applied to almost any form of dead brood that was not foul brood. In a rather general way, it seems to cover, then, any form of brood that is dead from some natural causes not related to disease of any sort.

This quotation suggests that a number of conditions are most likely included under the term "pickled brood" as it is popularly used. Brood dead of starvation and that found dead before capping and not dead of an infectious disease seem to be referred to especially by the name.

Beekeepers sending samples of disease to the laboratory have been asked the question: "What disease do you suspect?" In the replies received more than one disease was sometimes suggested as being suspected. Out of 189 replies received from beekeepers sending samples of sacbrood, European foulbrood was suggested in 55 replies, pickled brood in 39, foulbrood in 19, blackbrood in 15, poisoned brood in 7, chilled brood in 5, starved brood in 6, American foulbrood in 13, dead brood in 3, neglected brood in 1, scalded brood in 1, suffocated brood in 1, and in 24 cases the reply was: "Don't know." These replies show that beekeepers generally had not learned to recognize the disorder which is now called sacbrood by any one name.

It is natural to suppose that sacbrood would have been one of the conditions occasionally referred to under the term "pickled brood."

As sacbrood has been proved, however, to be a distinct disease and different from all other disorders, naturally it is incorrect to use the terms "sacbrood" and "pickled brood" synonymously, either in the popular or in the technical sense.[1]

APPEARANCE OF HEALTHY BROOD AT THE AGE AT WHICH IT DIES OF SACBROOD.

By comparing the appearance of healthy brood with that of brood dead of a disease, both the description and the recognition of the symptoms of the disease are often materially aided. Before discussing the symptoms of sacbrood, therefore, a description of the healthy brood at the age at which it dies of sacbrood will be given. In this description the same method will be used and similar terms employed as will be found in the description of the symptoms of the disease.

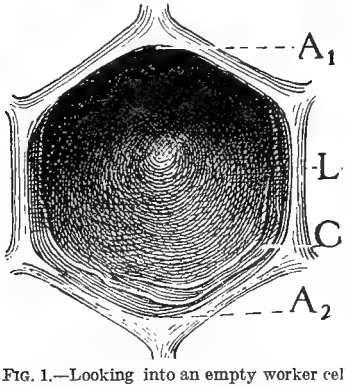


FIG. 1.—Looking into an empty worker cell uncapped by bees. The uppermost angle (A_1), the lowermost angle (A_2), the lateral wall (L), and the wrinkling of the inner surface of the cell near the opening, indicating the presence of a mass of cocoons (C), are shown. Enlarged about 8 diameters. (Original.)

It will be recalled by those who are at all familiar with healthy comb in which brood is being reared that the brood is arranged in such a way that capped and uncapped areas occur alternately and in more or less semicircular fashion. Practically all cells in the uncapped areas will be without caps while practically all in the capped areas will be capped.

Since the brood that dies of sacbrood, with but few exceptions, does so in capped cells, a description of such brood involves the form, size, and position of these cells.

A cell (figs. 1 and 2) may be described as having six side walls, a bottom or base, and a cap. (The cap has been removed by the bees from the cells from which these figures were drawn.) In general the six side walls are rectangular and equal. These walls form six equal obtuse angles within the cell (fig. 1). The angle which is uppermost in the cell (A_1) is formed by two sides which together may be termed the roof of the cell. The angle which is lowermost (figs. 1 and 2, A_2) is formed by two sides which with equal propriety may together be termed the floor of the cell (fig. 2, F). When a cell is cut along its long axis

[1] For the purpose of an explanation for those who may have learned to refer to sacbrood by the term "pickled brood," it might be felt advisable by some to continue for a while in some way a reference to the latter term. In such an event, the expression "so-called pickled brood" is suggested as being more nearly accurate than the term "pickled brood."

the cut surface of the older ones shows the presence of a varying number of old cocoons (fig. 2, C). Near the mouth of the cell on the side walls (figs. 1 and 2, C) will often be noted a wrinkling of the surface. This wrinkling is caused by the presence of old cocoons. The two remaining walls are parallel and will be referred to as the lateral walls (fig. 1, L). The bottom is concave on the inside. The cap

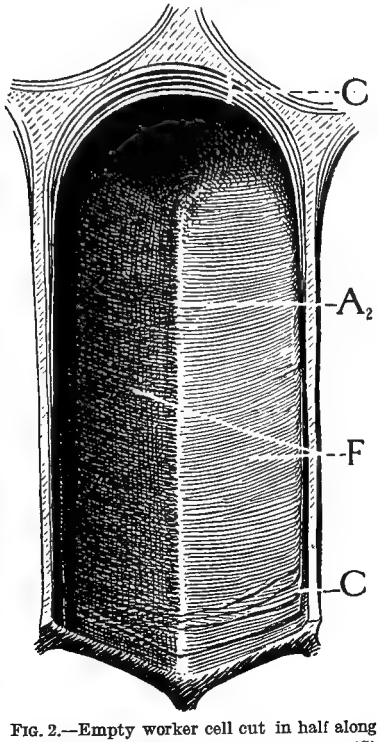


FIG. 2.—Empty worker cell cut in half along the long axis of the cell, showing cocoons (C) at the base and near the mouth of the cell, and the lowermost angle (A_2) formed by the two walls which constitute the floor (F) of the cell. Enlarged about 8 diameters. (Original.)

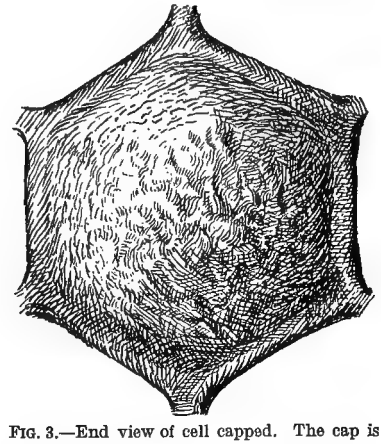


FIG. 3.—End view of cell capped. The cap is convex, being recently constructed. (Original.)

is also concave on the inside, making it convex on the outside.

When freshly constructed the surface of the cap (fig. 3) is smooth and and entire and shows considerable convexity. Later, not infrequently it is found to be less convex and somewhat irregular. The cap should remain normally for the most part entire (fig. 8). While this is the rule, there are exceptions to it. The beekeeper is familiar with the appearance which suggests that it had not been entirely completed (fig. 11; Pl. II, *b*).

The long axis of the cell is nearly horizontal, the bottom of the cell being normally only slightly lower than the mouth. The long axis measures approximately one-half inch, while the perpendicular distance between any two diametrically opposite side walls is approximately one-fifth of an inch. The side walls are each approximately one-tenth of an inch wide. It is in such a cell, then, that the brood of the age at which it dies of sacbrood is found.

APPEARANCE OF A HEALTHY LARVA AT THE AGE AT WHICH IT DIES OF SACBROOD.

The symptoms which differentiate sacbrood from the other brood diseases are to be found primarily in the post-mortem appearances of the larvæ dead of the disease. As an aid in interpreting the description of these appearances a description of the healthy larvæ is first made.

Larvæ[1] that die of sacbrood do so almost invariably after capping and at some time during the four days just preceding the change in form of the maturing bee to that of a true pupa.

During the first two days of this prepupal period the larva moves about more or less in the cell and spins a cocoon. It is then comparatively quiet for about two days, lying on its dorsal side and ex-

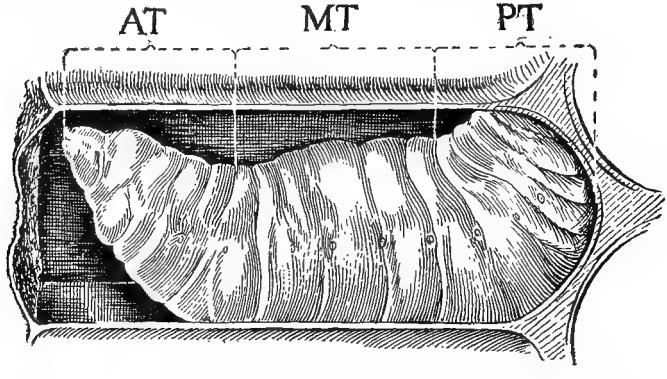


FIG. 4.—Lateral view of healthy worker larva showing the normal position within the cell. For convenience of description the length is divided into thirds—anterior third (AT), middle third (MT) and posterior third (PT). Enlarged about 8 diameters. (Original.)

tended lengthwise in the cell. At the close of this two-day period of rest, as a result of the metamorphosis going on, the larva changes very rapidly to a true pupa, assuming the outward form of an adult bee.

Although many larvæ die of sacbrood during the first two days or active period, of the 4-day prepupal period, by far the greater number of deaths occur during the last two days, the period of rest. A healthy larva at this resting period of its development is chosen, therefore, for description. As dead worker larvæ are the ones usually encountered in sacbrood and the ones almost invariably chosen in discussing the symptoms of the disease, the worker larva is here described.

The normal larva lies extended in the cell (fig. 4) on its dorsal side, motionless, and with its head pointing toward the mouth of the cell. Its posterior or caudal end lies upon the bottom of the cell,

[1] As beekeepers usually refer to the brood at this age as "larvæ," the term is used here to designate the developing bee at this stage of its growth.

while its extreme anterior or cephalic end extends almost to the cap and roof. The length of the larva is approximately one-half inch, being nearly that of the cell. Its two lateral sides cover about one-half each of the two lateral walls. The width of the larva is approximately one-fifth of an inch, being the distance between the two lateral walls of the cell.

The dorsal portion of the larva lies against the floor of the cell, being more or less convex from side to side and also from end to end. Its ventral surface is convex from side to side, and is, generally speaking, concave from end to end. Considerable empty space is found between the larva and the roof of the cell. The spiracles are visible. The glistening appearance, characteristic of a larva before capping, very largely disappears after capping. Although larvæ at this age might be thought of as white, they are in fact more or less bluish white in color. It is possible to remove a healthy larva at this age from the cell without rupturing the body wall, but care is required in doing so.

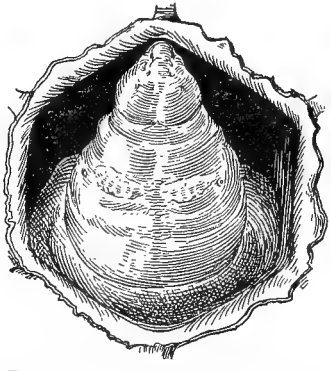


FIG. 5.—End view of healthy worker larva in normal position in the cell. Cap torn and turned aside with forceps. Enlarged about 8 diameters. (Original.)

For purposes of description it is convenient to divide the length of the larva into three parts. These may be denominated the anterior (AT), middle (MT), and posterior thirds (PT).

Anterior third.—On removing the cap from a cell the anterior cone-shaped portion of the larva is seen (fig. 5; Pl. II, *g*). The apex of this cone-shaped third is directed upward toward the angle in the roof of the cell, but is not in contact with the roof or the cap. Transverse segmental markings are to be seen. Along a portion of the median dorsal line there is frequently to be observed a narrow transparent area. A cross section of this third is circular in outline. The anterior third passes rather abruptly into the middle third. At their juncture on each lateral side, owing to a rapid increase in the width of the larva at this point, there is presented the appearance of a "shoulder."

Middle third.—This third (figs. 6 and 4; Pl. II, *m*) lies with its dorsal portion upon the floor of the cell, its axis being nearly horizontal. The ventral surface is convex from side to side, and is considerably below the roof of the cell. This upper surface is crossed from side to side by well-marked furrows and ridges representing segments of the larva. These furrows and ridges produce a deeply notched appearance at the lateral margins. In some of the segments a transverse trachea may be seen appearing as a very fine, scarcely per-

ceptible, white line. Sometimes there may be seen a narrow area along the median line of the ventral surface that is more nearly transparent than the remaining portion of the surface. This area may extend slightly into the anterior and posterior thirds. It is similar in appearance to the one on the dorsal side, but less distinct. A cross section of this third is slightly elliptical in outline. The middle third passes more or less gradually into the posterior third. The juncture on the ventral surface is indicated by a wide angle formed by the ventral surfaces of these two thirds.

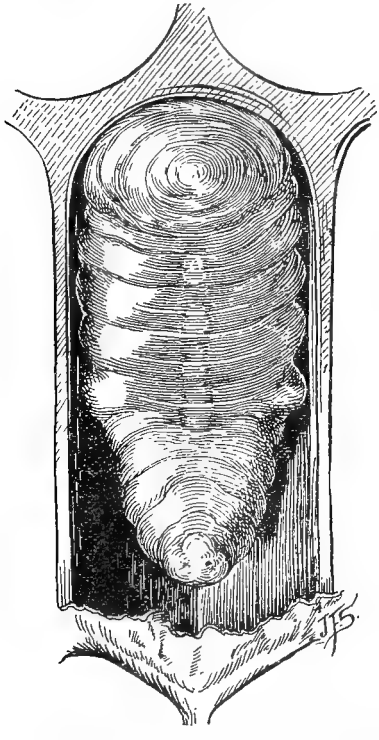


FIG. 6.—Healthy larva and cell viewed from above and at an angle. (Original.)

Posterior third.—In form the posterior third (figs. 6 and 4) is an imperfect cone, the axis of which is directed somewhat upward from the horizontal. This third occupies the bottom portion of the cavity of the cell. Its dorsal surface lies upon the bottom wall, with the extreme caudal end of the larva extending to the roof of the cell (fig. 4). The third is marked off into segments by ridges and furrows similar to, but less regular than, those of the middle third.

TISSUES OF A HEALTHY LARVA AT THE AGE AT WHICH IT DIES OF SACBROOD.

Upon removing a larva in the late larval stage and puncturing its body wall lightly, a clear fluid almost water-like in appearance flows out. This fluid consists chiefly of larval blood. By heating it, or by treating it with any one of a number of different reagents, a coagulum is formed in it. Upon rupturing the body wall sufficiently, the tissues of the larva flow out as a semiliquid mass. The more nearly solid portion of the mass appears almost white. This portion is suspended in a thin liquid, chiefly blood of the larva. A microscopic examination shows that the cellular elements of the mass are chiefly fat cells. Many fat globules suspended in the liquid tend to give it a milky appearance.

SYMPTOMS OF SACBROOD.

The condition of a colony depends naturally upon the condition of the individual bees of which it is composed. In the matter of diseases in practical apiculture the beekeeper is interested primarily in the

colony as a whole, and not in individual bees. Therefore, in describing the symptoms of a bee disease, the colony as a whole should be considered as the unit for description, and not the individual bee. A symptom of disease manifested by an individual bee, broadly considered, is, in fact, also a colony symptom. The symptoms of sacbrood as described in this paper are, therefore, those evidences of disease that are manifested by a colony affected by the disease.

It has been found that sacbrood can be produced in a healthy colony by feeding it a suspension in sirup of crushed larvæ dead of the disease. With sacbrood thus produced in experimental colonies the symptoms of the disease have been studied, and the description of these symptoms given here is based chiefly upon observations made in these experimental studies. The facts thus obtained are in accord with those observed in numerous samples of the disease sent by beekeepers from various localities in the United States for diagnosis. They are in accord, furthermore, with the symptoms as they have been observed in colonies in which the disease has appeared, not through experimental inoculation but naturally.

The symptoms of sacbrood which would ordinarily be observed through a more or less casual examination of the disease will first be considered. It must be remembered that the brood is susceptible to the disease, but that the adult bees are not.

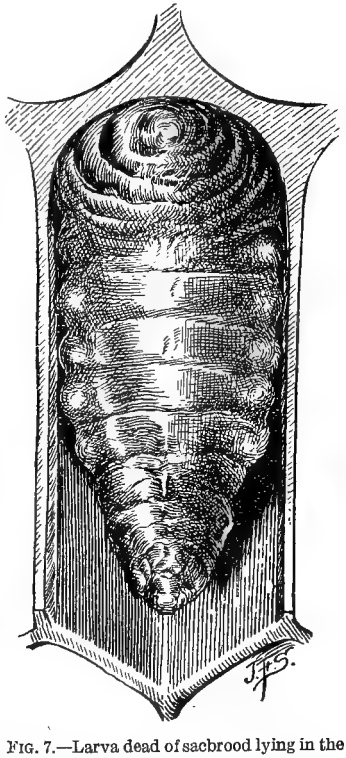


FIG. 7.—Larva dead of sacbrood lying in the cell as viewed from above and at an angle. It may have been dead a month. Cap of cell removed by bees. Enlarged about 8 diameters. (Original.)

SYMPTOMS AS OBSERVED FROM A CASUAL EXAMINATION.

The presence of dead brood is usually the first symptom observed. An irregularity in the appearance of the brood nest (Pl. I, figs. 1 and 2; Pl. IV) frequently attracts attention early in the examination. The strength of a colony in which the disease is present is often not noticeably diminished. Should a large amount of the brood become affected, however, the colony naturally becomes weakened thereby, the loss in strength soon becoming appreciable. Brood that dies of the disease does so almost invariably in capped cells, but before the pupal

stage is reached. It is rare to find a pupa dead of sacbrood (Pl. II, *zz*). The larvæ that die (fig. 7) are found lying extended lengthwise with the dorsal side on the floor of the cell. They may be found in capped (fig. 8) cells or in cells which have been uncapped (fig. 9), as bees often remove the caps from cells containing dead larvæ. Caps that are not removed are more often entire, yet not infrequently they are found to have been punctured by the bees. Usually only one puncture is found in a cap (Pl. II, *d*), but there may be two (fig. 10) or even more (Pl. II, *f*). The punctures vary in size, sometimes approximating that of a pinhead, although usually smaller, and are often irregular in outline. Sometimes a cap (fig. 11, Pl. II, *b*) has a hole through it which suggests by its position and uniform circumference that it has never been completed. Through such an opening (fig. 11; Pl. II, *e*) or through one of the larger punctures the dead larva may be seen within the cell.

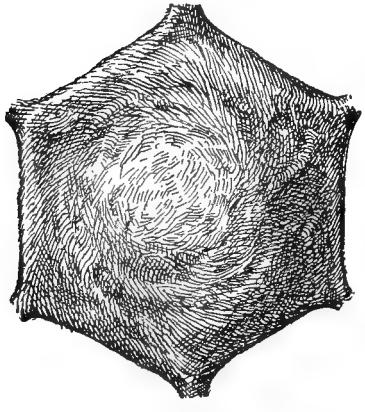


FIG. 8.—End view of capped cell which contains a larva dead of sacbrood, being similar to the one shown in figure 9. The cap here is not different from a cap of the same age over a healthy larva. (Original.)

A larva recently dead of sacbrood is slightly yellow. The color in a few days changes to brown. The shade deepens as the process of decay continues, until it appears in some instances almost black. Occasionally for a time during the process of decay the remains present a grayish appearance.

In sacbrood, during the process of decay, the body wall of the dead larva (figs. 7 and 9) toughens, permitting the easy removal of the remains intact from the cell. The content of the saclike remains, during a certain period of its decay, is watery and granular in appearance. Much of the time the form of the remains is quite similar to that of a healthy larva. If the dead larva is not removed, its surface, through evaporation of its watery content, becomes wrinkled, distorting its form. Further drying results in the formation of the

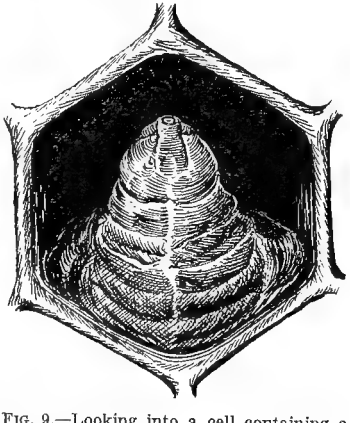


FIG. 9.—Looking into a cell containing a larva dead of sacbrood. The stage of decay is about the same as in figure 8. (Original.)

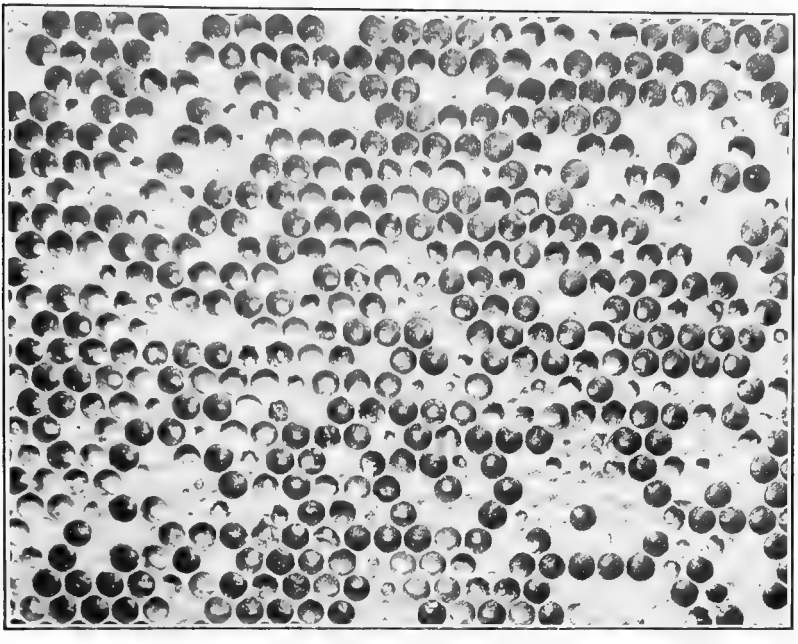


FIG. 1.—MARKED SACBROOD INFECTION. SIZE SLIGHTLY LESS THAN NATURAL. (ORIGINAL.)

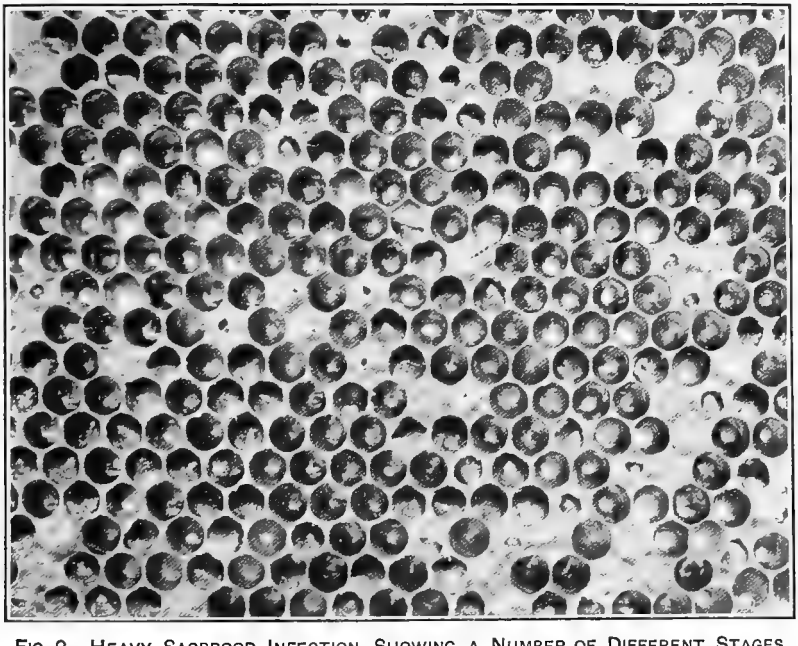
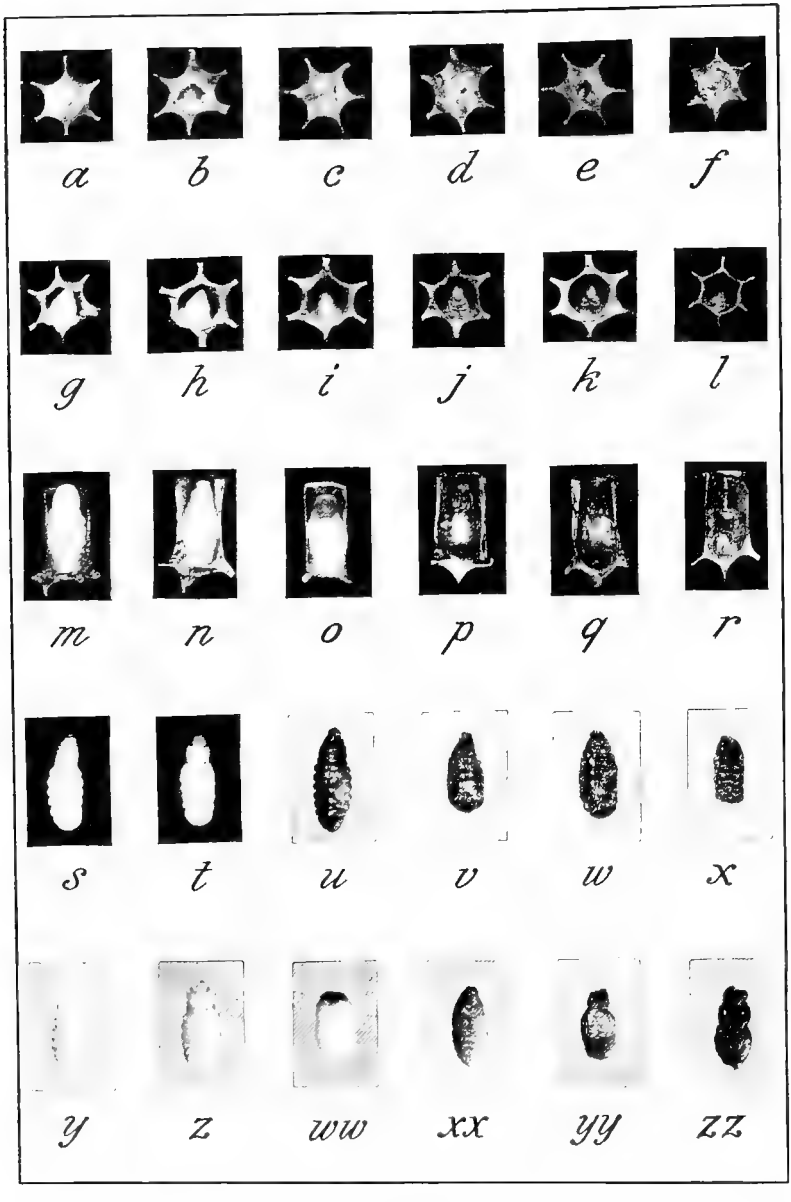


FIG. 2.—HEAVY SACBROOD INFECTION, SHOWING A NUMBER OF DIFFERENT STAGES OF DECAY OF LARVÆ. EGGS, YOUNG LARVÆ IN DIFFERENT STAGES OF DEVELOPMENT, AND DISEASED LARVÆ IN SAME AREA. NATURAL SIZE. (ORIGINAL.)

SACBROOD PRODUCED BY EXPERIMENTAL INOCULATION.



COMPARISON OF A HEALTHY LARVA AND THE REMAINS OF LARVÆ DEAD OF SACBROOD.

a, A cap of a healthy larva; *b*, *c*, *d*, *e*, and *f*, caps over larvæ in first, second, third, fourth, and fifth stages of decay, respectively; *g*, a healthy larva, end view; *h*, *i*, *j*, *k*, and *l*, an end view of the five stages of decay; *m*, a healthy larva viewed from above; *n*, *o*, *p*, *q*, and *r*, corresponding view of the five stages of decay; *s* and *y*, healthy larva removed from the cell; *t*, *u*, *v*, *w*, and *z*, larval remains in different stages of decay removed from the cell; *ww*, a larva recently dead of sacbrood with the anterior third removed by the bees; *x*, a scale removed from the cell; *xx*, larval remains from which a small portion has been removed by bees; *yy*, almost a pupa; *zz*, a pupa dead of sacbrood which had only recently transformed. (Original.)

"scale" (figs. 22, 23; Pl. II, *l*, *r*, and *x*). This scale is not adherent to the cell wall.

In sacbrood the brood combs may be said to have no odor. Larvæ undergoing later stages of decay in the disease, however, when crushed in a mass and held close to the nostrils are found to possess a disagreeable odor.

From a superficial or casual examination alone of a case of sacbrood it may be mistaken for some other abnormal condition of the brood. A careful study of the post-mortem appearances of larvæ dead of the disease, however, will make it possible to avoid any such confusion. A more careful study of the dead larvæ is therefore justified.

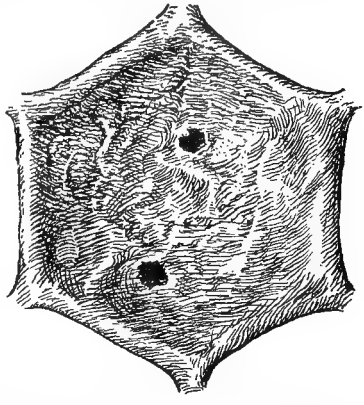


FIG. 10.—Cap of cell containing the remains of a larva dead of sacbrood. The cap is slightly sunken and bears two perforations made by the bees. (Original.)

APPEARANCE OF LARVÆ DEAD OF SACBROOD.

No signs in a larva dying of sacbrood have yet been discovered by which the exact time of death may be determined. As the larvæ in this disease usually die during the time when they are motionless, lack of movement can not be used as an early sign of death. In this description it is assumed that the larva is dead if it shows a change in color from bluish-white to yellowish or indications of a change from the normal turgidity to a condition of flaccidity.

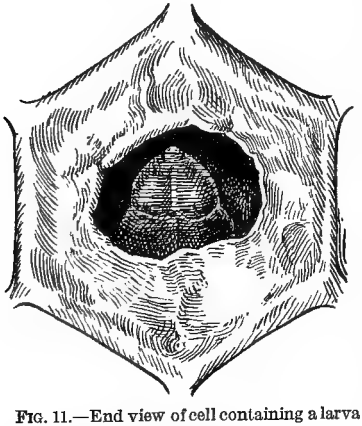


FIG. 11.—End view of cell containing a larva dead of sacbrood, with a cap which has the appearance of never having been completed. (Original.)

The appearance of a larva dead of sacbrood varies from day to day, changing gradually from that of a living healthy larva to that of the dried residue—the scale. A description that would be correct for a dead larva on one day, therefore, may and probably would be incorrect for the same larva on the following day. Moreover, all larvæ dead of the disease do not undergo the same change in appearance, causing another considerable range of variation. For convenience of description, this gradual and continual change in appearance is here considered in five more or less arbitrary stages. As the

same plan will be followed and similar terms will be used in describing these stages as were employed in the description of a healthy larva of the same age, the interpretation of the description will be aided if the appearance of a healthy larva as described above is borne in mind.

First Stage.

Uncapping a larva showing the first symptoms of the disease, it will be observed that it has assumed a slightly yellowish appearance.

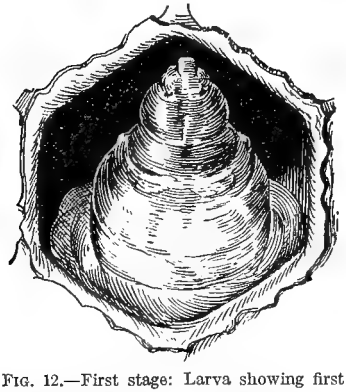


Fig. 12.—First stage: Larva showing first symptoms of sacbrood and presenting the dorsal view of the anterior third. Cap removed artificially. (Original.)

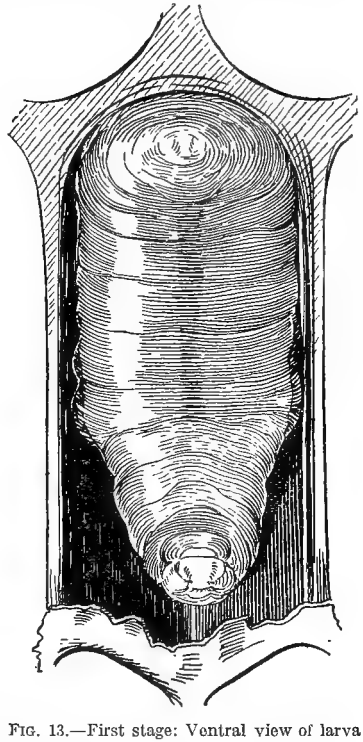


Fig. 13.—First stage: Ventral view of larva dead of sacbrood as seen from above and at an angle, giving a ventral view of all three thirds. Cap torn across. (Original.)

This shade deepens somewhat during the stage, but does not become a deep yellow.

Anterior third.—The lateral margins and extreme cephalic end of the anterior third (fig. 12; Pl. II, *b*, *h*) may have assumed, and frequently do assume, a more or less transparent appearance (represented in the figure by shading). The position and the surface markings of the anterior third are approximately those of the normal larva. When a change in the position is observed, however, the extreme anterior end of the larva—the apex of this cone-like third—having settled somewhat, does not approach so near the roof of the cell as does that of a healthy larva. It is sometimes found also that this cone-like third is deflected more or less to one side or the other.

Middle and posterior thirds.—The changes from the normal that have taken place in these two thirds are similar and can, therefore, be described together. The yellowish tint is here observed. The transverse ridges and furrows are still well marked (fig. 13). The trans-

verse tracheæ under slight magnification may be distinctly seen. The narrow, somewhat transparent area present along the ventral median line of the healthy larva is still to be seen in this stage of the decay. The lateral and posterior margins are still deeply notched and are frequently found to appear quite transparent. This appearance is due to a watery looking fluid beneath the cuticular portion of the body wall.

Sometimes only the remnant of a larva (fig. 14; Pl. II, *ww*) dead of sacbrood is found in the cell. Such remnants vary in size. The

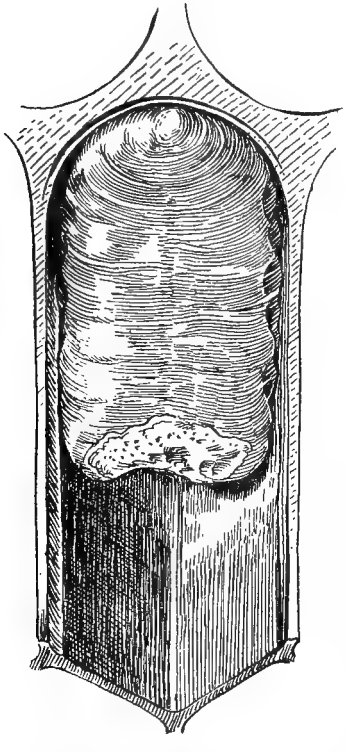


FIG. 14.—First stage: Portion of a larva dead of sacbrood, showing a more or less transverse roughened surface from which the bees have removed a portion of the larva piecemeal. (Original.)

FIG. 15.—Second stage: Dorsal view of anterior third of a larva dead of sacbrood. (Original.)

surface left from the removal of tissues is somewhat roughened, indicating that the removed portion has been taken away piecemeal, and is more or less transverse to the larva.

Consistency of the larva in the first stage.—The cuticular portion of the body wall, which chiefly constitutes the sac that characterizes the disease sacbrood, is less easily broken at this time than in the healthy larva. When the body wall is broken the tissues of the larva, which constitute the contents of the sac, flow out. This fluid tissue mass is less milky in appearance than that from a normal larva. The granular character of the contents of the sac which is marked in later stages of decay is already in evidence. By microscopic examination the granular appearance is found to be due chiefly to fat cells.

Condition of the virus in the first stage.—When larvæ of this stage are crushed, suspended in sirup, and fed to healthy bees, a large

amount of sacbrood is readily produced, showing that the larval remains in this stage are particularly infectious. This is an important fact, as it is the stage of decay at which the larva is frequently removed piecemeal from the cell.

SECOND STAGE.

The color of the decaying larva has changed from the yellowish hue of the first stage to a brownish tint. The yellow, however, has not yet in all cases entirely disappeared.

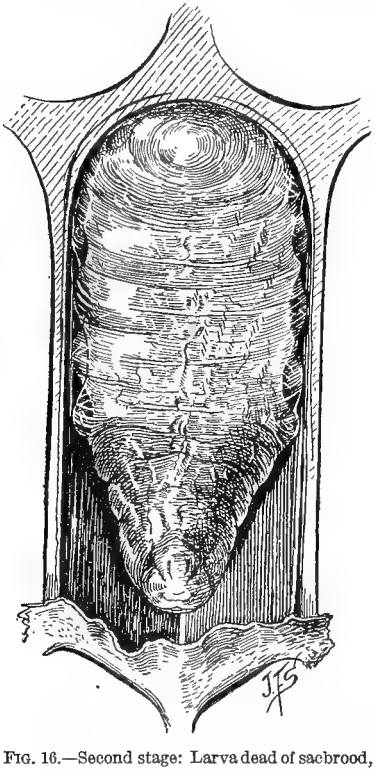


FIG. 16.—Second stage: Larva dead of sacbrood, ventral view. (Original.)

FIG. 17.—Third stage: Dorsal view of anterior third of larva dead of sacbrood. (Original.)

Anterior third.—The shade of brown is deeper in the anterior third (fig. 15; Pl. II, *i*) as a rule than in the other two thirds. On the ventral surface of the anterior third there are sometimes present minute, very dark, nearly black areas, appearing little more than mere points. Upon dissecting away the molt skin, these areas are found to be associated with the developing head and thoracic appendages of the bee. The position of the anterior third in this stage has changed only slightly from that observed in the preceding one. The apex is farther from the roof of the cell (Pl. II, *i*). The deflection is more marked and is seen in a greater number of larvæ. The surface markings have not changed materially.

Middle and posterior thirds.—The changes that have occurred in each of these two thirds are still similar and can, therefore, again be described together.

The ventral surface of these two thirds (fig. 16, Pl. II, *o*) is less convex from side to side. The ridges and furrows, representing the segments, are less pronounced. The lateral margins are still deeply notched. The prominent angle seen on the ventral side of a healthy larva, at the juncture of the middle and posterior thirds, has given place to a wider one in this stage of decay. The clear subcuticular fluid frequently observed at the lateral and posterior margins of larvæ dead of this disease is here increased in quantity.

Consistency of the contents of the sac.—The cuticular sac is now more readily observed and less easily broken. The decaying contents consist of a more or less granular-appearing mass suspended in a watery appearing fluid, the mass possessing a slightly brownish hue. The microscopic examination shows that the granular appearance is due to the presence of decaying tissue cells, chiefly fat cells, which are changing slowly as the decay of the larva goes on.

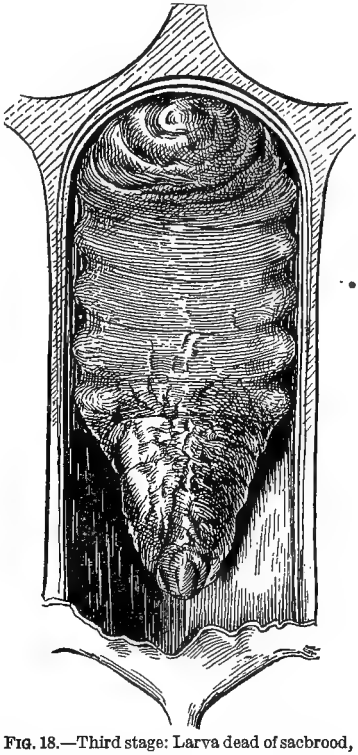


FIG. 18.—Third stage: Larva dead of sacbrood, ventral view. (Original.)

Condition of the virus.—The results of inoculations show that the remains of larvæ at this stage of decay are still in some instances infectious. The amount of infection produced when such larvæ are used in making inoculations is very much less, however, than when larvæ in the first stage are used.

THIRD STAGE.

The color of the dead larva of this stage is quite brown, that of the anterior third being a deeper shade than that of the other two thirds. An indication that the remains are drying is observed in the wrinkling of the surface that is beginning to be in evidence.

Anterior third.—The color of the anterior third is a deep brown. This third still preserves its conelike form (figs. 17 and 9; Pl. II, *j*), the distance of the apex from the roof of the cell being still further increased. This may equal one-fourth or more of the diameter of the mouth of the cell. The surface markings are still quite similar to those of a healthy larva with the exception that evidences of drying are present.

Middle third.—While the color of the middle third is similar to and often approaches in its shade that of the anterior, very frequently it is considerably lighter. The ventral surface of this third (figs. 18 and 7) is less convex from side to side than in the preceding stage, and the segmental markings, while still plainly visible, are less pronounced. The notches along the lateral margins are also less pronounced.

Posterior third.—The color of the posterior third (figs. 18 and 7; Pl. II, *p*) equals or exceeds in depth of shade that of the middle third and sometimes equals that of the anterior third. The surface markings are still pronounced and much resemble those of the normal larva.

That the watery content of the sac is being lessened through evaporation is evidenced by the diminution of the quantity of the watery-

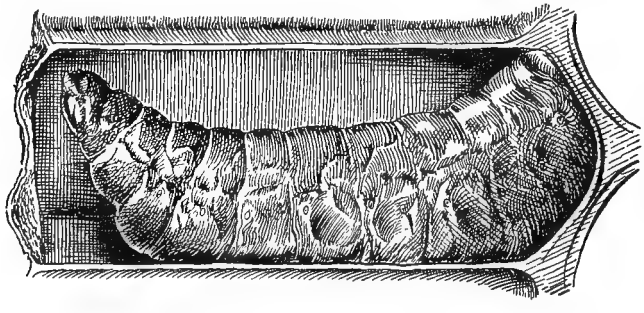


FIG. 19.—Third stage: Larva dead of sacbrood, lateral view. (Original.)

appearing substance seen at the lateral margins of the middle and posterior thirds and by the wrinkling of the cuticular sac. These wrinkles are small and numerous.

The lateral view of the larva in the third stage (fig. 19) shows that it still maintains, in a general way, the form and markings of the normal larva (fig. 4). The turgidity is gone, although the position in the cell is very much as it is in the healthy larva.

Consistency of the sac and its contents.—It is the appearance of the remains of the larva in the third stage of the decay that best characterizes the disease, sacbrood. The cuticular sac is now quite tough, permitting the removal of the larva from the cell with considerable ease and with little danger of its being torn. The content of the sac is a granular mass, brownish in color and suspended in a comparatively small quantity of a more or less clear watery-appearing fluid. Upon microscopic examination the mass is found to consist of decaying tissues, chiefly fat cells.

Condition of the virus in the third stage.—When the larval remains in this stage of decay are crushed and fed in sirup to healthy colonies no sacbrood is produced, indicating that the dead larvæ at this stage

are not infectious. The status of the virus in this stage is not definitely known, but the facts thus far obtained indicate that it is probably dead.

FOURTH STAGE.

The brown color of the larval remains has further deepened, the anterior third being much darker as a rule than the other two-thirds. The marked evidence of drying now present might be said to characterize this stage.

Anterior third.—The color is a very deep brown, often appearing almost black. As a result of drying, the apex of this conelike third

FIG. 20.—Fourth stage: Remains of larva dead of sacbrood. (Original.)

is often nearer the roof of the cell in this stage than in the preceding one. As a result it has also been drawn inward from the mouth of the cell. The surface markings seen in the normal larva are in this stage (fig. 20; Pl. II, *k*) of decay almost obliterated through the wrinkling of the surface, due to drying.

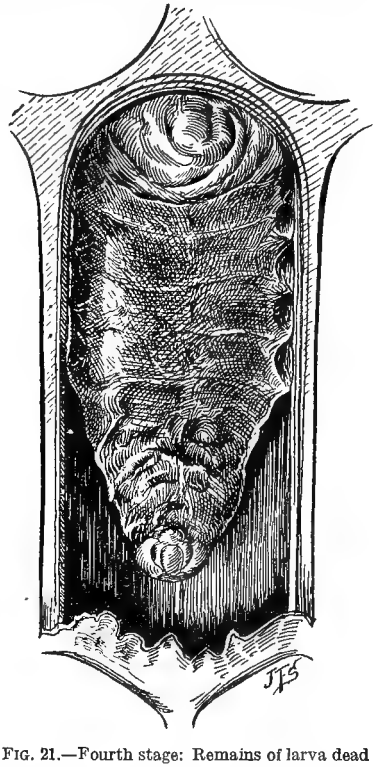


FIG. 21.—Fourth stage: Remains of larva dead of sacbrood, ventral view. (Original.)

Middle third.—This third is decidedly brown, but lighter in shade than the anterior third. The ventral surface (fig. 21; Pl. II, *q*) is slightly concave from side to side. The segmental markings are still to be seen, but are not at all prominent. The notched lateral margins extend upon the side walls of the cell. The subcuticular fluid so noticeable in some of the earlier stages has disappeared through evaporation. The effect of drying is very noticeable, causing a marked wrinkling of the surface.

Posterior third.—The posterior third (Pl. II, *q*) may or may not be darker than the middle third, but it is not darker than the anterior

third. The effect of the drying on this third is quite perceptible also. The surface markings and notched margin of the normal larva are still indicated in the decaying remains, but are much less pronounced. The subcuticular fluid is no longer in evidence.

Consistency of the contents of the sac.—Upon tearing the sac, the contents are found to be less fluid than in preceding stages. The decaying tissue mass is still granular in appearance. As the drying proceeds further the contents of the sac become pastelike in consistency.

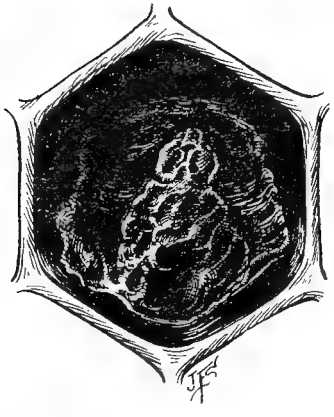


FIG. 22.—Fifth stage: Scale, or larval remains, in sacbrood as seen on looking into the cell. (Original.)

Condition of the virus in the fourth stage.—As in the preceding stage, the larval remains in the fourth stage do not seem to be infectious.

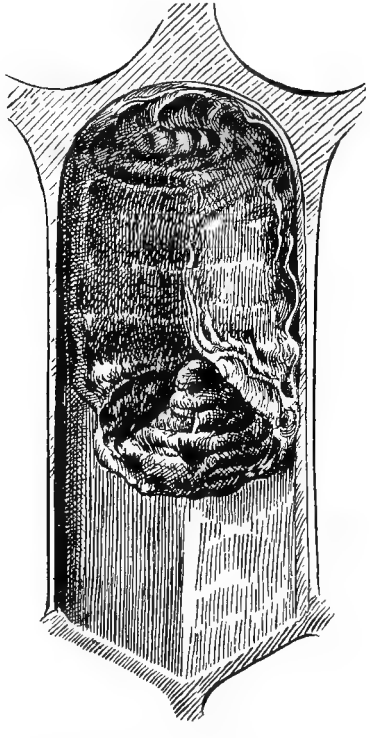


FIG. 23.—Fifth stage: Scale, or larval remains, in sacbrood viewed at an angle from above. (Original.)

FIFTH STAGE.

The dead larva in this last stage has lost by evaporation all of its moisture, leaving the dry, mummylike remains known as the "scale."

Anterior third.—The anterior third (fig. 22; Pl. II, *l*) through drying is retracted from the mouth of the cell, with the apex drawn still deeper into the cell and raised toward its roof. This third is greatly wrinkled, and, being of a very dark-brown color, presents often an almost black appearance.

Middle third.—The middle third (fig. 23; Pl. II, *r*), is deeply concave from side to side and may show remnants of the segmental markings of the larva. The surface is often roughened through drying. Sometimes both longitudinal and transverse tracheæ are

plainly visible. The margin frequently presents a wavy outline corresponding to the original furrows and ridges of the lateral margin of the larva.

Posterior third.—The posterior third (figs. 23 and 24) extends upon the bottom of the cell, but does not completely cover it. A lateral view of the scale (fig. 24) shows that it is turned upward anteriorly and drawn somewhat toward the bottom of the cell. The ventral surface is concave, often roughened, and directed somewhat forward. This margin, like that of the middle third, has a tendency toward being irregular.

The scale.—The scale can easily be removed intact from the cell. (Pl. II, *x*.) Indeed, when very dry, many of them can be shaken from the brood comb. When out of the cell, they vary markedly in appearance. The anterior third is of a deeper brown than the the other two thirds as a rule. The dorsal side of the middle and

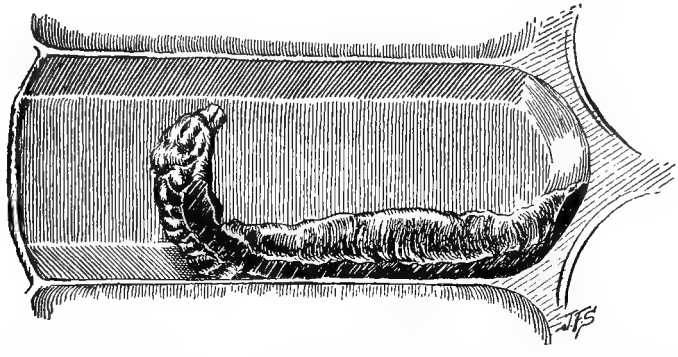


FIG. 24.—Scale, or larval remains, in position in cell cut lengthwise, lateral view. (Original.)

posterior thirds is shaped to conform to the floor of the cell, being in general convex, with a surface that is smooth and polished. The margin is thin and wavy. The anterior third and the lateral sides of the middle and posterior thirds being turned upward, the ventral surface being concave, and the posterior side being convex, the scale in general presents a boatlike appearance and could be styled "gondola-shaped." This general form of the scale has been referred to by beekeepers as being that of a Chinaman's shoe. When completely dry, the scale is brittle and may easily be ground to a powder.

Condition of the virus in the scale.—The scales in sacbrood, when fed to healthy bees, have shown no evidence of being infectious.

The length of time that dead larvæ are permitted by the bees to remain in the cells before they are removed varies. They may be removed soon after death, they may remain until or after they have become a dry scale, or they may be removed at any intervening stage in their decay. Not infrequently they are permitted to remain to or

through the stage described above as the third stage (figs. 7, 9, 17, and 18; Pl. II *j*, *p*). That the dead larvæ are allowed to remain in the cells often for weeks is in part the cause of the irregularity observed in the appearance of the brood combs (p. 11). (Pls. I, IV.)

APPEARANCE OF THE TISSUES OF A LARVA DEAD OF SACBROOD.

The gross appearance of a larva during its decay after death from sacbrood has just been described. The saclike appearance of the remains, with its subcuticular watery-like fluid and its granular content, can better be interpreted by knowing something of the microscopic structure of the dead larva.

A section through a larva (fig. 25, A) dead of sacbrood shows that the fat tissue constitutes the greater portion of the bulk of the body. The fat cells (FC) are comparatively large. In the prepared section when considerably magnified (C) they are seen to be irregular in outline, with an irregular-shaped nucleus (Nu). Bodies stained black, more or less spherical in form and varying in size, are found in them. The presence of these cells is the chief cause for the granular appearance of the contents of larvæ dead of sacbrood. This appearance has often been observed by beekeepers and is a well-recognized symptom of sacbrood.

In the section (A) may be seen a molt skin (C_2), which is at a considerable distance from the hypodermis (Hyp). Another cuticula (C_1) is already quite well formed and lies near the hypodermis. Between these two cuticulæ (C_2 and C_1) during the earlier stages of decay there is a considerable space ("intercuticular space") (IS). This space is filled with a watery-looking fluid. That the fluid is not water, but that it is of such a nature that a coagulum is formed in it during the preparation of the tissues for study, is shown by the presence of a coagulum in the sections.

The body (B, A) wall of the larva is composed of the cuticula (C_1), the hypodermis (Hyp) and the basement membrane (BM). The hypodermal cells may be present in the mass content of the larval remains. These cells are comparatively small. Similar ones are to be found in the tracheal walls (Tra). These cells, however, make up only a small portion of the contents of the sac.

There are many other cellular elements to be found in the decaying mass of larval tissues, some of which contribute to this granular appearance. Among these are the œnocytes (Oe), cells (D) larger than the fat cells, but comparatively few in number. These are found among the fat cells, especially in the ventral half of the body. The œnocytes in the prepared tissues are irregular in outline, having a nucleus regular in outline. The cytoplasm is uniformly granular and does not contain the black staining bodies found in the fat cells (C).

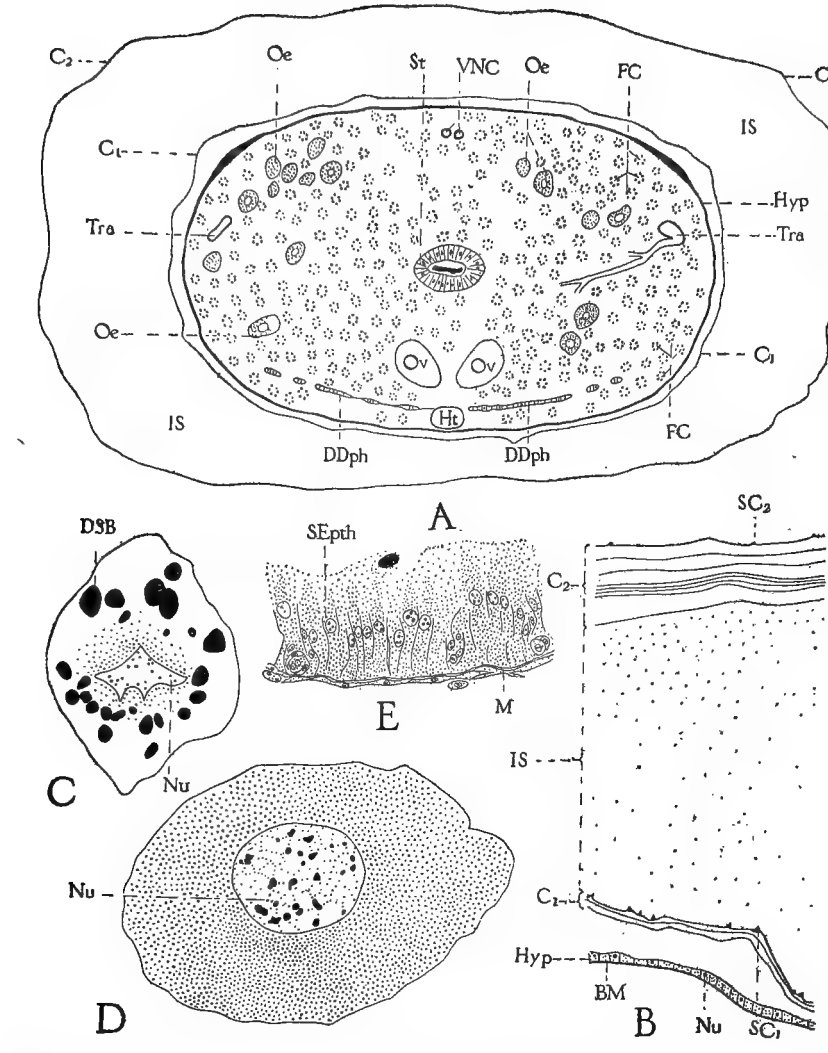


FIG. 25.—The tissues of a worker larva after being dead of sacbrood about one week. A, cross section, semidiagrammatic, of the abdomen in the region of the ovaries, showing a recently cast cuticula, or molt skin (C_2), a newly formed cuticula (C_1), the hypodermis (Hyp), the stomach (St), the ovaries (Ov), the heart (Ht), the ventral nerve cord (VNC), the dorsal diaphragm (DDph), tracheæ (Tra), œnocytes (Oe), and fat cells (FC). Between the cuticula C_2 and the cuticula C_1 is a considerable intercuticular space (IS). B represents the body wall in this pathological condition, showing the cuticula C_2 and the cuticula C_1, both bearing spines (SC_2 and SC_1), and the intercuticular space (IS) in which is found evidence of a coagulum formed from the fluid filling the space by the action of the fixing fluids. The remainder of the body wall, the hypodermis (Hyp), and the basement membrane (BM) are also shown. C, fat cell with irregular outline, irregular nucleus (Nu), and deep staining bodies (DSB). D, œnocyte with uniformly staining cytoplasm, and with a nucleus (Nu) having a uniform outline. E, a portion of the stomach wall showing the epithelium (SEpth) during metamorphosis, it being at this time quite columnar in type, and the musculature (M). (Original.)

The molt skin (C_2) is probably the one that is shed normally about three days after the larva is capped. The cuticula (C_1), already quite well formed, is probably the one which normally would have entered into the formation of the molt skin that is cast at the time the larva or semipupa changes to a pupa. The molt skin (C_2) constitutes for the most part the sac which is seen to inclose the decaying larval mass in sacbrood, the cuticula (C_1) probably assisting somewhat at times. The presence of the subcuticular fluid is made more intelligible by these facts. Larvæ dying of sacbrood at an earlier or later period in their development will present an appearance varying somewhat from that just described.

Contrasted with the stomach (midintestine or midgut) of a feeding larva, the stomach (A, St) of a larva at the age at which it dies of sacbrood is small. The cells lining the wall of the organ vary considerably in size and shape, depending upon the exact time at which death takes place. In contrast to the low cells of the stomach wall in younger larvæ, the cells (E, SEpth) at this later period are much elongated. These cells would also at times be found in the decaying granular mass present in the larval remains.

The various organs of the body contribute to the cellular content of the decaying larval mass. At the period at which the larva dies of sacbrood, the cellular changes accompanying metamorphosis are particularly marked. This condition introduces various cellular elements into the decaying larval mass.

The granular mass from the larval remains in sacbrood is, therefore, a composite affair. Upon examining the mass microscopically, it will be found that the granular appearance is due for the most part to fat cells suspended in a liquid. The liquid portion seems to be chiefly blood of the larva, or, at least, derived from the blood, although augmented most probably by other liquids of the larva and possibly by a liquefaction of some of the tissues present. The granular mass suspended in a watery fluid, as a symptom of sacbrood, is by these facts rendered more easily understood.

CAUSE OF SACBROOD.

Doolittle (1881), Jones (1883), Simmins (1887), Root (1892 and 1896), Cook (1902), Dadant (1906), and others through their writings have pointed out the fact that there are losses sustained from sacbrood. There has been no consensus of opinion, however, as to the infectiousness of the disease. On this point Dadant (1906) writes:

> Whatever may be the cause of this disease (so-called Pickled Brood), and although it is to a certain extent contagious, it often passes off without treatment. But, as colonies may be entirely ruined by it, it ought not to be neglected.

In the quotation Dadant expresses the belief that the disease is an infectious one. This view has been proved by recent studies to be the correct one. Since the disease is one of a somewhat transient nature, often subsiding and disappearing quickly without treatment, and is quite different in many ways from the foulbroods, it is not strange that some writers should have held that it is not infectious.

PREDISPOSING CAUSES.

Beekeepers have known for many years certain facts concerning the predisposing causes of sacbrood. Recent studies have added others relative to sex, age, race, climatic conditions, season, and food as possible predisposing factors in the causation of the disease.

Age.—The results of the studies suggest that adult bees are not directly susceptible to the disease. Pupæ are rarely affected (Pl. II, *zz*). If one succumbs to the disease, it is quite soon after transformation from the larval stage. Primarily it is the larvæ that are susceptible. When a larva dies of the disease, it does so almost invariably after capping, and usually during the 2-day period immediately preceding the time for the change to a pupa.

Sex.—Worker and drone larvæ may become infected. Queen larvæ apparently are also susceptible, although this point has not yet been completely demonstrated.

Race.—No complete immunity against sacbrood has yet been found to exist in any race of bees commonly kept in America. That one race is less susceptible to the disease than another may be said to be probable, although the extent of such immunity has not been established.

The question: "What race of bees is there in the diseased colony?" was asked beekeepers sending samples of diseased brood. Out of 140 replies received from those sending sacbrood samples, 53 reported hybrids, 49 reported Italians, 21 reported blacks, and 17 reported Italian hybrids. These replies show that the bees commonly kept by American beekeepers are susceptible, although their relative susceptibility is not shown.

The bees which have been inoculated in the experimental work on sacbrood have been largely Italians or mixed with Italian blood. Blacks have also been used. No complete immunity was observed in any colony inoculated. That the blacks are more susceptible than strains having Italian blood in them is suggested by some of the results. Facts concerning the problem of immunity as relating to bees are yet altogether too meager to justify more definite statements.

Climate.—Historial evidence strongly suggests that sacbrood is found in Germany (Langstroth, 1857), England (Simmins, 1887),

and Switzerland (Burri, 1906). Beuhne (1913) reports its presence in Australia, and Bahr (1915) has encountered a brood disorder among bees in Denmark which he finds is neither of the foul broods. He had examined 10 samples of it but had not studied it further. He says it may be sacbrood.

About 400 cases of sacbrood have been diagnosed by Dr. A. H. McCray and the writer among the samples of brood received for examination at the Bureau of Entomology. A few of these were obtained from Canada. Whether the disease occurs in tropical climates or the coldest climates in which bees are kept has not yet been completely established.

The mountains and coast plain of the eastern United States, the plains of the Mississippi Valley and the mountains, plateaus, and coast plain of the western portion of the country have contributed to the number of samples examined. It occurs in the South and the North.

Its occurrence in such widely different localities is proof that sacbrood is of such a nature that it can appear under widely different climatic conditions. The relative frequency of the disease, furthermore, is not materially different in the different sections of the country. It must be said, however, that the extent, if any, to which the disease is affected by climate has not yet been determined.

The practical import of these observations regarding climate, of particular interest here, is that the presence of sacbrood in any region can not be attributed entirely to the prevailing climatic conditions.

Season.—It has long been known that sacbrood appears most often and in the greatest severity during the spring of the year. As is shown by the results obtained in the diagnosis of it in the laboratory, the disease may appear at any season of the year at which brood is being reared. In the inoculation experiments sacbrood has been produced with ease from early spring to October 21. While it is thus shown that the brood is susceptible to sacbrood at all seasons, various factors together cause the disease to occur with greater frequency during the spring.

Food.—Before it was known that sacbrood is an infectious disease the quantity or quality of food was not infrequently mentioned by beekeepers as being the cause of the disease. Since a filterable virus has been shown to be the exciting cause of the disease, it is left to be considered whether food is a predisposing cause. The distribution of the disease mentioned above, under the heading "Climate," here again serves a useful purpose. Since it occurs in such a wide range of localities, wherein the food and water used by the bees vary as greatly almost as is possible in the United States, the conclusion may be drawn that its occurrence is not dependent upon food of any restricted character. Furthermore, sacbrood is found in colonies having an abundant supply of food, as well as in colonies having a

scarcity. It has been produced experimentally in colonies under equally varying conditions in regard to the quantity of food.

While it is possible that the quantity or quality of food may influence somewhat the course of the disease in the colony, the rôle played by food in the causation of sacbrood must be slight, if indeed it contributes at all appreciably to it. Practically, therefore, for the present it may be considered that neither the quality nor quantity of food predisposes to this disease.

EXCITING CAUSE OF SACBROOD.

That sacbrood is an infectious disease was demonstrated by the writer (1913) through experiments performed during the summer of 1912. This was done by feeding to healthy colonies the crushed tissues of larvæ dead of sacbrood, suspended in sugar sirup. The experiments were performed under various conditions, and it was found that the disease could be produced at will, demonstrating thereby that it was actually an infectious one.

In the crushed larval mass no microorganisms were found either microscopically or culturally to which the infection could be attributed, although the experiments had proved that the larva dead of the disease did contain the infecting agent. This led to the next step in the investigation, which was to determine whether the virus was so small that it had not been observed, and whether its nature would permit its passage through a filter. The first filter used for this purpose was the Berkefeld.

The process by which the filtration is done is briefly this: Larvæ which have been dead of sacbrood only a few days are picked from the brood comb and crushed. The crushed mass is added to water in the proportion of 1 part larval mass to 10 parts water. A higher dilution may be used. This aqueous suspension is allowed to stand for some hours, preferably overnight. To remove the fragments of the larval tissues still remaining, the suspension is filtered, using filter paper. The filtrate thus obtained is then filtered by the use of the Berkefeld filter[1] (fig. 26) properly prepared. The filtering in the case of the coarser filters especially can be done through gravity alone.

To determine whether any visible microorganisms are present in this last filtrate, it is examined microscopically and culturally. When found to be apparently free from such microorganisms, a quantity of it may be added to sirup and the mixture fed to healthy colo-

[1] The Berkefeld filter consists of a compact material (infusorial earth) in the form of a cylinder. A glass mantel (A) in which is fixed the filter forms a cup for holding the fluid to be filtered. Having filtered the aqueous suspension of crushed sacbrood larvæ through paper, the filtrate is then filtered by allowing it to pass through the walls of the Berkefeld cylinder (B). The filtrate from this filtration is collected into a sterile flask (F) through a glass tube (D) with its rubber connection (C). In filtering in this instance gravity is the only force used.

nies. When all this is properly done, sacbrood will appear in the inoculated colonies. This shows that the virus [1] of this disease, to a

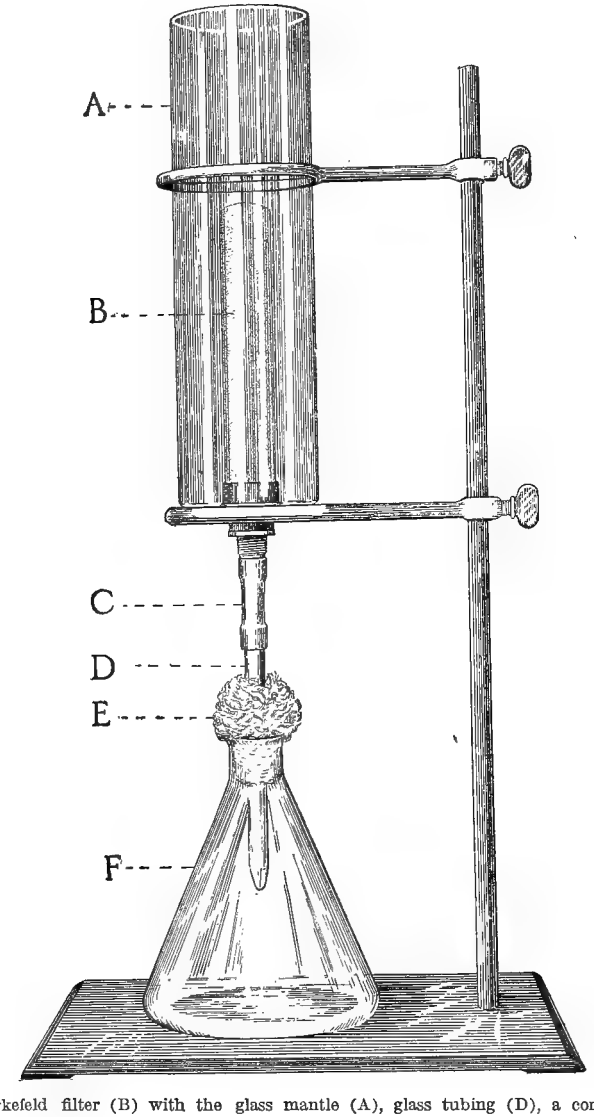


FIG. 26.—Berkefeld filter (B) with the glass mantle (A), glass tubing (D), a connecting rubber tubing (C), and a flask (F) with a cotton plug (E). (Original.)

certain extent, at least, passes through the Berkefeld filter. With this filter the virus is therefore filterable.

[1] In referring to the infecting agent in sacbrood, the term "virus" is preferable to the terms "germ" or "parasite." In relation to the disease, however, its meaning is the same as that conveyed by the latter terms.

In the study of the virus of sacbrood use has been made also of the Pasteur-Chamberland filter [1] (fig. 27). This is a clay filter, the pores of which are much finer than those of the Berkefeld used. In using this filter, an aqueous suspension of larvæ dead of the disease is prepared as before. This is filtered by the aid of pressure obtained

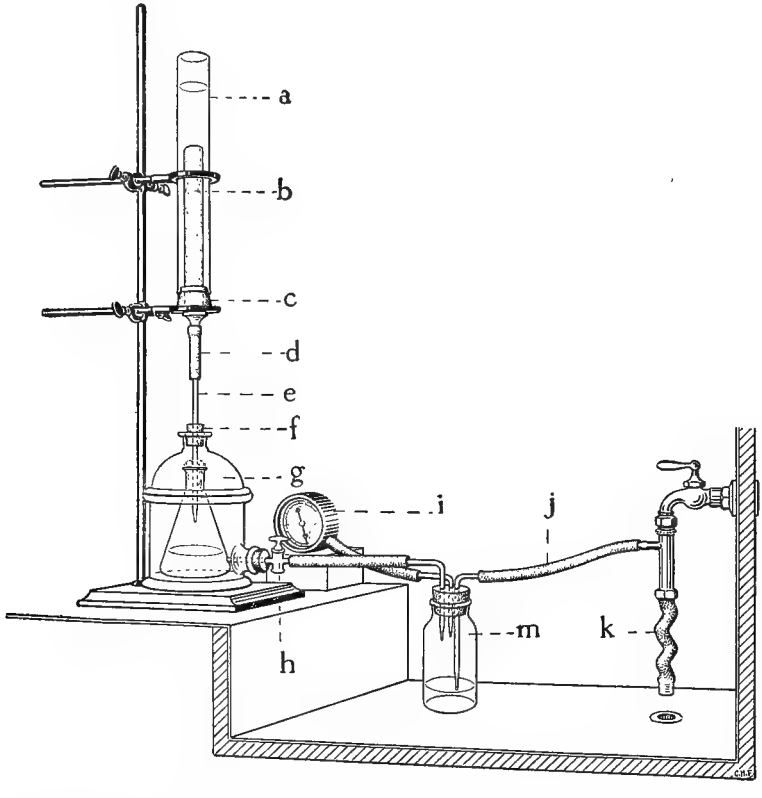


FIG. 27.—A convenient apparatus which can be employed in using the Pasteur-Chamberland, Berkefeld, and other filters. Pasteur-Chamberland filter (b) with a glass mantle (a), a rubber stopper (c) through which passes the filter, a connecting rubber tubing (d), glass tubing (e), a perforated rubber stopper (f), a vacuum jar (g), designed by the writer, in which is placed a cotton-stoppered and sterilized flask, a glass stopcock (h), a vacuum gauge (i), a reservoir (m) with pressure-rubber connections (j), and a vacuum pump (k). (Original.)

by means of a partial vacuum in an apparatus devised for this purpose. Filtrates obtained from this filter when fed to healthy colonies produced the disease. Since the virus of sacbrood will pass through

[1] The Pasteur-Chamberland filter consists of clay molded in the form of a hollow cylinder and baked. This is used with a glass cylinder (a) fitted with a rubber stopper (c). In the use of this filter, force is employed. This was obtained for these experiments through the use of a jar (g) devised by the writer in which a partial vacuum can be produced. In this jar, is placed a flask plugged with cotton and sterilized. Connections are made as shown in the illustration, the vacuum being produced through the use of the pump (k). In less than half an hour usually a half-pint of filtrate can be obtained with this apparatus.

the pores of the Pasteur-Chamberland filter also, it is therefore filterable and is very properly referred to as a "filterable"[1] virus.

In considering the virus of sacbrood it is suggested that the beekeeper think of it as a microorganism [2] which is so small or of such a nature that it has not been seen, and which will pass through the pores of fine clay filters. This conception of it will at least make it more easily understood.

WEAKENING EFFECT OF SACBROOD UPON A COLONY.

The first inoculations in proving that sacbrood is an infectious disease were made on June 25, 1912. Two colonies were used, each being fed with material from a different source. The inoculation feedings were made on successive days. Sacbrood having been produced in the colonies, the inoculations were continued at intervals throughout July and August. During this period, a large amount of sacbrood was present in both colonies. By the end of July these colonies had become noticeably weakened, and by the end of August they had become very much weakened, as a result of the sacbrood present in them. On September 5 one of the colonies swarmed out.

The brood (Pl. IV) of this colony, large in quantity, was practically all dying of sacbrood. The other colony, when examined on September 16, was found to be very weak. At this time, however, most of the dead brood had been removed and healthy brood was being reared. This colony increased in strength and wintered successfully.

The results obtained from the inoculation of these two colonies demonstrated not only that sacbrood is an infectious disease, but also that the disease in a colony tends to weaken it. The results indicate also that a colony may be destroyed by the disease, or it may recover from it, gain in strength, and winter successfully.

Each year since 1912 two or more colonies have been fed sacbrood material at intervals during the brood-rearing season for the purpose of obtaining disease material for experimental purposes. The inoculated colonies in all instances have shown a tendency to become weakened as a result of the inoculations.

The death of the worker larvæ is the primary cause for the weakness resulting from the disease in a colony. Another point to be thought of is that dead sacbrood larvæ remaining in the cells for weeks, as they not infrequently do, reduce the capacity of the brood nest for brood rearing, which has a tendency also to weaken the colony.

[1] In searching the tissues of larvæ dead of sacbrood and the filtrates obtained from them nothing has been discovered by the aid of the microscope, or culturally, which has yet been demonstrated as being the infecting agent. This being true, the virus could be spoken of tentatively as an "ultramicroscopic virus." It is preferable, for the present, however, to refer to it simply as a filterable virus.

[2] There is some question whether, in the case of diseases having a virus which is filterable, the infecting agent is in every instance a microorganism. The evidence is strong, however, that it is.

AMOUNT OF VIRUS REQUIRED TO PRODUCE THE DISEASE, AND THE RAPIDITY OF ITS INCREASE.

Assuming the virus of sacbrood to be a very minute microorganism, the number of germs present in a larva dying of the disease must be considered as exceedingly large. Whether a single germ taken up by a larva will produce the disease in every instance, or in any instance, is not known. If the disease does result at any time from the ingestion of a single germ, all of the conditions, it may be assumed, must be especially favorable for the production of the disease. From what is known of diseases of other animals and of man, and from the results thus far obtained in the study of sacbrood, it is well, at present, to assume that the number of sacbrood germs taken up by a larva may be so small that no disease results.

It is certain, however, that a comparatively small number of sacbrood germs ingested by a larva about two days old are sufficient to produce the disease. That the few germs thus taken up can increase within the larva during an incubation period of five or six days to such a vast number as is assumed to be present in a larva dying of the disease indicates the extreme rapidity with which the germs are able to multiply.

The minimum quantity of virus necessary to produce a moderate infection in a colony has not been definitely determined. It was found by experiments, however, that the virus contained in a single larva recently dead of the disease was sufficient to produce a large amount of sacbrood in a colony.

As a very rough estimate, it may be said that the quantity of virus in a single larva dead of sacbrood is sufficient, when suspended in half a pint of sirup and fed to a healthy colony, to produce infection in and death of at least 3,000 larvæ. Starting then with the virus contained in a single larva, in less than one week it would easily be possible to have 3,000 larvæ dead of the disease, which means that the virus has been increased 3,000-fold within one week. This latter amount of virus would be sufficient to produce an equal amount of infection in 3,000 colonies, increasing the amount of virus again 3,000-fold. In less than two weeks, therefore, theoretically it would be possible to produce a sufficient amount of virus to infect 9,000,000 colonies, more colonies probably than are to be found at present in the United States. Carrying the idea somewhat further, within three weeks, theoretically enough virus could be produced to inoculate every colony in existence.

These facts are sufficient to indicate somewhat the enormous rapidity with which the virus of sacbrood is capable of increasing.

METHODS USED IN MAKING EXPERIMENTAL INOCULATIONS.

The laboratory study of bee diseases being new, it has been necessary in many instances to devise new methods. In the experimental inoculations of bees the methods used have undergone revision from time to time. Those now employed have proved quite satisfactory.

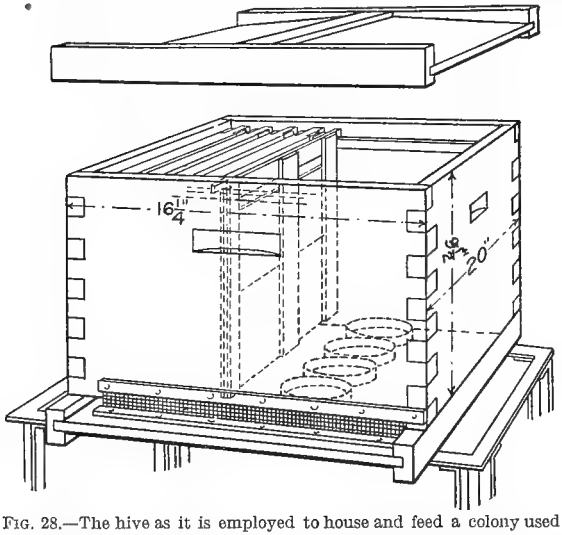


FIG. 28.—The hive as it is employed to house and feed a colony used for experimental inoculations. Here are shown four Hoffman frames, a division board, four open Petri dishes as feeders, and the entrance nearly closed with wire cloth, the opening being on the side of the hive body occupied by the colony. The dimensions indicated are approximate. The angle at which the hive was photographed for this drawing caused its length to appear foreshortened. (Original.)

As the virus of sacbrood has not been cultivated in the laboratory artificially, it has been necessary in these investigations to inoculate a large number of colonies. A nucleus of bees that could be accommodated on from 3 to 6 brood frames was found to serve very satisfactorily the purpose of an experimental colony. The queen should always be clipped. The frames are placed in one side of a 10-frame hive body (fig. 28). Over the entrance to the hive is placed wire cloth, leaving a small space of about 1 inch in length on the side occupied by the brood frames. Petri dishes[1] (fig. 29) serve well the purpose of a feeder. Both halves of the dish are used as receptacles. These are placed, preferably about four of the halves, within the hive on the bottom board on the side not occupied by frames. The hives of the experimental apiary (Pl. III) are arranged chiefly in pairs, with the entrances of consecutive rows pointing in opposite directions. The space occupied by the apiary should be

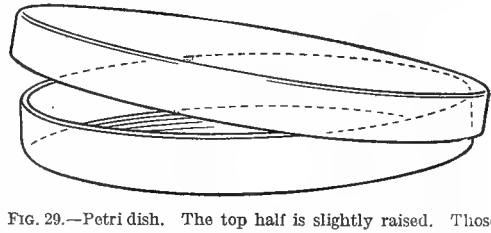
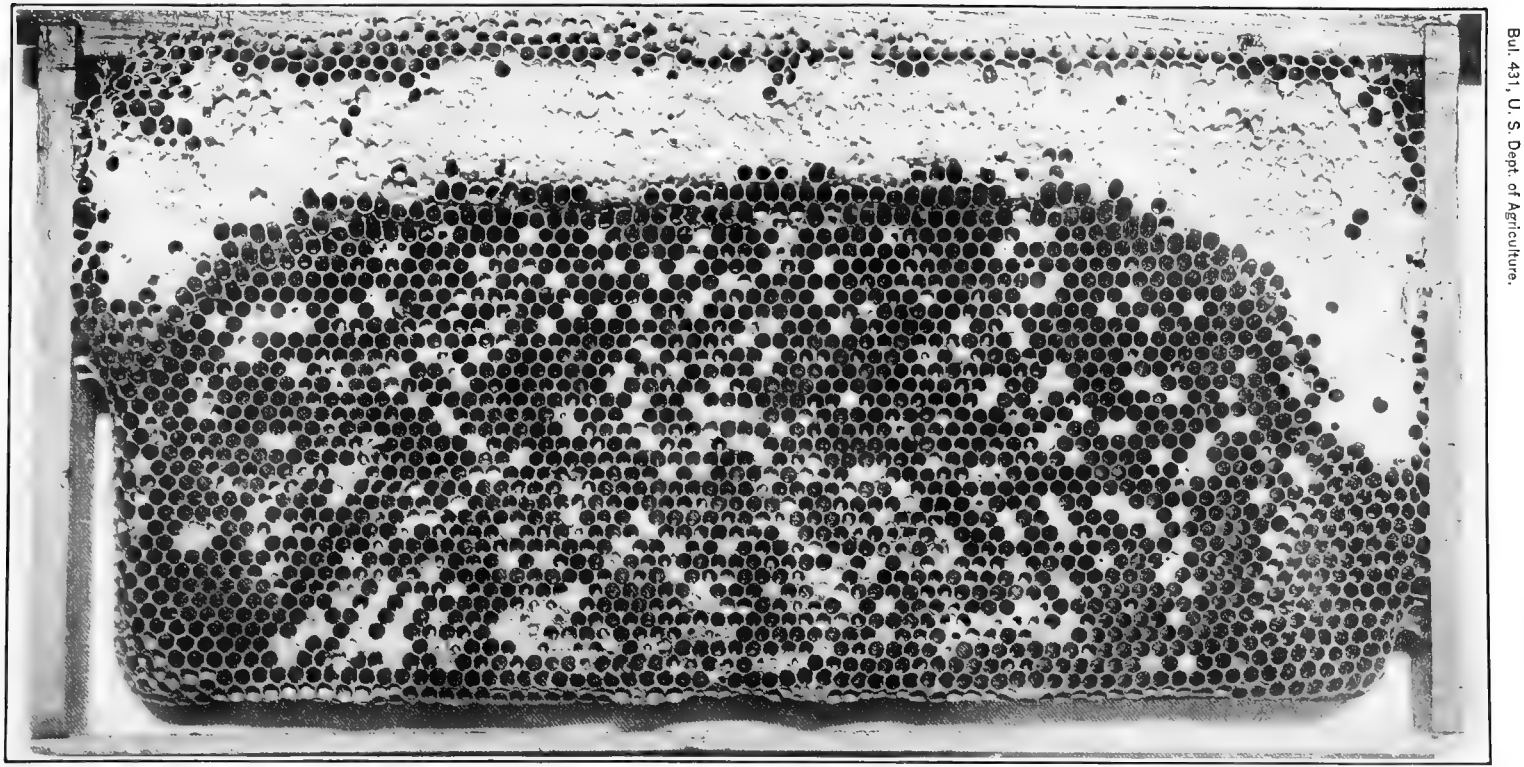


FIG. 29.—Petri dish. The top half is slightly raised. Those used here are 4 inches in diameter. (Original.)

[1] A Petri dish, a much-used piece of apparatus in a laboratory, is simply a shallow, circular, glass dish with a flat bottom and perpendicular sides. It consists of two halves, a bottom and a top. These are very similar. The top half, being slightly larger, fits over the bottom one when the two halves are placed together.

A View of the Experimental Apiary of 54 Colonies in which the Inoculation Experiments made During the Summer of 1915 were Conducted. (Original.)

Plate III.



BROOD FRAME CONTAINING SACBROOD, TAKEN FROM AN EXPERIMENTAL COLONY IN WHICH THE DISEASE WAS PRODUCED BY FEEDING THE VIRUS OF THE DISEASE. (ORIGINAL.)

broken up, preferably by trees or shrubbery. By these means, it will be observed, there is a tendency to minimize the likelihood of robbing, swarming, absconding, and accidental straying or drifting of bees to foreign colonies.

In preparing the material with which the colony is inoculated, larvæ in early stages of the disease are picked from the brood frames, crushed, and added to sugar sirup. The crushed mass from 10 or more sacbrood larvæ, suspended in somewhat more than half a pint of sugar sirup, has been found to be a suitable quantity of the infective material to use in making an inoculation. The suspension may be fed to the bees as one feeding or more. The inoculation feedings should be made as a rule toward evening to avoid the tendency to rob, which may be noticed during a dearth of nectar. Inoculations should not be made when the tendency to rob is at all marked.

FIG. 30.—Capillary pipette. A piece of glass tubing drawn to capillary size at one end. Reduced to three-fourths of the size used. (Original.)

Before a colony is inoculated it should be determined that its activities are normal. A colony should not be inoculated for several days after it has been made by division, or immediately after its removal from a foreign location. An experimental colony when inoculated should have larvæ of all ages, and a queen doing well.

Between five and six days after a colony has been inoculated with sacbrood virus, the first symptoms of the disease are to be expected. The finding of capped larvæ having a slightly yellowish hue (fig. 12; Pl. II, *b*, *h*) is the best early symptom by which the presence of the disease may be known.

Another method of inoculation may be used and under certain circumstances is desirable. The method is more direct than the one just described. The crushed tissues of a diseased larva are suspended in a small amount of water or thin sugar sirup. With a capillary pipette (fig. 30) made from small glass tubing, a very small amount of the suspension is added directly to the food which surrounds the healthy larva in the cell. This is easily done. Having drawn some of the suspension into the pipette, carefully touch the food in the cell surrounding the larva with the point of the pipette. A small amount of the suspension will flow out and mix with the food. Larvæ approximately two days of age should be selected for feeding. A dozen

or more should be fed in making an inoculation. The area of brood inoculated may be designated by marking on the brood frame, or by removing the brood from around the area inoculated, thus marking it off.

MEANS FOR THE DESTRUCTION OF THE VIRUS OF SACBROOD.

Although the virus of sacbrood may increase with great rapidity, fortunately it is quite as readily destroyed. Nature supplies many means by which this may be accomplished. While theoretically a sufficient amount of virus may be produced within one month to inoculate all the bees in existence, within another month, if left to natural means alone, practically all such virus would be destroyed. This latter fact constitutes one of the chief reasons for the comparatively rapid self-recovery of colonies from this disease.

It was observed in the experiments that larvæ dead of sacbrood when left in the brood comb ceased to be infectious in less than one month after death.

HEATING REQUIRED TO DESTROY SACBROOD VIRUS WHEN SUSPENDED IN WATER.

Approximate results have been published (White, 1914) relative to the heating that is necessary to destroy the virus of sacbrood when it is suspended in water. In the following table are given some results which have been obtained:

TABLE I.—*Effect of heating on the virus of sacbrood suspended in water.*[1]

Date of inoculation.	Temperature.		Time of heating.	Results of inoculation.
	°F.	°C.	*Minutes.*	
Aug. 6, 1913	122	50	30	Sacbrood produced.
Sept. 10, 1913	131	55	10	Do.
Sept. 9, 1913	131	55	20	Do.
Sept. 18, 1913	135	57	15	Do.
June 30, 1915	136	58	10	Do.
Sept. 10, 1913	136	58	10	No disease produced.
Aug. 28, 1915	138	59	10	Do.
Sept. 10, 1913	140	60	15	Do.
Aug. 28, 1915	142	61	10	Do.
Aug. 26, 1913	149	65	15	Do.
Do	158	70	15	Do.
Do	167	75	15	Do.
Do	176	80	15	Do.

[1] Fractions will be omitted in this paper, the nearest whole number being given.

It will be observed from Table I that 138° F. (59° C.) maintained for 10 minutes was sufficient to destroy the virus of sacbrood in the inoculation experiments recorded. Technically, in view of the variable factors which must be considered in experiments of this kind, this result, as representing the thermal death point of the sacbrood virus, should be considered as being only approximate. For practical purposes, however, it is sufficient.

In performing these experiments a crushed mass, representing from 10 to 20 larvæ recently dead of the disease, is diluted to about 10 times its volume with tap water. About one-half ounce of this suspension is placed in a test tube (fig. 31), almost filling it. The tube is stoppered with a perforated cork, bearing a short glass tube of small caliber and drawn at one end to capillary size. This is all immersed in water at a temperature to which it is desired that the virus shall be heated. It requires nearly five minutes for the temperature of the suspension in the tube to reach that of the water outside. After reaching the degree desired the temperature is maintained for 10 minutes, after which the tube is removed and the contents added to about one-half pint of sirup. The suspension is then fed to a healthy colony. If by such a feeding no sacbrood is produced, the virus is considered as having been destroyed by the heating. On the other hand, if the disease is produced it follows naturally that the virus had not been destroyed.

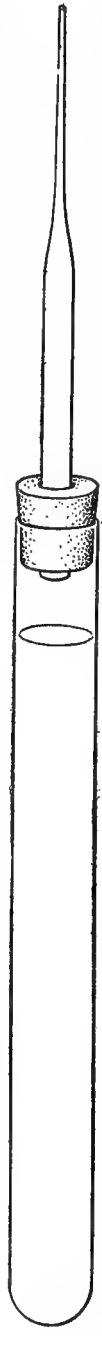


FIG. 31.—Tube in which a suspension of sacbrood larvæ is placed for heating. It consists of a test tube one-half inch in diameter supplied with a perforated stopper through which passes a short piece of glass tubing drawn at one end to capillary size. (Original.)

HEATING REQUIRED TO DESTROY SACBROOD VIRUS WHEN SUSPENDED IN GLYCERINE.

In determining the amount of heating that is necessary to destroy the virus of a disease when it is suspended in a liquid, the results should always be given in terms of at least the three factors, (1) degree of temperature, (2) time of heating, and (3) the medium in which the virus is suspended.

With the virus of sacbrood the results vary markedly, depending upon the nature of the liquid in which the suspension is made. To illustrate this point the results of a few inoculation experiments are given here in which the virus was heated while suspended in glycerine.

TABLE II.—*Effect produced by heating the virus of sacbrood suspended in glycerine.*

Date of inoculation.	Temperature.		Time of heating.	Results of inoculation.
	°F.	°C.	*Minutes.*	
June 25, 1915	140	60	10	Sacbrood produced.
June 24, 1915	149	65	10	Do.
June 25, 1915	158	70	10	Do.
Aug. 28, 1915	160	71	10	Do.
Do	163	73	10	No disease produced.
Aug. 7, 1915	167	75	10	Do.

In these inoculations it will be observed that a temperature somewhat greater than 158° F. (70° C.) maintained for 10 minutes was necessary to destroy the virus of sacbrood when it was suspended in glycerine, while a temperature somewhat less than 140° F. (60° C.) is sufficient to destroy it when suspended in water (p. 34). The same technique was employed when glycerine was used as the suspending medium as was employed when water was used as the medium. The same strain of virus was used in both instances. The point here illustrated is of special interest in connection with the heating of honey containing the virus of sacbrood.

HEATING REQUIRED TO DESTROY SACBROOD VIRUS WHEN SUSPENDED IN HONEY.

From the results obtained by heating the virus of sacbrood in glycerine as given above it might be expected that a higher temperature would be necessary to destroy the virus when it is suspended in honey than when it is suspended in water.

In determining the heating necessary to destroy the virus when suspended in honey the technique followed was similar to that employed when water and glycerine suspensions were used. The virus used in the inoculations bearing the date 1915 was of the same strain in all instances.

TABLE III.—*Results obtained when the virus of sacbrood was heated in honey.*

Date of inoculation.	Temperature.		Time of heating.	Results of inoculation.
	°F.	°C.	*Minutes.*	
June 1, 1915	140	60	10	Sacbrood produced.
June 11, 1915	145	63	10	Do.
Do	149	65	10	Do.
June 4, 1915	154	68	10	Do.
June 24, 1915	156	69	10	Do.
Do	158	70	10	Do.
June 1, 1915	158	70	10	No disease produced.
June 18, 1915	158	70	10	Do.
July 3, 1915	160	71	10	Do.
Aug. 28, 1915	160	71	10	Do.
Aug. 7, 1915	163	73	10	Do.
Aug. 28, 1915	163	73	10	Do.
June 1, 1915	167	75	10	Do.
Aug. 7, 1915	167	75	10	Do.
June 1, 1915	176	80	10	Do.

As shown by the results recorded in Table III, the virus of sacbrood when suspended in honey was destroyed in 10 minutes at a temperature very near 158° F. (70° C.). This temperature is more than 18° F. (10° C.) greater than the temperature required to destroy in the same time the virus when suspended in water and approximately equal to that necessary to destroy it when suspended in glycerine.

RESISTANCE OF SACBROOD VIRUS TO DRYING AT ROOM TEMPERATURE.

In the experiments made for the purpose of determining the amount of drying which the virus of sacbrood will withstand, larvæ recently dead of the disease were used. These are crushed, strained through cheesecloth, and the crushed mass poured into Petri dishes (fig. 32) to the extent of a thin layer for each dish, the material in each being the crushed remains of about 30 larvæ. These are placed in a drawer, shielding the larval material from the light. The drying then proceeds at the temperature of the room. This temperature varied greatly from day to day, sometimes being as high as 93° F. (34° C.).

At intervals, reckoned in days, after the preparation of the virus, colonies are inoculated. An aqueous suspension is made of the drying larval content contained in a Petri dish. This is added to sirup, and the sirup suspension is fed to a healthy colony. The experiments gave the following results:

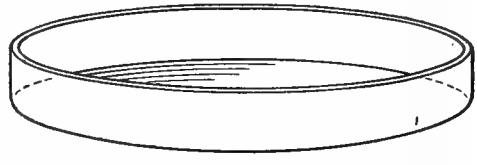


FIG. 32.—Open Petri dish. One-half of Petri dish, either top or bottom. (Original.)

TABLE IV.—*Resistance of sacbrood virus to drying at room temperature.*

Date of inoculation.	Time of drying.	Results of inoculation.
Aug. 8, 1914	3 days	Sacbrood produced.
Aug. 14, 1914	7 days	Do.
Sept. 6, 1915	13 days	Do.
July 1, 1915	16 days	Do.
Sept. 28, 1915	18 days	Do.
July 6, 1915	20 days	Do.
Sept. 3, 1915	22 days	No sacbrood produced.
Sept. 27, 1915	26 days	Do.
Oct. 9, 1914	28 days	No.
July 29, 1915	28 days	Do.
Sept. 3, 1915	35 days	Do.
Do	45 days	Do.
May 22, 1915	7 months 12 days	Do.
Do	7 months 21 days	Do.

From the results recorded in Table IV it will be noted that the virus of sacbrood in the experiment referred to withstood drying at room temperature for approximately three weeks.

The inoculations made during the third week indicated, by the reduced amount of sacbrood produced, that much of the virus had already been destroyed. Obtaining negative results from the use of larval material which had been drying more than seven months tends toward eliminating the possibility that the virus possesses a resting stage.

Similar preliminary experiments made to determine the amount of drying which the virus of sacbrood will withstand at outdoor temperature and at incubator temperature (about 99° F. [37° C.]) gave results approximately those obtained from drying at room temperature, the time being somewhat less in the case of drying at incubator temperature.

Preliminary experiments indicate also that when the virus is mixed with pollen and allowed to dry the period for which it remains virulent is increased only slightly.

RESISTANCE OF SACBROOD VIRUS TO DIRECT SUNLIGHT WHEN DRY.

In the experiments made to determine the amount of sunlight which the virus of sacbrood is capable of resisting, Petri-dish preparations similar to those made in the drying experiment were prepared. After drying a few hours in the room the uncovered dish is exposed to the direct rays of the sun. At different intervals, measured in hours, inoculations of healthy colonies are made similar to those in the drying experiments. The following results were obtained:

TABLE V.—*Resistance of the virus of sacbrood, when dry, to direct sunlight.*

Date of inoculation.	Time of exposure to sun's rays.	Results of inoculation.
	Hours.	
Sept. 17, 1915	2	Sacbrood produced.
July 29, 1915	2½	Do.
Sept. 17, 1915	3	Do.
Sept. 16, 1915	4	Do.
Do	5	Do.
Do	6	Do.
Aug. 25, 1915	6	Do.
Sept. 10, 1915	4	No disease produced.
Do	5	Do.
Sept. 9, 1915	7	Do.
Do	9	Do.
Aug. 19, 1915	12	Do.
July 16, 1915	13	Do.
Aug. 20, 1915	18	Do.
Sept. 11, 1915	21	Do.

The results recorded in Table V show that the virus of sacbrood in the experiments made was destroyed in from four to seven hours' exposure to the direct rays of the sun. The results obtained also indicate that much of the virus was destroyed in a 2-hour exposure to the sun.

It will be readily appreciated that the time that the virus will resist the sun's rays will depend a great deal upon the intensity of the rays at the time of its exposure and the thickness of the layer of the infective larval material in the Petri dish. The drying that

would naturally take place during the exposure to the sun would tend also to destroy the virus, but as the resistance to drying is better given in weeks than days, this factor may be disregarded here.

RESISTANCE OF SACBROOD VIRUS TO DIRECT SUNLIGHT WHEN SUSPENDED IN WATER.

In the experiments made for the purpose of determining the resistance of the virus of sacbrood to the direct rays of the sun when suspended in water, Petri dishes were again used. About 1½ ounces of the aqueous suspension containing the crushed tissues of 30 larvæ is poured into the dish and exposed to the direct rays of the sun. After intervals reckoned in hours the inoculations of healthy colonies are made. The contents of a single Petri dish are added to about one-half pint of sirup and the suspension fed to a healthy colony. The following results were obtained from the experiments:

TABLE VI.—*Resistance of sacbrood virus to the direct rays of the sun when suspended in water.*

Date of inoculation.	Time of exposure to sun's rays.	Results of inoculation.
	Hours.	
Sept. 10, 1915	1	Sacbrood produced.
Aug. 20, 1915	2	Do.
Sept. 14, 1915	2	Do.
Aug. 24, 1915	2	Do.
Aug. 18, 1915	3	Do.
Sept. 9, 1915	4	Do.
Sept. 10, 1915	4	Do.
Aug. 24, 1915	5	Do.
Do	4	No disease produced.
Aug. 16, 1915	5	Do.
Sept. 8, 1915	5	Do.
Do	6	Do.
Sept. 9, 1915	7	Do.
Do	8	Do.
Aug. 25, 1915	10	Do.
Aug. 20, 1915	12	Do.
July 13, 1915	13	Do.
Aug. 26, 1915	13	Do.

From Table VI it will be seen that when suspended in water the virus of sacbrood was killed in from four to six hours.

The aqueous suspensions in the Petri dishes in these experiments did not reach by several degrees the temperature 138° F. (59° C.) at which the virus is destroyed readily by heating (p. 34). Naturally experiments of the nature of those in this group will vary in all cases with the intensity of the sun's rays to which the virus is exposed. The exposures were made in these experiments between 9 and 4 o'clock, the sun's rays toward the middle of the day being most often used.

RESISTANCE OF SACBROOD VIRUS TO DIRECT SUNLIGHT WHEN SUSPENDED IN HONEY.

The crushed and strained tissue mass of larvæ dead of sacbrood was suspended in honey and exposed to the direct rays of the sun. To prevent robbing by bees, closed Petri dishes were used. At intervals reckoned in hours healthy colonies were inoculated, each with the virus from a single Petri dish. The exposures were made during the day between 9 and 4 o'clock, preference being given to the hours near midday. The group of experiments conducted on this point gave the following results:

TABLE VII.—*Resistance of the sacbrood virus to direct sunlight when suspended in honey.*

Date of inoculation.	Time of exposure to sun's rays.	Results of inoculation.
	Hours.	
Aug. 24, 1915	1	Sacbrood produced.
Do	2	Do.
Aug. 18, 1915	4	Do.
Sept. 9, 1915	4	Do.
Sept. 10, 1915	4	Do.
Aug. 24, 1915	5	Do.
Aug. 16, 1915	5	No disease produced.
Aug. 25, 1915	5	Do.
Sept. 8, 1915	5	Do.
Do	6	Do.
Sept. 9, 1915	7	Do.
Do	8	Do.
Aug. 25, 1915	10	Do.
Sept. 11, 1915	12	Do.
Aug. 26, 1915	13	Do.
Sept. 11, 1915	18	Do.

From the results of the experiments recorded in Table VII it will be observed that the virus of sacbrood when suspended in honey was destroyed by the direct rays of the sun in from five to six hours. These figures represent the time for destruction of all of the virus used in each experiment. The results obtained from the experiments indicate, however, that much of it was destroyed earlier.

LENGTH OF TIME THAT SACBROOD VIRUS REMAINS VIRULENT IN HONEY.

In devising methods for the treatment of sacbrood it is of particular interest to know the length of time that the virus will remain virulent when it is in honey. Experiments have been made to gain data on this point. Larvæ recently dead of sacbrood are crushed, strained, and suspended in honey. About one-half pint of the suspension, representing the virus from about 30 dead larvæ, is placed in each of a number of glass flasks. These are allowed to stand at room temperature, being shielded from the light by being placed in a closed cabinet.

After periods reckoned in days inoculations of healthy colonies are made. The following results have been obtained:

TABLE VIII.—*Length of time the virus of sacbrood remains virulent in honey.*

Date of inoculation.	Time virus was in honey.		Results of inoculation.
	Mos.	*Days.*	
June 17, 1915	0	20	Sacbrood produced.
June 4, 1915	0	23	Do.
Oct. 2, 1915	0	30	Do.
Sept. 3, 1915	0	24	No disease produced.
July 29, 1915	0	29	Do.
June 30, 1915	0	33	Do.
Do	0	35	Do.
July 17, 1915	0	36	Do.
Oct. 21, 1915	0	49	Do.
Sept. 8, 1915	0	70	Do.
May 13, 1915	[1] 7	10	Do.
May 6, 1915	7	20	Do.
May 4, 1915	8	2	Do.
May 18, 1915	8	21	Do.
Sept. 3, 1915	12	1	Do.

[1] The dead brown larval remains were not crushed before being introduced into the honey.

The experiments recorded in Table VIII show that the virus of sacbrood when suspended in honey at room temperature remained virulent for three weeks, but was entirely destroyed before the end of the fifth week. It is most likely that the virus in most instances is destroyed by the end of one month at this temperature.

The experiments in which the virus had been allowed to remain in the honey for more than seven months suggest that there is probably no resting stage of the virus to be considered in this connection. The facts tend to indicate that the virus does not receive any marked amount of protection by being in honey. From the dates of the experiments in this group it will be noted that the virus was subjected to summer temperature. The evidence at hand indicates that it remains virulent somewhat longer when the temperature is lower.

RESISTANCE OF SACBROOD VIRUS TO THE PRESENCE OF FERMENTATIVE PROCESSES.

Fermentation and putrefaction [1] are other means by which the virus of sacbrood may be destroyed in water. A crushed and strained mass of tissue from larvæ recently dead of the disease is suspended in a 10 per cent sugar (granulated or cane sugar) solution.

[1] "Fermentation" has reference here particularly to the breaking up of carbohydrate substances by the growth of microorganisms, the sugars in honey being naturally the carbohydrates especially of interest in these discussions. The process results in the formation of a large number of substances—acids, alcohols, etc. The odor accompanying such a process could not be called offensive. By the term "putrefaction" is meant the breaking up of nitrogenous organic substances by microorganisms. These have a chemical composition quite different from the carbohydrates. When broken up the resulting substances are more often alkaline in nature. The odor from a suspension in which putrefactive processes are going on is usually distinctly offensive.

A small quantity of soil is added to inoculate the suspension further. This is then distributed in test tubes (fig. 33), the quantity in each tube representing the virus from about 15 larvæ. These suspensions are allowed to remain at room temperature, shielded from the light. Under these conditions fermentation goes on rather rapidly.

FIG. 33.—Test tube bearing a cotton plug, used in testing the effect of fermentation, putrefaction, and disinfecting agents on the virus of sacbrood. (Original.)

After intervals reckoned in days colonies free from the disease are inoculated, each with the suspension from a single tube. Results from such inoculations are given in the following table:

TABLE IX.—*Resistance of sacbrood virus to fermentation in a 10 per cent sugar solution at room temperature.*

Date of inoculation.	Period of fermentation.	Results of inoculation.
	Days.	
Sept. 9, 1915	1	Sacbrood produced.
Sept. 11, 1915	2	Do.
Do	3	Do.
Sept. 13, 1915	4	Do.
July 14, 1915	3	No disease produced.
July 22, 1915	5	Do.
Sept. 14, 1915	5	Do.
Sept. 22, 1915	7	Do.
July 10, 1915	9	Do.
June 10, 1915	13	Do.
July 7, 1914 [1]	34	Do.
Aug. 27, 1914	51	Do.
Do	85	Do.
Do	87	Do.
Do	90	Do.
Do	244	Do.

[1] The results recorded for 1914 were obtained with a suspension of crushed larvæ, in various stages of decay, in sirup made from about equal parts water and sugar.

From the results of experiments recorded in Table IX it will be noted that the virus of sacbrood was destroyed in from three to five days in the presence of fermentation in 10 per cent cane sugar (saccharose) at room temperature.

As the rapidity of fermentative processes varies with the temperature present, it is natural to suppose that the time required for the destruction of the virus will vary. From experiments it is found that at incubator temperature the time is slightly less, and at outdoor temperature it is somewhat greater than at room temperature.

RESISTANCE OF SACBROOD VIRUS TO FERMENTATION IN DILUTED HONEY AT OUTDOOR TEMPERATURE.

Employing the egg test [1] as used by beekeepers in diluting honey for the purpose of making vinegar, it is found that it requires about

[1] This test is applied in the following manner: Water is added to honey until an egg placed in the mixture is nearly submerged, the surface remaining above the liquid being only about as large as a 10-cent piece.

four volumes of water to one of ripened honey to obtain the strength recommended. The honey solution by volume, therefore, is about 20 per cent honey.

A suspension of the virus of sacbrood in such a solution is distributed in test tubes placed in an empty hive body and allowed to ferment at outdoor temperature. After periods reckoned in days colonies are inoculated as was done in case of the sugar solutions described above. The following results were obtained from the experiments performed:

TABLE X.—*Resistance of sacbrood virus to fermentative processes in a 20 per cent honey solution at outdoor temperature.*

Date of inoculation.	Time of fermentation.	Results of inoculation.
	Days.	
Sept. 11, 1915	3	Sacbrood produced.
Sept. 13, 1915	4	Do.
Sept. 14, 1915	5	No disease produced.
Aug. 4, 1915	6	Do.
Sept. 15, 1915	6	Do.
Sept. 14, 1915	7	Do.
Sept. 22, 1915	7	Do.
Sept. 17, 1915	8	Do.
Sept. 8, 1915	40	Do.

In the presence of fermentative processes taking place in a 20 per cent honey solution at outdoor temperature it will be observed that the virus of sacbrood in the experiments recorded in Table X was destroyed in six days. The outdoor temperature during these experiments was quite warm. Had it been cooler, the time for the destruction of the virus would have been somewhat increased. In the making of vinegar it may be concluded that the virus of sacbrood, should it be present in the honey used, would be destroyed in a comparatively short time as a result of fermentation.

RESISTANCE OF SACBROOD VIRUS TO THE PRESENCE OF PUTREFACTIVE PROCESSES.

Larvæ containing the virus of sacbrood are crushed and suspended in water. A small quantity of soil is added. The suspension is strained and distributed in test tubes. These are allowed to stand at room temperature in a state of putrefaction. After periods reckoned in days colonies free from the disease are inoculated, each with the contents of a single tube added to sirup. From experiments of this kind the results following have been obtained.

TABLE XI.—*Resistance of sacbrood virus to putrefaction.*

Date of inoculation.	Time of putrefaction.	Results of inoculation.
	Days.	
Aug. 6, 1914	1	Sacbrood produced.
Aug. 7, 1914	2	Do.
Aug. 10, 1914	3	Do.
July 20, 1915	3	Do.
Sept. 13, 1915	4	Do.
Sept. 14, 1915	5	Do.
July 22, 1915	5	Do.
July 8, 1915	7	Do.
May 22, 1915	9	Do.
Sept. 22, 1915	7	No disease produced.
Aug. 18, 1915	10	Do.
Sept. 16, 1914	14	Do.
Sept. 25, 1914	14	Do.
July 1, 1915	16	Do.

From Table XI it will be noted that the virus of sacbrood was destroyed in the experiments recorded in from 7 to 10 days. As in the case of fermentation, so in the case of putrefaction, it is to be expected that the time for the destruction of the virus will vary appreciably with the temperature at which the putrefactive processes take place.

RESISTANCE OF SACBROOD VIRUS TO CARBOLIC ACID.

Larvæ recently dead of sacbrood are crushed and strained. This larval mass is diluted with carbolic acid in aqueous solution. About 10 parts of carbolic acid to 1 part of the larval mass is used. This suspension is distributed in test tubes and allowed to stand at room temperature. Each tube contains the virus from about 15 larvæ. After periods, reckoned in days, colonies free from disease are inoculated, each with the contents of a single tube added to sirup.

Carbolic acid solutions of ½, 1, 2, and 4 per cent were used in making the suspensions. The following results were obtained from the experiments:

TABLE XII.—*Resistance of sacbrood virus to carbolic acid.*

Date of inoculation.	Strength of carbolic acid used.	Time in suspension.	Results of inoculation.
	Per cent.	*Days.*	
Sept. 3, 1914	½	1	Sacbrood produced.
Sept. 18, 1914	½	16	Do.
Sept. 3, 1914	½	24	Do.
Sept. 17, 1914	½	38	No disease produced.
Aug. 12, 1915	½	50	Do.
Aug. 20, 1915	½	50	Do.
May 14, 1915	½	238	Do.
Sept. 3, 1914	1	1	Sacbrood produced.
Sept. 18, 1914	1	16	Do.
June 23, 1915	1	25	Do.
Sept. 17, 1915	1	38	No disease produced.
Aug. 12, 1915	1	50	Do.
Aug. 21, 1915	1	50	Do.
June 4, 1915	1	251	Do.

TABLE XII.—*Resistance of sacbrood virus to carbolic acid*—Continued.

Date of inoculation.	Strength of carbolic acid used.	Time in suspension.	Results of inoculation.
	Per cent.	*Days.*	
Sept. 3, 1914	2	1	Sacbrood produced.
Sept. 18, 1914	2	16	Do.
June 23, 1915	2	25	Do.
Sept. 17, 1915	2	38	No disease produced.
Aug. 12, 1915	2	42	Do.
Aug. 21, 1915	2	50	Do.
		Hours.	
June 23, 1915	4	3	Sacbrood produced.
July 1, 1915	4	7	Do.
		Days.	
June 23, 1915	4	25	No disease produced.
Aug. 12, 1915	4	50	Do.

From the preliminary results recorded in Table XII it will be observed that the virus of sacbrood shows a marked resistance to the disinfecting power of carbolic acid. Under the conditions of the experiments the virus resisted its action for more than three weeks in ½, 1, and 2 per cent aqueous solutions.

These results lead naturally to a consideration of the effect of drugs on the virus of sacbrood in the treatment of the disease. On this point complete data are yet wanting.

While the disinfecting power of a compound, as shown in experiments such as those described above for carbolic acid, may indicate something as to the value of the compound as a drug, it does not necessarily prove its value. More definite proof is gained through feeding colonies with the virus suspended in honey medicated with the drug, and then continuing to feed the inoculated colonies with honey similarly medicated daily thereafter until the time for the appearance of the disease.

To illustrate the nature of experiments which are being conducted to determine the value of drugs in the treatment of sacbrood, experiments with quinine and carbolic acid are here referred to. A colony was fed the virus of sacbrood suspended in honey and water, equal parts, to which was added 5 grains of the bisulphate of quinine to one-half pint of diluted honey, and on each of the five days following the inoculation the same colony was fed diluted honey containing no virus, but medicated with quinine in the same way. On the seventh day following the inoculation with the virus there was found to be a large quantity of sacbrood produced in the colony so inoculated and treated.

A similar experiment in which carbolized honey was used gave like results. These experiments, although not furnishing conclusive proof, do indicate something of what might be expected from the use of quinine or carbolic acid as a drug in the treatment of sacbrood.

Technically the foregoing studies should be thought of as being preliminary. Questions relating to virulence of the virus, resistance of the bees, technique, and many other factors contribute to make results such as these vary. For practical purposes, however, they are sufficiently complete. In estimating the time necessary for the destruction of the virus in practical apiculture by any of the foregoing tables of results it should be emphasized that the time element should be somewhat increased, inasmuch as the conditions present in the experiments were more favorable for its destruction than would ordinarily be the case in practice.

MODES OF TRANSMISSION OF SACBROOD.

The transmission of a brood disease must be thought of as taking place (1) from diseased to healthy brood within a colony and (2) from a diseased colony to a healthy one. The manner in which sacbrood is spread naturally depends directly upon the modes by which the virus of the disease is transmitted.

As is shown experimentally, the virus of sacbrood produces the disease when it is added directly to the food of young larvæ or when it is mixed with sirup and fed to a colony. From this fact it is fair to assume that sacbrood may result whenever the food or water used by the bees contains the living virus of the disease.

Bees have a tendency to remove diseased or dead larvæ from the cells. When the removal is attempted about the time of death, it is done piecemeal. Each fragment removed from such a larva, if fed to a young healthy larva within a week, would most likely produce sacbrood in the larva. Within the hive, therefore, the disease may be transmitted to healthy larvæ more or less directly in this way.

Just what becomes of these bits of tissue removed from the diseased larvæ, however, is not known. If it were the rule that the tissues of the dead larva after being removed in fragments were fed unaltered to the young healthy larvæ within two weeks after its removal, it would seem that the disease would increase rapidly in the colony as a result. Such an increase, however, is unusual, the tendency in a colony being in most cases toward a recovery from the disease.

This fact leads one to think of other possibilities regarding the destiny of the infected tissues removed as fragments from the diseased larvæ. If the infective material were fed to the older larvæ, death probably would not result. Should it be used by adult bees as food for themselves, the likelihood of the transmission of the disease under such circumstances would apparently be very materially reduced. If the infective material were stored with the honey and

did not reach the brood within a month or six weeks, it is not probable that the disease would be transmitted under such circumstances (p. 41). Should the dead larvæ or any fragments of them be carried out of the hive, the virus would have to be returned to the hive, as a matter of course, before further infection of the brood could take place from such infective material.

It is left to be considered in what way the infective material if removed from the hive might be returned to the brood and infect it. Should any material containing the virus reach the water supply of the bees, or the flowers visited by the bees, it is within the range of possibility that some of the living virus might be returned to the hive and reach healthy young larvæ.

While out of the hive, however, the virus must withstand certain destructive agencies in nature. Under more or less favorable circumstances it would withstand drying alone for a few weeks (p. 37), but if exposed to the sun it might be destroyed in a few hours. (p 38). If the virus were subjected to fermentation it might be destroyed within a week (p 43), and if subjected to putrefaction, within two weeks (p. 44).

The experimental evidence indicates that the virus, once out of the hive and freed from the adult bees removing it, during the warmer seasons of the year, at least, has but little chance of being returned to the hive and producing any noticeable infection. In the experimental apiary (Pl. III) a large number of colonies have been heavily infected with sacbrood through experimental inoculation, and no infection was observed to have resulted in the uninoculated colonies. If throughout the main brood-rearing season the usual source of infection were the flowers or the water supply, a quite different result would be expected.

Tentatively it may be concluded, therefore, that the probability of the transmission of the virus of sacbrood by way of flowers visited by bees, practically considered, is quite remote, being, however, to a limited extent theoretically possible.

It would seem that there is a greater likelihood of the water supply being a source of infection than flowers. The chances for infection from this source, should it occur at all, would be greater in the spring, as at such a time the quantity of infective material in diseased colonies is greater, increasing the chances that some of it might be carried to the water supply and contaminate it, and furthermore, the destructive agencies in nature are at this time less efficient.

Bees drifting or straying from infected colonies to healthy ones must be thought of as possible transmitters of the disease. That the disease is not spread to any great extent in this way is evidenced

by the fact that colonies in the apiary that were not inoculated experimentally remained free from disease, although many colonies in the apiary were heavily infected at the time.

Sacbrood has a tendency to weaken a colony in which it is present. Frequently this weakness is noticeable and often marked. Robbing, which occurs not infrequently at such a time, results in the transmission of the virus, to some extent at least, directly to healthy colonies. Robbing, therefore, must always be considered as a probable means of transmission.

The modes of transmission of sacbrood within the colony and from colony to colony, as will be seen, are not by any means completely determined. In what way the sacbrood virus is carried over from one brood-rearing season to another is one of the many problems concerning this disease that are yet to be solved. The foregoing facts, accompanied by the brief discussions, it is hoped, will throw some light upon this important phase of the study—the transmission of this disease—and will serve as an aid to later researches.

DIAGNOSIS OF SACBROOD.

The diagnosis of sacbrood can be made from the symptoms already described (p. 10). The colony may or may not be noticeably weakened. The adult bees are normal in appearance. Scattered here and there on the brood frame among the healthy brood are found dead larvæ in the late larval stage. Usually there are only a few of them, yet sometimes there are many. These larvæ may be in capped or uncapped cells. When found in uncapped cells, however, the cappings had already been removed by the bees after the death of the larvæ. The cap over a dead larva in a cell may be found punctured or not. The brood possesses no abnormal odor, or practically none.

The post-mortem appearances of larvæ dead of the disease are especially valuable in making the diagnosis. The larva is found extended lengthwise in the cell and on its dorsal side. Throughout the period of decay it will be found to maintain much of the form and markings of a healthy larva of the age at which it died. Soon after death the larval remains are slightly yellow. After a period they assume a brownish tint. Since the brown color deepens as the process of decay and drying takes place, the remains may be found having any one of a number of shades of brown. They may appear at times almost black.

After death the cuticular portion of the body wall becomes toughened, permitting the easy removal of the larva intact from the cell. When removed, the saclike appearance of the remains becomes easily apparent. Upon rupturing the cuticular sac the contents are found to be a brownish, granular-appearing mass suspended in a compara-

tively small quantity of more or less clear liquid. The scales formed by the drying of the decaying remains are easily removed from the cells. After becoming quite dry many of them indeed can be shaken from the brood comb.

Upon crushing larvæ which have been found dead for some time but not yet dry, a marked unpleasant odor will be noticed if the crushed mass is held near the nostrils.

Microscopically no microorganisms are to be found in the decaying remains of the larvæ. Cultures made from them are also negative.

Differential diagnosis.—Sacbrood must be differentiated from the other brood diseases.

American foulbrood may be recognized by the peculiar odor of the brood combs when the odor is present. The body wall of the larval and pupal remains is easily ruptured, and the decaying mass becomes viscid, giving the appearance popularly referred to as "ropiness." The scale adheres quite firmly to the floor of the cell. The presence of *Bacillus larvæ* in the brood dead of the disease is a positive means by which it may be differentiated from sacbrood.

European foulbrood may be recognized by the fact that the larvæ as a rule die while coiled in the cell and before an endwise position is assumed. In the majority of instances, therefore, death takes place before the cells are capped. The saclike appearance characterizing the dead larvæ in sacbrood is absent. The granular consistency of the decaying mass is absent also. Microscopically, a large number of bacteria are found in larvæ dead of European foulbrood, but are absent in larvæ dead of sacbrood. The presence of *Bacillus pluton* is a positive means by which European foulbrood may be recognized. *Bacillus alvei* and other species may also be present.

Sacbrood must also be differentiated from other conditions referred to as chilled brood, overheated brood, and starved brood, which occasionally are encountered. This can be done by a comparison of the symptoms presented by these different conditions with the symptoms of sacbrood, and the history of the cases. Some of the larvæ dead from these conditions will be found to have died while yet coiled in the cell. This fact suggests some condition other than sacbrood. When dying later, the saclike remains characterizing sacbrood are not present in conditions other than sacbrood.

PROGNOSIS.

The tendency in a colony affected with sacbrood is to recover from the disease. Colonies which during the spring months show the presence of more or less disease, by midsummer or earlier may, and very

frequently do, contain no diseased brood. Experimentally it is possible to destroy a colony by feeding it repeatedly the virus of sacbrood, and beekeepers report that the disease sometimes destroys colonies in their apiaries. The percentage of colonies, however, that actually die out as a direct result of the disease is small. The weakening of the colony in the spring of the year not only reduces or entirely eliminates the profits on it for the season, but may also cause it to be in a weakened condition on the approach of winter.

Whether a larva once infected ever recovers from the disease is not known. Reasoning from what is known of the diseases of other animals and man, one would expect that a larva may recover from sacbrood infection. It is known that many larvæ, both worker and drone, do die. From the information thus far obtained it does not appear that a queenless colony would be likely to remain so as a consequence of the disease.

As to the prognosis of the disease in a colony it may be said, therefore, that it is very favorable for the continued existence of the colony. As to the economic losses to be expected from the disease, the present studies suggest that they may vary from losses that are so light as not to be detected upon examination to losses that may equal the entire profits of the colony for the year. Indeed, at times the death of the colony takes place as a result of the disease.

RELATION OF THESE STUDIES TO THE TREATMENT OF SACBROOD.

An earlier paper (White, 1908) contains a brief general discussion of the relation existing between the cause of bee diseases and the treatment of them. The general remarks made in it apply also to sacbrood. No doubt the beekeeper in studying the results given here has already observed relations existing between them and points which should be incorporated in methods for treatment. Mentioning a few of them here may serve to suggest still others.

That the weakness resulting in a sacbrood colony is due to the death of worker larvæ; that adult bees are not susceptible to the disease; that queenlessness is rarely to be expected as a sequence of the disease; that the disease may be produced with ease at any time of the year that brood is being reared; that it occurs at all seasons, but is more frequently encountered in the spring; that it is found in localities differing widely as to food and climatic conditions; and that no complete racial immunity to the disease has yet been found are facts concerning the predisposing causes of sacbrood which beekeepers will at once recognize as bearing a close relation to the methods by which the disease should be treated.

As sacbrood can not occur in the absence of its exciting cause (a filterable virus), a knowledge of this cause is of special importance in the treatment of the disease.

That sacbrood is very frequently encountered; that it is infectious, but that it is more benign in character than malignant; that it does not spread rapidly from one colony to another; that colonies manifest a strong tendency toward self-recovery from the disease; that this tendency is stronger after midsummer; that the disease may so weaken a colony during the early brood-rearing season that the profits from it may be much reduced, or even rendered nil; and that the disease may indeed destroy the colony are facts which must be considered in devising logical methods for its treatment.

That the virus of sacbrood remains virulent in larvæ dead of the disease for less than one month; that it remains virulent in honey approximately one month; that when mixed with pollen it ceases to be virulent after about one month; and that in drying no virulence is to be expected after one month, are facts that account in a large measure for the strong tendency to recover from the disease manifested by the colony and that furnish information concerning the use of combs from sacbrood colonies. From the results it may be concluded that it is better, theoretically, to store combs from sacbrood colonies for one or two months before they are again used, provided such storing entails no particular inconvenience or financial loss to the beekeeper.

Further experiments show that brood frames from badly-infected colonies may be inserted into strong, healthy ones, and cause thereby very little infection and consequently only a slight loss. This is especially true after the early brood-rearing season of the year is past. Since this can be done, it is quite probable that the practical beekeeper will find that this disposition of the combs will be the preferable one to make. At any event, it is comforting to know that it is never necessary to destroy the combs from sacbrood colonies on account of the disease.

The experimental results here given regarding the destruction of the virus through heating, fermentation, putrefaction, drying, and direct sunlight should assist materially in the solution of the problem of the transmission of sacbrood, and should be found helpful in devising efficient methods for the treatment of the disease.

Toward disinfecting agents it is shown that the virus of sacbrood possesses, in some instances at least, marked resistance. These and other experimental results thus far obtained indicate that the use of any drug in the treatment of the disease should not be depended upon until such a drug has been proved to be of value.

No fear need be entertained in practical apiculture that the disease will be transmitted by the hands or clothing of the operator, by the tools used about the apiary, through the medium of the wind, or by the queen. It would seem at all times superfluous in the case of sacbrood to flame or burn the inside of the hive or to treat the ground about a hive containing an infected colony.

There is but little danger that the disease will be transmitted by way of flowers visited by bees from sacbrood colonies and later from healthy ones.

Theoretically, it is possible that the disease may be transmitted through a contamination of the water supply by bees from sacbrood colonies. Whether infection ever takes place in this way, however, is not yet known. If the disease is ever transmitted in this way, it would seem that it is more likely to take place in the spring of the year than at any other season.

While there is yet much to be learned about sacbrood, it is hoped that by carefully considering these studies the beekeepers will be aided in devising efficient and economical methods for its treatment.

SUMMARY AND CONCLUSIONS.

The following summary and statements of conclusions seem to be justified as a result of the investigations recorded in this paper:

(1) Sacbrood is an infectious disease of the brood of bees.

(2) Adult bees are not susceptible to the disease.

(3) The infecting agent causing sacbrood is of such a nature that it passes through the pores of a fine clay filter. It is therefore a filterable virus.

(4) A colony may be inoculated by feeding it sirup or honey containing the virus.

(5) The quantity of virus contained in a single larva recently dead of the disease is sufficient to produce quite a large amount of sacbrood in a colony.

(6) The period from time of inoculation to the appearance of the first symptoms of the disease—the incubation period—is approximately six days, being frequently slightly less.

(7) By inoculation the disease may be produced at any season of the year that brood is being reared.

(8) The disease is more often encountered during the first half of the brood-rearing season than during the second half.

(9) It occurs among bees in localities having as wide a range of climatic conditions, at least, as are found in the United States.

(10) The course of the disease is not greatly affected by the character or quantity of the food obtained and used by the bees.

(11) Larval remains recently dead of the disease prove to be very infectious when fed to bees. Dead larvæ which have been in the brood comb more than one month are apparently noninfectious.

(12) Colonies possess a strong tendency to recover from the disease without treatment.

(13) The virus of sacbrood suspended in water and heated to 138° F. (59° C.) was destroyed in 10 minutes. Considering the varying factors which enter into the problem, the minimum temperature necessary to destroy this virus when applied for 10 minutes should

be found at all times to lie somewhere between the limits of 131° F. (55° C.) and 149° F. (65° C.).

(14) When the virus of sacbrood is suspended in honey it may be destroyed by heating the suspension for 10 minutes at approximately 158° F. (70° C.).

(15) The virus resisted drying at room temperature for approximately three weeks.

(16) The virus when dry was destroyed by the direct rays of the sun in from four to seven hours.

(17) The virus when suspended in water was destroyed by the direct rays of the sun in from four to six hours.

(18) The virus when suspended in honey was destroyed by the direct rays of the sun in from five to six hours.

(19) The virus when suspended in honey and shielded from direct sunlight remained virulent for slightly less than one month at room temperature during the summer.

(20) The virus was destroyed in approximately five days in the presence of fermentative processes taking place in 10 per cent sugar solution at room temperature.

(21) In the presence of fermentative processes going on in 20 per cent honey solution at outdoor temperature the virus of sacbrood was destroyed in approximately five days.

(22) In the presence of putrefactive processes the virus remained virulent for approximately 10 days.

(23) The virus will resist ½ per cent, 1 per cent, and 2 per cent aqueous solutions of carbolic acid, respectively, for more than three weeks, 4 per cent being more effective.

(24) Neither carbolic acid nor quinine as drugs should at present be relied upon in the treatment of sacbrood.

(25) Varying factors entering into many of the problems discussed in this paper tend to vary the results obtained. In such problems the results here given must be considered from a technical point of view as being approximate only. They are sufficiently exact for application by the beekeeper, but to insure the destruction of the virus in practical apiculture the time element indicated from these experiments as sufficient should be increased somewhat.

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United States Department of Agriculture,

BUREAU OF ENTOMOLOGY.

L. O. HOWARD, Entomologist and Chief of Bureau.

THE BROOD DISEASES OF BEES.

By E. F. PHILLIPS, Ph. D.,

In Charge of Bee Culture.

In view of the widespread distribution of infectious brood diseases among bees in the United States, it is desirable that all bee keepers learn to distinguish the diseases when they appear. It frequently happens that an apiary becomes badly infected before the owner realizes that any disease is present, or it may be that any dead brood which may be noticed in the hives is attributed to chilling. In this way disease gets a start which makes eradication difficult.

There are two recognized forms of disease of the brood, designated as European and American foul brood, which are particularly virulent. In some ways these resemble each other, but there are certain distinguishing characters which make it possible to differentiate the two. Reports are sometimes received that a colony is infected with both diseases at the same time, but this is contrary to the experience of those persons most conversant with these conditions. While it may be possible for a colony to have the infection of both diseases at the same time, it is not by any means the rule, and such cases are probably not authentically reported. Since both diseases are caused by specific bacilli, there is absolutely no ground for the idea held by some bee keepers that chilled or starved brood will turn to one or the other of these diseases. Experience of the best practical observers is also in keeping with this. For a discussion of the causes of these diseases the reader is referred to Technical Series, No. 14, of the Bureau of Entomology, "The Bacteria of the Apiary, with Special Reference to Bee Diseases," by Dr. G. F. White.

AMERICAN FOUL BROOD.

American foul brood (often called simply "foul brood") is distributed through all parts of the United States, and from the symptoms published in European journals and texts one is led to believe that it is also the prevalent brood disease in Europe. Although it is found in almost all sections of the United States, there are many localities entirely free from disease of any kind.

The adult bees of an infected colony are usually rather inactive and do little toward cleaning out infected material. When the larvæ are first affected they turn to a light chocolate color, and in the advanced stages of decay they become darker, resembling roasted coffee in color.

Usually the larvæ are attacked at about the time of capping, and most of the cells containing infected larvæ are capped. As decay proceeds these cappings become sunken and perforated, and, as the healthy brood emerges, the comb shows the scattered cells containing larvæ which have died of disease, still capped. The most noticeable characteristic of this infection is the fact that when a small stick is inserted in a larva which has died of the disease, and slowly removed, the broken-down tissues adhere to it and will often stretch out for several inches before breaking. When the larva dries it forms a tightly adhering scale of very dark brown color, which can best be observed when the comb is held so that a bright light strikes the lower side wall. Decaying larvæ which have died of this disease have a very characteristic odor which resembles a poor quality of glue. This disease seldom attacks drone or queen larvæ. It appears to be much more virulent in the western part of the United States than in the East.

EUROPEAN FOUL BROOD.

European foul brood (often called "black brood") is not nearly as widespread in the United States as is American foul brood, but in certain parts of the country it has caused enormous losses. It is steadily on the increase and is constantly being reported from new localities. It is therefore desirable that bee keepers be on the watch for it.

Adult bees in infected colonies are not very active, but do succeed in cleaning out some of the dried scales. This disease attacks larvæ earlier than does American foul brood, and a comparatively small percentage of the diseased brood is ever capped. The diseased larvæ which are capped over have sunken and perforated cappings. The larvæ when first attacked show a small yellow spot on the body near the head and move uneasily in the cell. When death occurs they turn yellow, then brown, and finally almost black. Decaying larvæ which have died of this disease do not usually stretch out in a long thread when a small stick is inserted and slowly removed. Occasionally there is a very slight "ropiness," but this is never very marked. The thoroughly dried larvæ form irregular scales which are not strongly adherent to the lower side wall of the cell. There is very little odor from decaying larvæ which have died from this disease, and when an odor is noticeable it is not the "glue-pot" odor of the American foul brood, but more nearly resembles that of soured dead brood. This disease attacks drone and queen larvæ very soon after the colony is infected. It is as a rule much more infectious than American foul brood and spreads more rapidly. On the other hand, it sometimes happens that the disease will disappear of its own accord, a thing which the author never knew to occur in a genuine case of American foul brood. European foul brood is most destructive during the spring and early summer, often almost disappearing in late summer and autumn.

TREATMENT OF INFECTIOUS DISEASES.

The treatment for both American foul brood and European foul brood is practically the same. It is impossible to give minute directions to cover every case, but care and common sense will enable any bee keeper successfully to fight diseases of brood.

Drugs.—Drugs, either to be given directly in food or to be used for fumigating combs, can not be recommended for either of these diseases.

Shaking treatment.—To cure a colony of either form of foul brood it is necessary first to remove from the hive all of the infected material. This is done by shaking the bees into a clean hive on clean frames with small strips of comb foundation, care being taken that infected honey does not drop from the infected combs. The healthy brood in the infected combs may be saved, provided there is enough to make it profitable, by piling up combs from several infected hives on one of the weakest of the diseased colonies. After a week or ten days all the brood which is worth saving will have hatched out, at which time all these combs should be removed and the colony treated. In the case of box hives or skeps the bees may be drummed out into another box or preferably into a hive with movable frames. Box hives are hard to inspect for disease and are a menace to all other bees in the neighborhood in a region where disease is present.

The shaking of the bees from combs should be done at a time when the other bees in the apiary will not rob and thus spread disease, or under cover. This can be done safely in the evening after bees have ceased to fly, preferably during a good honey flow. Great care should be exercised to keep all infected material away from other bees until it can be completely destroyed or the combs rendered into wax. Wax from diseased colonies should be rendered by some means in which high heating is used, and not with a solar wax extractor. The honey from a diseased colony should be diluted to prevent burning and then thoroughly sterilized by hard boiling for at least half an hour, if it is to be fed back to the bees. If the hive is again used, it should be very thoroughly cleaned, and special care should be taken that no infected honey or comb be left in the hive.

It is frequently necessary to repeat the treatment by shaking the bees onto fresh foundation in new frames after four or five days. The bee keeper or inspector must determine whether this is necessary, but when there is any doubt it is safer to repeat the operation rather than run the risk of reinfection. If repeated, the first new combs should be destroyed. To prevent the bees from deserting the strips of foundation the queen may be caged in the hive or a queen-excluding zinc put at the entrance.

Treatment with bee escape.—The shaking treatment may be modified so that instead of shaking the bees from the combs the hive is moved from its stand, and in its place a clean hive with frames and foundation is set. The queen is at once transferred to the new hive, and the field bees fly there when they next return from the field. The infected hive is then placed on top of or close beside the clean hive and a bee escape placed over the entrance of the hive containing disease, so that the younger bees and those which later emerge from the cells may leave the hive but can not return. They therefore join the colony in the new hive.

Fall treatment.—If it is desirable to treat a colony so late in the fall that it would be impossible for the bees to prepare for winter, the treatment may be modified by shaking the bees onto combs with plenty of honey for winter. This will be satisfactory only after brood rearing has entirely ceased. In such cases disease rarely reappears.

In the Western States, where American foul brood is particularly virulent, it is desirable thoroughly to disinfect the hive by burning the inside or by chemical means before using it again. This is not always practiced in the Eastern States, where the disease is much milder. Some persons recommend boiling the hives or disinfecting them with some reliable disinfectant such as carbolic acid or corrosive sublimate. It is usually not profitable to save frames because of their comparatively small value, but if desired they may be disinfected. Great care should be exercised in cleaning any apparatus It does not pay to treat very weak colonies. They should either be destroyed at once or several weak ones be united to make one which is strong enough to build up.

Recently some new "cures" have been advocated in the bee journals, particularly for European foul brood, with a view to saving combs from infected colonies. The cautious bee keeper will hardly experiment with such methods, especially when the disease is just starting in his locality or apiary, but will eradicate the disease at once by means already well tried.

In all cases great care should be exercised that the bee keeper may not himself spread the infection by handling healthy colonies before thoroughly disinfecting his hands, hive tools, and even smoker. Since it takes but a very small amount of infected material to start disease in a previously healthy colony, it is evident that too much care can not be taken. In no case should honey from unknown sources be used for feeding bees. Care should also be exercised in buying queens, since disease is often transmitted in the candy used in shipping cages. Combs should not be moved from hive to hive in infected apiaries.

"PICKLE BROOD."

There is a diseased condition of the brood called by bee keepers "pickle brood," but practically nothing is known of its cause. It is characterized by a swollen, watery appearance of the larva, usually accompanied by black color of the head. The larvæ usually lie on their backs in the cell, and the head points upward. The color gradually changes from light yellow to brown after the larva dies. There is no ropiness, and the only odor is that of sour decaying matter, not at all like that of American foul brood. In case the larvæ are capped over, the cappings do not become dark, as in the case of the contagious diseases, but they may be punctured. So far no cause can be given for this disease, and whether or not it is contagious is a disputed point. Usually no treatment is necessary beyond feeding during a dearth of honey, but in very rare cases when the majority of larvæ in a comb are dead from this cause the frame should be removed and a clean comb put in its place to make it unnecessary for the bees to clean out so much dead brood.

CHILLED, OVERHEATED, AND STARVED BROOD.

Many different external factors may cause brood to die. Such dead brood is frequently mistaken, by persons unfamiliar with the brood diseases, for one or the other of them. Careful examination will soon determine whether dead brood is the result of disease or merely some outside change. If brood dies from chilling or some other such cause, it is usually soon carried out by the workers, and the trouble disappears. No treatment is necessary. Brood which dies from external causes often produces a strong odor in the colony, but wholly unlike that of American foul brood, merely that of decaying matter. The color of such brood varies, but the characteristic colors of the infectious diseases are usually absent, the ordinary color of dead brood being more nearly gray.

Approved:

JAMES WILSON,
Secretary of Agriculture.

WASHINGTON, D. C., *October 3, 1906.*

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UNITED STATES DEPARTMENT OF AGRICULTURE

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L. O. HOWARD, Chief

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A STUDY OF THE BEHAVIOR OF BEES IN COLONIES AFFECTED BY EUROPEAN FOULBROOD[1]

By ARNOLD P. STURTEVANT
Specialist in the Bacteriology of Bee Diseases

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INTRODUCTION

The brood diseases of bees cause annually large losses of bees and consequently of the honey crop. The predominant attitude among beekeepers has long been how best to eradicate an invading bee disease after the attack has been made. They depend upon this procedure, because little is known with any degree of certainty concerning the natural conditions which might prevent or control the onslaught of the disease. As a result of this attitude, much more importance has been placed on the significance of apiary inspection and police-power laws and of purely remedial treatment, the reasons for which in many cases are imperfectly understood. But the old adage "an ounce of prevention is worth a pound of cure" has yet to be refuted, particularly with regard to beekeeping. In the realm of human

[1] A series of investigations was started in the spring and summer of 1918 by the Office of Bee-Culture Investigations, Bureau of Entomology, for the purpose of making an intensive study of European foulbrood of bees, primarily from the standpoint of insect behavior in relation to the disease, correlated with the facts and practical observations already known to the beekeeper. This paper, which was submitted for publication January 13, 1919, is a preliminary report on the beginning of the investigation.

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medicine, for the last two decades at least, this precept has been gaining strength so that to-day preventive medicine stands on a par with, if not above, most of the other branches of medicine. Why is it not logical to apply this principle to the control of bee diseases?

Ever since European foulbrood of bees was first recognized (in 1894), in New York State, as a distinct brood disease, there have been much controversy and speculation concerning the etiology of the disease, the means of transmission, the method of spread, and, resulting therefrom, the question of control. From the laboratory standpoint, the etiology of the disease has been worked out quite definitely bacteriologically (12)[1]. But as yet *Bacillus pluton*, the accepted cause of European foulbrood, never has been grown in pure culture on artificial media, although it has been definitely identified as the cause of the disease. This precludes any further advance along this line of attack for the time being.

From the side of practical experience, there have been recorded large numbers of observations, many of them of a similar nature. These observations have led to many accepted practices, as, for instance, the use of Italian bees and strong colonies in combating the disease. Although the weight of numbers tends to give substantiation to observations, the scientific explanation of how these things are true never has been studied carefully and coordinated with the practical side into an epidemiological study of the colony under disease conditions in European foulbrood.

The history of bee diseases has developed mainly along two lines. The scientific side has been concerned principally with determining the causes of the various diseases microbiologically, the method of diagnosis, and conclusively differentiating them. These facts have been described sufficiently in various bulletins of the Bureau of Entomology and will not be discussed here. From the practical side, countless observations have been recorded, largely in the bee journals, in which various manifestations of the disease and experiences with methods of treatment have been discussed. But in all this literature, particularly with regard to European foulbrood, there are few observations on the disease and on the behavior of the bees in relation to it beyond simple description of symptoms.

Early in the experience with European foulbrood it was learned by careful observers that strong colonies are essential in successfully combating the disease. Later the value of Italian bees was discovered. West (11), a New York State apiary inspector, in giving what is one of the best early descriptions of European foulbrood, makes some pertinent observations on the disease. He states that when diseased brood is placed above a strong, healthy colony, with a queen excluder between, so that any healthy brood may emerge,

[1] Reference is made by number in parenthesis to "Literature cited," p. 28.

the diseased larvæ are cleaned out as this is taking place. The union with a healthy colony and the strength gained by the emergence of so many young bees gives the colony the stimulus to eliminate the disease. He notes, as have many other beekeepers since, that in August, when the buckwheat honey flow begins, the stronger of the diseased colonies are stimulated to clean up.

Alexander (1) published a method of treatment for European foulbrood, the principle of which, after many varying failures and successes, is now the basis for the present method of treatment most used; that is, requeening with Italian stock. Alexander mentions the need of three factors: First, the necessity of requeening with young yellow Italians, as hybrids of Italian and black bees are prone to contract the disease in the first place and also are more likely to succumb to it; second, particularly emphasized, a period (at least 27 days, according to Alexander) of queenlessness in which to allow the bees properly to clean up the cells and polish them, preparatory for eggs of a new queen; third, a factor which is mentioned only casually but which is equally important with the other two, the direction to unite and strengthen diseased colonies before treating. So little emphasis was placed on this that the majority of beekeepers overlooked it in using Alexander's treatment and therefore condemned the treatment as unsuccessful except in rare cases.

In an editorial (8) in the same issue of the journal in which Mr. Alexander was writing, the question was raised as to why the period of broodlessness caused by winter, which is much longer than 27 days, does not always prevent a recurrence of the disease. Mr. Alexander answered this question by explaining that when the queen stops laying in the fall, the bees do not polish up the cells as they do earlier in the season, and that some of the dried-down material may remain until the next spring. The opinion also is given in this editorial that Italians are more able to resist the disease than hybrids because they do more thorough work in house cleaning and are less inclined to rob.

Phillips (6) makes the statement that "European foulbrood is more destructive during the spring and early summer than at other times, often entirely disappearing during the late summer and early autumn, or during a heavy honey flow," but gives no indication as to how this takes place. The same year Miller (2) published his theory of the relation of the nurse bees to the spread of European foulbrood. He believes that the nurse bees suck up the juices of a freshly diseased larva which has not become offensive, and then transmit the disease when feeding the healthy larvæ. On this supposition he believes that if egg laying ceases for 5 or 6 days ("the period the larvæ remain unsealed in their cells") there will no longer be larvæ in the proper condition for nurse bees to feed upon,

nor healthy unsealed larvæ to receive the infection, and the disease will thereby come to an end.

Dr. Miller has been using a 10-day period of queenlessness in his treatment of European foulbrood since his accidental discovery that 10 days were sufficient, but in a later article (3) in enlarging upon his nurse-bee theory he assumes that the larva is fed during a period of 5 days but is not effective as a carrier of infection during the whole time as probably no larvæ are torn open until they are 2 or 3 days old; thus making it possible to shorten the queenless period even more. He admits that not all the dead, partially dried larvæ will be cleaned out, but believes that it is only the fresh yellow ones which are infectious. He also states that nurse bees are not inclined to travel far on the combs, a fact which may explain why the disease may be found confined to one comb for several days before spreading farther. Dr. Miller seems to have overlooked several important factors which will be discussed later.

Quite an extensive piece of investigation was carried on during the summers of 1915 and 1916 by the author at the Massachusetts Agricultural Experiment Station upon the effect of requeening diseased colonies with various strains of Italian bees. At that time the importance of strong colonies with the requeening had not been emphasized so strongly and less attention was paid to that factor. The records show, however, that in a total of 50 colonies observed, covering two seasons, of 10 strong colonies only 2 showed recurrence, while 1 was doubtful; of 20 medium-strength colonies, 10 showed recurrence with 2 doubtful; of 14 weak colonies, 8 showed recurrence. In all these cases the new queen was not introduced until the colony was nearly or entirely clean. In the case of several of the weaker colonies it was necessary to strengthen them before requeening was possible, in order to save the colony. One or two of these, which were united and requeened with Italian stock, were the best colonies the next spring.

Adding some strength to at least part of Miller's theory is a statement in a letter by G. C. Matthews, formerly of this bureau, who wrote in February, 1918, concerning his observations in California in 1914. He found that where the hives stood in rows of pairs the disease continued to spread down each row to corresponding members of each pair. This ceased when he rearranged his apiary so that the rows of hives were at least 10 feet apart, and alternate pairs of hives were turned at right angles. No pair was allowed to remain close to another facing the same way. This prevented the drifting of nurse bees, which he believes to be the method of spreading the disease. Furthermore, he found by introducing one Italian queen into the middle colony of an isolated row of hybrid bees that there was considerable drifting of nurse bees. Seven days after the brood

from the Italian queen began to emerge, yellow bees were found on either side in several of the hybrid colonies. Speaking of uniting weak diseased colonies and requeening, Matthews writes:

> After two or three were put together, each stack of brood was given an Italian cell. When young queens commenced to lay there was still disease in many of those hives, but as the queens increased in laying the bees cleaned out an ever-increasing sphere of comb for a brood nest until they had the hives free of disease. But in no case, however long a hive might be queenless, did I see the disease cleaned out before a virgin appeared in the hive. In other words, a virgin had to be present before the bees would commence their job of cleaning up. Therefore, I see little to commend the practice of keeping diseased colonies queenless 21 days.

A new bulletin by Phillips (7) has been issued recently by the Department of Agriculture. The fundamental idea emphasized is that "in keeping European foulbrood under control it is far more important to prevent the disease from getting a foothold in a colony than it is to eradicate the disease afterward." This bulletin, aside from discussing symptoms and methods of treatment, states concisely for the first time the facts observed in apiary practice on which successful treatment is based, and without an understanding of which it is difficult for a beekeeper to use preventive measures with any success.

The analysis of these factors of response in behavior to treatment, as stated by Phillips, has been used to some extent as a foundation for the present work on the behavior of the colony in relation to disease, in an endeavor to substantiate, with data obtained under controlled conditions, these facts that are constantly observed in apiary practice and, if possible, to eliminate confusion in methods of treatment.

PROCEDURE

Shortly after the middle of May, 1918, experiments were started in Ithaca, N. Y., at the Cornell Agricultural College. Through the kindness of Prof. J. G. Needham, head of the department of entomology, and others associated with him, the use of a small, isolated yard of bees and also of laboratory facilities was offered for the purpose of carrying on these investigations. This small apiary had been used previously in fruit-pollination studies and had no record of disease. The yard was admirably located in a naturally well-protected hollow beyond the college fruit orchards, about a mile and a half from the main college apiary or other apiaries, with high ground and woods intervening. The author and the Office of Bee-Culture Investigations are under deep obligations to the Cornell authorities for the assistance so cordially extended.

Being in the buckwheat district, the general locality was well adapted to the work because of the desire for as late a main honey

flow as possible in order not to have the influence of a heavy honey flow until other factors had been studied. At Ithaca the main honey flow is generally from buckwheat, coming from the 1st to the middle of August. Rather unfortunately for the best results from the experiments, however, the summer of 1918 was unusual in this section, for the abnormally heavy honey flow from clover necessitated finishing the work earlier than had been planned, owing to the great difficulty of artificially infecting colonies during the heavy honey flow.

There were seven colonies in the original experimental apiary. At first it was intended to work on a larger scale, but the trend of the observations soon led to the plan of working in more detail and on a smaller scale. These colonies were moved some distance apart to prevent drifting and robbing. Some were divided and some were strengthened in an effort to make a series of experiments on colonies of different strengths. The colonies were designated by letter and the combs of each colony by number. From time to time some of these colonies were artificially infected with diseased European foulbrood larvæ from samples sent to the laboratory for diagnosis. Similar colonies were held intact and uninfected for controls. The infection was made by feeding diseased larvæ macerated in sugar solution (about 50 per cent). For the preliminary experiments 10 larvæ were fed in about 250 c. c. of sirup. Later, after the heavy honey flow had begun, it was necessary greatly to increase this dose in order to start the infection. The infected sirup was fed to the bees in sterilized glass petri dishes, placed on top of the frames and protected by an empty comb-honey super placed on the regular hive body with the cover on top.

At the time of inoculation, the condition of each colony was noted as to age, race, condition and appearance of the queen, proportion of nurse bees to old field bees, the number of frames of brood with the amount in each, its age, sealed or unsealed; in other words, the condition of the colony with regard to factors known to be significant in resisting disease. In two colonies the infected sirup was slightly colored with harmless eosin dye to determine where the fresh sirup was placed and its ultimate disposition. At first daily observations were made to determine the earliest appearance of disease, the period of incubation, the symptoms exhibited, and the rate of increase.

By holding up each comb in bright sunlight so that the light shone directly on the larvæ, it was easy to detect the first symptoms of the disease. All the healthy larvæ had the characteristic firm, well-rounded, pearly-white, glistening appearance. The first effect of the disease, besides an abnormal uneasy movement, was a loss of the

glistening character and a slight tinge of grayish or creamy discoloration which would not be noticed except in direct sunlight. These larvæ showed only *Bacillus pluton* present when examined microscopically, as will be mentioned later. Soon after these first symptoms, however, the more noticeable symptoms appeared, such as a larva with its back out, the increase of the light grayish yellow color, and, later, the moist, melting appearance.

A statistical record was kept of the number of larvæ showing new disease at each observation, the number previously diseased that had been cleaned out in the interval since the previous observation, and those remaining over in the cells uncleaned for more than one period between observations. At various times observations were made of the behavior and types of bees engaged in cleaning up and the fate of the material removed. Great care was necessary in these observations to disturb the colony as little as possible. On good days it was sometimes possible to remove a comb carefully from the hive and to watch the bees continuing at their work, and even to watch the queen laying eggs. An eight-frame observation hive containing a strong healthy colony was given a diseased comb from time to time and the bees were observed as they worked on it.

One of the difficulties of the work was to find a satisfactory method of recording the desired data for each comb. At first the diseased cells were marked on the comb by a circle of red celloidin around the entrance of the cell. Although this dried rapidly, it proved unsatisfactory, as the bees, in their attempt to remove the foreign material, seemed to remove both diseased and healthy larvæ indiscriminately. Next small pins were used, inserted in the cell above the one showing disease. In this case the bees tore down the surrounding cells and completely removed the pins, many of which were found on the bottom board. Finally a method of plotting the diseased cells in a comb was adopted. An empty frame was laid off in inch squares by means of heavy black thread. This, used as a templet superimposed on a comb, aided in the location of the diseased and cleaned out cells, so that they could be recorded on a correspondingly ruled card (fig. 1). Placing this over the comb, it was easy to locate exactly each cell and to determine how long the diseased material remained, thus aiding in following the course of the disease throughout its various stages. The only difficulty with this method was the tediousness of the observations. Therefore, after the disease had become definitely established, daily observations of each colony were considered unnecessary. Longer periods showed just as well what was happening in the colony. Also, after the disease had developed enough so that it could be definitely predicted whether the colony would recover or gradually be exterminated, observations of behavior under treatment were

started, the method and degree of house cleaning being watched after the colony had been dequeened, strengthened, and requeened with good Italian stock. Note was also made of any recurrence of disease and

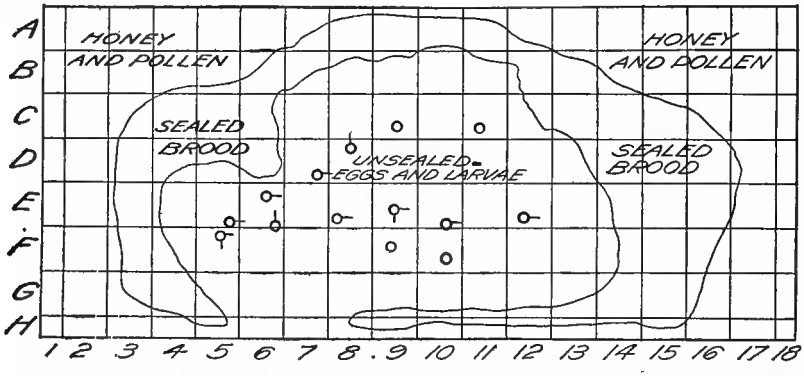

FIG. 1.—Method of plotting the location and history of diseased bee larvæ in the combs. ○ Freshly diseased larvæ. ○- Cells that have been cleaned out. ♀-Cells that have been cleaned out and filled with nectar. ♁ Larvæ remaining in the cells more than one observation period. The area of sealed brood was the amount present at the time of infection of the colony.

apparent reason therefor. In other words, a complete study was made of the cycle of the disease and of the activities of the bees during its course.

OBSERVATIONS

COLONY G

Race.—Hybrid.

Queen.—1917, dark and poor.

Bees.—Workers and drones very dark, almost black, very excitable.

Condition of colony at time of infection.—Brood in four frames, a little less than half sealed, besides two frames of eggs. Bees covering about eight frames, medium strength. Slightly more field bees than nurse bees, because of having divided this colony, old bees returning from the division.

Date of first infection.—May 28, 1918.

Material used.—Ten diseased larvæ from sample No. 5863, macerated in 250 c. c. of a 50 per cent sugar sirup.

First appearance of disease noted.—May 31, 1918, three days after inoculation.

Age of larvæ first attacked.—Three to four days after hatching from the eggs.

Colony G (fig. 2), hybrids, soon succumbed to the infection, the first diseased larva appearing three days after infection, the gross diagnosis being confirmed by the finding of *Bacillus pluton* on microscopic examination. The spread of the disease was rapid, the disease being present in only one comb on the third day and in seven combs on the seventh day. All of this early spread took place in brood unsealed

at the time of infection. The first high peak of the disease coming on the nineteenth day was followed by a slight improvement, when for a time the house cleaning exceeded the occurrence of fresh disease. This was probably due to the stimulus of the increasing honey

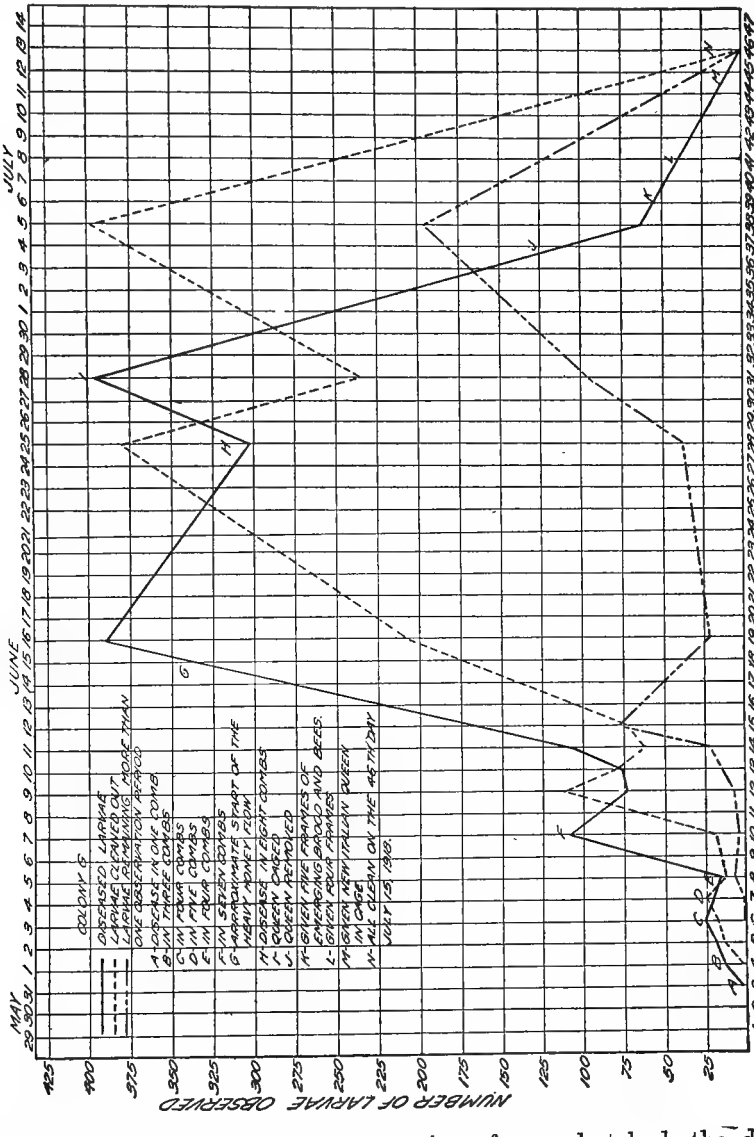

Fig. 2.—The course of European foulbrood in colony G.

flow. But as soon as the next series of eggs hatched, the disease again gained the upper hand, reaching another higher peak on the thirty-first day, at which time it was deemed necessary to start treatment. It had become evident that the colony was being overrun by the disease. More and more dead larvæ were being allowed to re-

main in the cells for several days without being cleaned out. Also more larvæ nearly ready for pupation were being affected. Most of these instead of remaining coiled were inclined to extend on the lower side wall in a brownish gray, slimy mass and exhibited a tendency to be viscid. At this stage of decomposition, when a stick is inserted the mass forms a coarse granular band for a short distance and then breaks so as to form droplike masses, but does not stretch out in a fine thread. These larval masses dried down to rubbery dark brown scales something like American foulbrood scales in appearance, but different in consistency. These scales could be removed quite easily and would bend like a piece of partially granular old rubber. They also lay irregularly placed in the cells, often spirally extended, while American foulbrood scales are uniformly on the lower side wall. The bacteriological explanation for this abnormal characteristic will be discussed later under bacteriological observations.

The predominance of these rubbery masses and scales increased as the disease progressed and the bees seemed to make little attempt to clean them out, even after the queen was caged on the thirty-first day, thus shutting off any increase of fresh larvæ, or even after the queen and all queen cells were removed on the thirty-seventh day. On the thirty-ninth and also on the forty-first day, five and four frames, respectively, of emerging brood and Italian bees were united with this colony, but it was not until a new Italian queen, confined in a cage, had been hung in on the forty-fifth day that a final complete cleaning up was made.

This new queen was not accepted, however, and a young queen was raised from the brood that was added to this colony, so that further observations were ended here although the virgin queen was killed and another Italian queen introduced. This colony was reported healthy, however, about the middle of August.

The hybrid bees seemed to lack ambition to fight the disease. When combs were removed from the colony, the bees never were observed to be working in the cells, and paid little attention to material partially drawn from the cells and crushed.

COLONY F

Race.—Italian, possibly with some slight hybrid blood.

Queen.—1917, fairly good condition.

Bees.—Workers, good color, fairly quiet, drones inclined to be darker.

Condition of colony at time of infection.—Brood in three frames, a little more than one-third sealed. Bees covering about six frames. Building up well. Proportion of field bees to nurse bees about equal.

Date of first infection.—May 31, 1918.

Material used.—Ten diseased larvæ from sample No. 5874, macerated in 250 c. c. of a 50 per cent sugar sirup.

First appearance of disease noted.—June 4, 1918, four days after infection.

Age of larvæ first attacked.—Four days after hatching from the egg.

Colony F (fig. 3), which was the next one to be infected, although not as strong as colony G, was of Italian stock and did not show the appearance of disease until one day later. On the fourth day one cell appeared in each of two combs. It was not until the twenty-

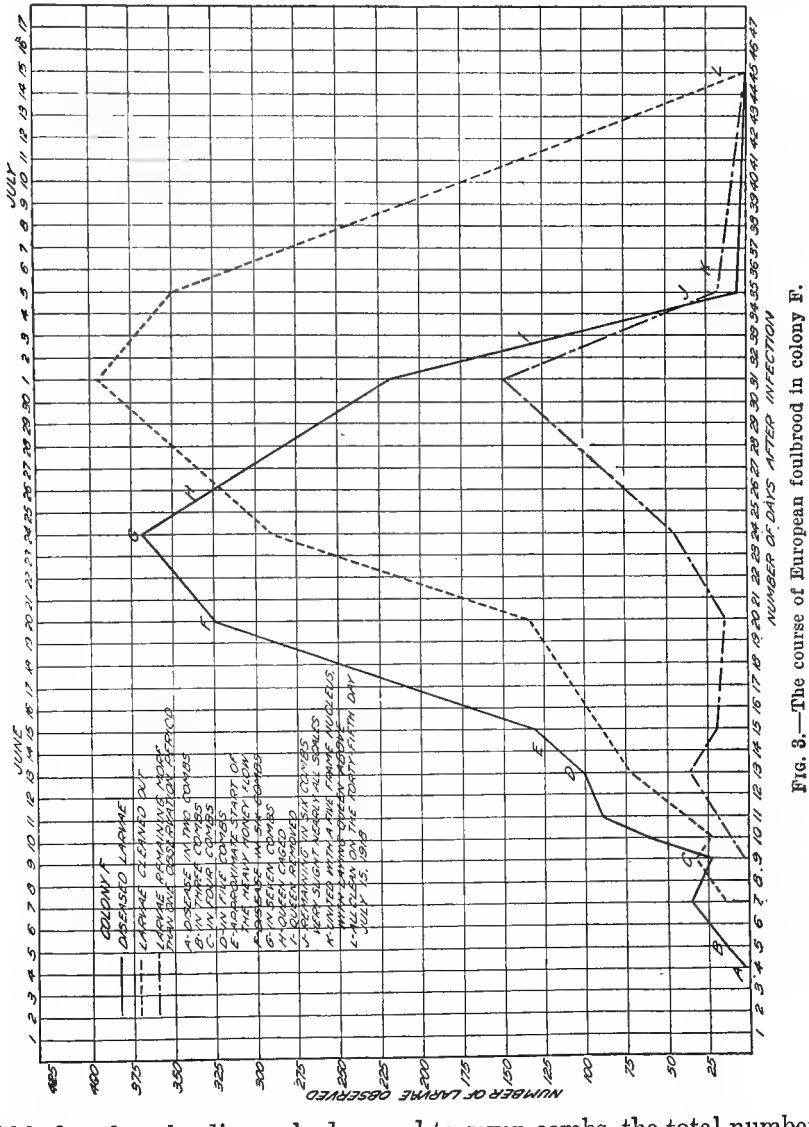

FIG. 3.—The course of European foulbrood in colony F.

fifth day that the disease had spread to seven combs, the total number of diseased larvæ being, as a whole, less than in the hybrid colony. There was not brood in all seven combs at the time of infection, but the brood increased faster than the disease spread. After the twenty-fourth day a permanent improvement began to be manifest.

This improvement continued after the queen was caged and became more marked after she was removed from the colony.

These bees were better house cleaners as well; the appearance of larvæ remaining over more than one observation period did not become evident until after the ninth day, compared with the sixth day in colony G. At no time were there as many of the larvæ nearly ready to pupate that were gummy or rubbery. Even though this colony was on the average weaker than colony G all the time, it handled the disease much better. It was 14 days before colony G had cleaned up to such an extent that it was deemed safe to introduce a new queen, while in colony F, with the Italian bees, the combs were so nearly cleaned of everything but a few old scales that a five-frame nucleus with a new Italian laying queen was united with this colony after a 10-day queenless period and in 9 days more everything was absolutely clean and the queen was laying in the combs that had had disease in them.

When an observation was made nine days after the new queen's eggs were first noted, it was found that there was a slight recurrence of disease in three of the combs. But, unfortunately, at the same time, queen cells and no eggs were found, denoting that for some reason this queen had not been accepted. Therefore the queen cells were all removed and a new queen was introduced. Although the author's observations ended of necessity soon thereafter, it was reported to him that this colony was doing nicely later in August and was perfectly healthy. If the first new queen had not disappeared, it is quite probable that as soon as a sufficient number of her bees had emerged they would have cleaned up the recurring disease in the same manner as was done in colony J, which will be mentioned later.

Several times in this colony, during the cleaning-up process, bees were watched in the act of sucking up juices of diseased larvæ that had been partially removed from the cells with the aid of forceps.

COLONY H

Race.—Hybrid, a division of Colony G, hybrid.

Queen.—1918. Of their own raising. Poor.

Bees.—Dark hybrids, almost black, excitable.

Condition of colony at time of infection.—Brood in three frames, a few eggs in one, only one small patch sealed, the remainder from eggs up to 4-day larvæ. Bees covering about five frames. Fairly good proportion of nurse bees.

Date of first infection.—July 1, 1918; second infection, July 8, 1918.

Material used.—20 old, dried, rubbery, diseased scales from sample No. 5898, macerated in 250 c. c. of a 50 per cent sugar sirup, colored with eosin.

First appearance of disease noted.—July 5, doubtful. Positive July 8, 7 days after infection.

Age of larvæ first attacked.—Four days after hatching from the egg.

Colony H (fig. 4) was treated as a double experiment. The infection of this colony was not started until after the honey flow had come on quite heavily. Also, instead of freshly diseased larvæ, old brown rubbery scales were used that showed *Bacillus pluton* present microscopically, but were heavily overgrown by *Bacillus alvei.* It was desired to learn whether these scales were still infectious, so that nurse bees working on them, cleaning them out, might get infective material on their feet and mouth parts which could be carried to healthy larvæ. This was noted later in the observation hive, where, under the magnifying glass, bees were seen trying to remove some of these rubbery scales, first moistening them with their tongues and then pulling at them with the mandibles and front feet.

This colony, which was marked hybrid and weak, was slow in developing the disease, partly because of the diluting effect of the

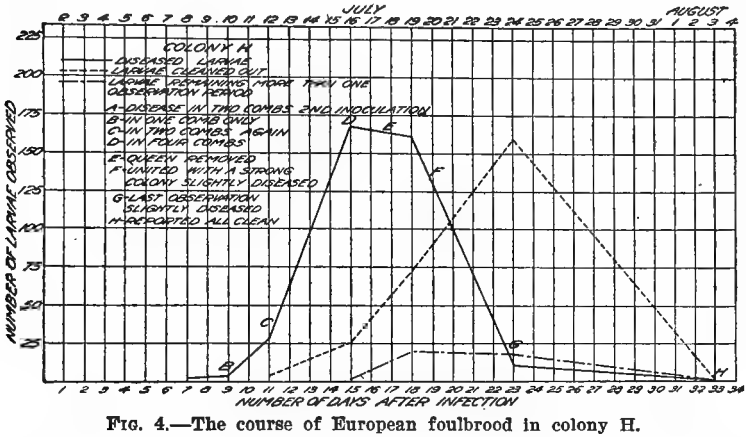

FIG. 4.—The course of European foulbrood in colony H.

heavy honey flow and probably partly because there was a smaller number of infectious organisms present in the scales than in fresh larvæ. This is explained by the fact that the secondary putrefactive invading organisms would tend to kill off the primary organism, because of the accumulation of the products of the putrefactive action.

On the seventh day before the disease was first noted, a second infection of scales macerated in sugar sirup was given this colony to counteract the effect of these retarding factors. However, later on the seventh day, diseased larvæ were found, and from then on the disease started to spread and increase irrespective of the heavy honey flow, exhibiting all the symptoms and tendencies shown in colony G, of which this colony was a division before infection.

On the seventeenth day it was necessary to remove the queen and start treatment, but what was taking place was evident. This removal of the queen did not seem to have a very marked effect on the house

cleaning until the colony was united with colony I, a slightly diseased Italian colony. They then began cleaning the H combs, and the combined colony was reported clean in August.

COLONY A

Race.—Italian with some possible slight hybrid blood.

Queen.—1918. Of their own raising.

Bees.—Workers, good color; fairly quiet. Drones, some slightly darker than pure Italians.

Condition of colony at time of infection.—Brood in seven frames about half sealed. Bees covering about nine frames with a good proportion of young nurse bees. Colony strong and building up.

Date of first infection.—July 2, 1918. Second infection, July 6, 1918.

Material used.—First, 20 diseased larvæ from sample No. 5937 macerated in 250 c. c. of a 50 per cent sirup, colored with eosin, abnormally heavy infection; second infection, 20 diseased larvæ from sample No. 5953 in 250 c. c. of uncolored sirup.

First appearance of disease noted.—July 8, 1918, in drone brood, six days after infection.

Age of larvæ first attacked.—Four days after hatching from the egg.

Colony A (fig. 5) was a fairly strong colony of Italians. Like colony H, it was infected after the heavy honey flow had started and was

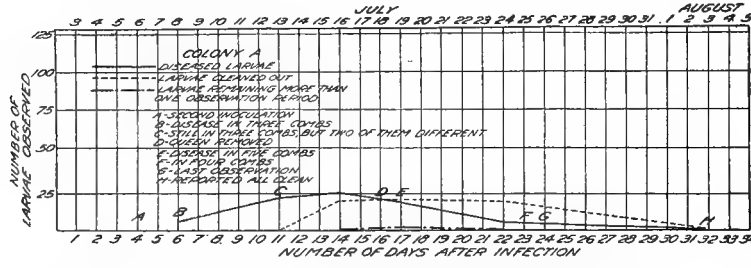

FIG. 5.—The course of European foulbrood in colony A.

given twice the amount of infective material colonies F and G received. Nothing having appeared on the fourth day, a second infection of the same amount was given. On the sixth day 6 diseased larvæ were seen in three combs. This colony, however, was so strong that the disease obtained very little foothold, and from the fourteenth day began to decline, or at least failed to make further gains. As a side experiment in this colony a comb of eggs laid by an Italian queen was placed in between two combs showing disease. If there is anything in the belief that Italian stock is more resistant to disease, the larvæ in this comb should not have developed the disease, or at least not so soon. However, on the sixth day one or two larvæ showed disease, increasing slightly in numbers for a few days until the observations were of necessity stopped. It was intended to perform this experiment with several variations, such as placing eggs laid by an Italian queen in a diseased hybrid colony and placing eggs from

a hybrid queen in a diseased Italian colony, but the presence of the heavy honey flow made it impracticable to carry the matter further.

This colony A cleaned up readily after removal of the queen and was reported all healthy in August. Although a new queen was given to them it is probable that a period of queenlessness and the returning of the same queen would have answered just as well.

COLONY I

Race.—Italian.

Queen.—1917, fairly good condition.

Condition of colony at time of infection.—Seven frames of emerging brood well covered with young bees. A strong 8-frame colony.

Date of first infection.—July 10, 1918.

Material used.—Thirty diseased larvæ from sample No. 5959, macerated in 250 c. c. of a 50 per cent sugar sirup. This was abnormally heavy infection of diseased material.

First appearance of disease noted.—July 15, 1918, five days after infection.

Age of larvæ first attacked.—Four days after hatching from the egg.

This colony was infected during the heavy honey flow, but although given a heavy infection it had sufficient strength, aided by the heavy honey flow, to prevent the disease from spreading. On July 15 there were a few diseased larvæ in two combs. On July 24, 14 days after inoculation, there were only a few diseased larvæ in three combs. This was after the queen had been removed on July 18 and the colony had been united with colony H on the 20th.

An interesting observation was that under the magnifying glass the methods of the nurse bees in sucking the juices from dead diseased larvæ and the pulling of the skins out to carry them away could be noted. No bee worked very long at a time on one larva. One after another worked until all was completed.

SUMMARY OF PREVIOUS EXPERIMENTS

Table I gives a partial summary of the data thus far described.

TABLE I.—*Showing the first appearance of disease noted after infection. Also the number of combs showing infection and the spread of the infection from comb to comb in the various colonies under observation*

Colony.	Date infected.	Days after infection.																									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
	1918	Number of combs.																											
G[1]	May 28			1	3		4	5	4		7																		8
F[1]	May 31				2	3				4				5							6				7				
H[2]	July 1																												
	July 8							2		1		2				4													
A[2]	July 2																												
	July 6						3											5						4					
I[2]	July 10					2									3	(Different from first two.)													

[1] Experiments started before the beginning of the heavy honey flow.
[2] Experiments started after the beginning of the heavy honey flow.

In the first group, colonies G and F, it is quite apparent that the Italian bees, colony F, made the better showing, even though the hybrids were the stronger colony in the beginning. As may be seen from a comparison of the two plots in figures 2 and 3, in colony G wherever there was a lag in the house cleaning there was marked increase in the number of larvæ remaining over more than one observation period, and these increased until strengthening treatment was started. On the other hand, in colony F these were removed almost entirely by the time the strengthening treatment was started. The Italians did not allow the disease to appear as soon or to spread as rapidly, cleaned house better, left fewer larvæ to dry down to the brownish rubbery scales, and responded to the increased honey flow and treatment much more readily.

In the second group, colonies H, A, and I, the Italian colonies A and I again made the best showing. With the added diluting effect of the honey flow, they allowed the disease to gain no foothold whatever, while the hybrids, though aided by the honey flow, soon succumbed and allowed the disease to gain on them. It is evident that the Italian bees are much more vigorous house cleaners. In several instances, toward the end of the egg laying of the old queen, and well along in the progress of the disease, cells were noted on these diagrams which had previously contained diseased larvæ, but which had been cleaned out, and then in which disease had reappeared after other eggs had been laid and hatched in them. They were cells in which fresh nectar had not been placed between the two series of larvæ.

It was also noted that as the honey flow increased and as the brood became more scattered from the effects of the disease, more and more fresh nectar was placed in the brood nest in cells from which dead larvæ had been removed. Most of this nectar, however, was moved up later, particularly after the bees began preparing the brood nest for a new queen in the process of treatment. That the advent of a heavy honey flow was effective in controlling the disease is evident, particularly in the length of time between the infection and the first appearance of disease. The data, however, show little difference in the resistance to infection, or so-called immunity, being slightly in favor of the Italians, if there is any difference at all.

Disregarding the effect of the honey flow, the period of incubation of the disease is apparently between 3 and 4 days. However, it was noted that after *Bacillus pluton* was first observed it was anywhere from 24 to 48 hours before many characteristically diseased larvæ were observed. Therefore, the actual period of incubation is probably from 24 to 48 hours.

SUPPLEMENTARY OBSERVATIONS

STUDY OF NATURALLY INFECTED COLONIES

As a supplementary study to the preceding artificial infection experiments, some observations were made upon the behavior of naturally infected colonies undergoing treatment. Through the kindness of W. L. Bean, of McGraw, N. Y., it was possible to make a series of such observations. In his apiary of about 30 colonies, all hybrids, the majority were diseased when observed June 8, 1918. Soon thereafter Mr. Bean kindly loaned two of these diseased colonies to be carried to Ithaca for closer observation. Mr. Bean at once started treating his bees, requeening with Italian stock by the method of introducing a queen cell almost ready to emerge. Apparently, this method was successful, for in the latter part of July Mr. Bean reported all treated colonies healthy and some 800 pounds of surplus honey.

COLONY J

Race.—Hybrid.
Queen.—Queenless at time of arrival at Ithaca. Was poor hybrid of own raising, probably reared while disease was present in the colony.
Strength in spring.—Weak.
Strength at time of treatment.—Scattered brood in eight frames. Weak in bees, particularly in nurse bees.
Approximate date of disease first noted.—May 31, 1918.
Date of start of treatment observations.—June 16, 1918.

This colony made no effort to clean up, even though they had lost their queen shortly before being brought to Ithaca. On the 18th of June six frames of Italian bees and emerging brood were placed on top of it. At once house cleaning started, a reduction of 50 per cent being noted in the fresh, moist, melting larvæ within 24 hours. In this colony it was interesting to watch the bees doing the house cleaning, particularly when diseased larvæ in various stages of decomposition were partially withdrawn from the cell with a pair of forceps. With the aid of a powerful hand magnifying glass it was easy to watch them suck up the juices of the dead larvæ, even those which had decomposed to the extent of being a coffee brown in color and viscid in consistency. No bee would work long on a larva but would back off and wipe her tongue thoroughly with her front feet. It is conceivable that this might contaminate her, making possible carriage of the infection to the next larva fed, even though the juices of the diseased larva were not actually fed to the healthy one. The majority of bees engaged in this work were the Italians. From these and other observations of a similar nature there is no doubt that the contamination of the mouth parts is the primary method of spreading the disease inside the colony.

On June 25 an Italian queen was introduced in a cage with candy even though a few scales were still present. This was fully 10 days after the colony had lost its queen, if not a little longer. On June 27 the queen was out and laying in one comb. Eight days later, on July 5, a recurrence of disease was noted, one larva being discolored and sunken, showing *Bacillus pluton* on microscopic examination. From that time on, for about 20 days after the first eggs of this queen were noted, one or two new diseased larvæ appeared at each observation, the number decreasing, however, until about the twenty-sixth day when they had all disappeared. As the new young Italian bees increased, the disease decreased, until a point was reached where they were in the predominance and had eliminated the disease by their activity. This was also observed in colony F.

COLONY K

Race.—Hybrid.

Queen.—1917. Dark hybrid of their own raising, probably from diseased stock.

Strength in spring.—Weak.

Strength at time of treatment.—Eight frames of scattered brood and hardly enough bees to cover them.

Approximate date of disease first noted.—May 31, 1918.

Date of start of treatment observations.—June 20, 1918, at which time the queen was removed.

Colony K, when it was brought to Ithaca, was so weak that it would soon have died. The bees made no attempt to clean out larvæ that had been partially pulled out of the cells with forceps and crushed. On June 26, six days later, they were still showing freshly diseased, moist, melting larvæ from eggs laid by the old queen, just before removal. At this time five frames of emerging brood and Italian bees were given this colony. On the 27th a new Italian queen was hung in with the cage closed. The presence of the new queen, however, seemed to give added impetus to the house cleaning so that by July 1, 11 days after removal of the queen, they were practically cleaned up and the cage was opened with candy in the opening. Further observations on this colony were ended because they refused to accept this queen. By the time another queen finally was accepted and was laying on July 18, it was too late, as the season's work was closed by the 23d.

BEHAVIOR OF BEES IN CLEANING CONTAMINATED CELLS

On June 6, 1918, a sample was received for diagnosis (No. 5898), consisting of an entire brood comb, containing quite an area of capped honey. About one-half of each side of the comb contained a large number of dead and diseased European foulbrood larvæ, in stages varying from the yellowish, moist, melting larvæ to dried rubbery scales of which there was quite a large proportion. This was

the same sample from which infectious dried scales were used to infect colony H. After this comb had remained in the laboratory, wrapped in paper for about 3 weeks, it was placed in the strong colony in the observation hive. The frame was first placed in the middle of the hive for about an hour and was then removed to the outside, where the work of the bees on it could be watched. A large number of what appeared to be young nurse bees were already hard at work on the dried diseased material. The bees, working on the dried gummy masses, would wet the mass with their tongues for a while and then tear at them with their mandibles, at times removing pieces large enough to be seen from the outside. Often these small pieces were apparently dropped to the bottom board. No one bee worked long at one place. Those bees working particularly on the fresh, moist material, when leaving, would carefully wipe their tongues with their front feet, thereby transferring some of the infection to them. Other bees were at work carrying away the larger, more easily removable dead masses. The entrance also was watched to see if any of this material was carried out. Several bees were observed carrying out portions of dead larvæ or pupæ. One bee carried a piece about 2 yards before dropping it. Others dropped what they were carrying soon after leaving the entrance, but on examining the surface of the ground about the entrance, very little material could be distinguished, so that apparently most of the material removed must have been carried some little distance before being dropped. After about an hour's work it was apparent that considerable progress had been made. This comb was removed before it was entirely cleaned and later placed in another healthy colony for observation. It was quickly cleaned up and quite a bit of nectar placed in it and, eventually, several square inches of brood. Observations, however, had to be stopped before any appearance of recurrence was noted. This same observation hive was given one or two other diseased combs to clean, but with the repeated probable infection from these sources the colony was so strong that no disease was noted in it during the entire season of observations.

POSSIBLE INFECTION THROUGH QUEEN

Colony M was a small nucleus made to receive the old queen from diseased colony K from McGraw, N. Y. The queen was introduced on June 20, 1918. For a while she laid fairly well, it being necessary to add one or two more combs. But later her brood became more and more scattered. Finally, on July 8, there was observed one dead larva, which looked suspicious, but which, on microscopic examination, proved to be negative. On July 10, however, one definite cell appeared and several other slightly yellowish, abnormally colored larvæ. This dead larva contained *Bacillus pluton*. From then on until this queen was killed and the colony united with another dis-

eased colony, more discolored larvæ appeared, showing definitely the development of the disease. As far as could be seen the only source of infection was the queen which had come from a diseased colony.

This occurrence had been observed previously by the author while employed at the Massachusetts Agricultural Experiment Station. During the summer of 1916 eight queens taken from diseased European foulbrood colonies were introduced into isolated, healthy nucleus colonies. Of these eight nuclei three developed European foulbrood, two were doubtful, and three remained healthy. Several such instances have been mentioned in the literature of beekeeping.

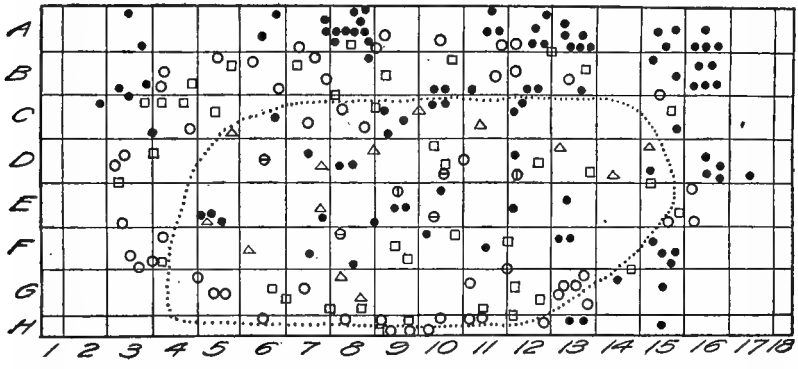

FIG. 6.—Distribution of cells containing infected sugar sirup and subsequent spread of the disease in a comb taken from colony H. . . . . Area covered by brood at time of infection, mostly unsealed. • Location of cells containing infected colored sugar sirup on July 3, 1918. ⊙ First positive diseased larvæ noted, 2 on July 8. ⊖ Number of new diseased larvæ (4) on July 10. △ Number of diseased larvæ (13) on July 12. □ Number of diseased larvæ (39) on July 16. ○ Number of diseased larvæ (52) on July 19.

DISTRIBUTION OF INTRODUCED INFECTED MATERIAL

An interesting experiment was carried out with sugar sirup, colored by a small amount of a harmless anilin dye, eosin, used as an indicator, which gave to the sirup a bright red color. The object of this experiment was to determine where the sirup, or, more important, where fresh nectar is first placed in the hive and combs. On May 27 two colonies were fed this colored sirup from above some time before the heavy honey flow from clover started. The results were striking, for in nearly every case the colored sirup was easily discernible in the cells and the greatest part of the sirup was located in quite a definite area. These colored cells were either scattered among the cells containing the larvæ or were placed in a ring of cells adjacent to the brood area toward the top of the comb, little being placed with the solid stores (fig. 6). Furthermore, for nearly 36 hours after the feeding practically all the young nurse bees showed a marked pinkish discoloration of the anterior end of their abdomens, denoting the dis-

tention of the honey stomachs by the retention of the colored sirup therein. About half the bees in the hives were discolored in this manner. After a day or so, however, this began to disappear. Also the number of cells showing the pink discoloration began to disappear. Evidently the sirup had been moved up, worked over, and mixed with other nectar or consumed.

Later, some time after the heavy honey flow had started, shortly after July 1, two more colonies were fed colored sirup, this time infected with diseased larvæ macerated therein.

In these cases the discolored abdomens were noted about as before, but the colored cells were less numerous and the color less striking. The location of the colored cells was similar to that in the former experiment; that is, mainly in the brood area or just contiguous to it and mostly above. The outside combs, containing considerable honey, showed scarcely any of the colored cells. This time these colored cells disappeared sooner, showing that the infected material must have been much diluted quite soon after being taken up from the feeding dishes.

Figure 6 shows the method of plotting the location of diseased larvæ in the combs and also the location of the cells containing the colored sugar sirup. As will be noted, a fairly large proportion of these cells are located within the area of brood at the time of feeding. It is interesting to note the tendency of diseased brood to form concentric circles, showing the two series of larvæ occurring between the dates noted. The spreading was from two cells at first to quite a large number at the last observation shown.

AGE AT WHICH LARVÆ ARE INFECTED

In previous observations it was constantly noted that the larvæ affected by European foulbrood were regularly at least 4 days old, the age at which the coiled larvæ completely fill the bottom of the cells. Occasionally a slightly younger and smaller larva would become diseased, but this was not the common occurrence. Furthermore, in the cases where the colored sirup was fed the bees, within 24 to 36 hours quite a number of larvæ averaging 4 days old could be seen discolored from having been fed this sirup, while it was noticeable that the younger larvæ under 3 days old never showed the discoloration. These colored larvæ were examined in a smear under a microscope, but the infecting organisms, being comparatively few in number, had not increased sufficiently at that time to be apparent.

The question now arises as to the age at which the larvæ first are fed nectar or infected material. There has been much controversy over the subject of composition and source of the larval food, but as yet no conclusive scientific evidence has been presented. Irrespective of the question whether the food at various stages originates from glands or is regurgitated, it is apparent from these observations that

there must be a difference between the food which larvæ younger than approximately 3 days old receive and that fed to older ones. Otherwise the younger larvæ would also show the pink coloration. Von Planta (9) by chemical analyses, of questionable exactness, however, makes a division in the feeding of the larvæ at the age of 4 days, at which time the high protein and low sugar content change to lower protein and higher sugar content. These analyses would tend to coincide with the above observations, only it is probable that the change begins earlier.

Additional data upon this subject are recorded in Tables II and III, although the observations were primarily for another purpose. In order to obtain further information relating to a possible difference in resistance to disease between Italian and hybrid bees, a careful record was made of the time when eggs were first noted in empty combs after the infection of the colony and when larvæ first showed disease thereafter. In the case of comb Special No. 2, the eggs were laid by an Italian queen in a healthy colony and then placed in a diseased colony. Colonies F, A, and I were of Italian stock while colonies G, H, and J were hybrid. In the recurrence of disease all were given new Italian queens. As has been mentioned before, as soon as the bees of the new Italian queens emerged in sufficient numbers the disease disappeared.

TABLE II

THE FIRST APPEARANCE OF DISEASE IN COMBS IN WHICH EGGS WERE LAID AFTER THE COLONY WAS INFECTED

Colony and comb No.	Number of days after eggs were first noted in comb.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
G 4										×				
G 6							×							
G 7						×								
G 8							×							
F 3							×							
F 4								×						
F 7						×								
H 3									×					
H 5									×					
A 2											×			
A 4											×			
A 5						×								
A 7								×						
A 8											×			
Spec. 2						×								
I 3									×					

RECURRENCE OF DISEASE AFTER EGGS OF A NEW QUEEN WERE FIRST NOTED IN THE COMBS

J 2														×
J 3														×
J 4							×							
J 5								×						
J 6a							×							
J 6b											×			
J 7										×				
J 8							×							
F 3									×					
F 4									×					
F 7									×					

TABLE III.—*Average time, under various conditions, in which disease becomes apparent in a colony after infection with European foulbrood. (Averages taken from Table II)*

	Before the heavy honey flow.
	Days.
Colony G, hybrid	7½
Colony F, Italian	7
Average of these two	7¼
	During the heavy honey flow.
	Days.
Colony H, hybrid	9
Colony A, Italian	8⅝
Colony I, Italian	9
Average of these three	8 11/12
	Recurrence of disease, after treatment, during honey flow.
	Days.
Colony J, hybrid originally	9½
Colony F, Italian originally	9
Average of these two	9⅛

The data shown, particularly in Table III, tend to disprove the theory that Italian bees have a natural immunity or resistance. If a larger number of observations could have been made, the variation would have appeared less. The effect of the honey flow is evident, however.

When it is a question of the age at which the larvæ are fed material that contains infection, these figures are significant. In the life history of the bee, 3 days are spent in the egg and from 5 to 6 days as larva before capping, making a period of 9 days in all. After 3 days in the egg and after having been fed predigested food for 3 days, with the additional 24 to 48 hour period of incubation, as was observed earlier in this paper, the larva ought to show disease from the fourth to the fifth day after hatching, or the seventh to eighth day of its existence, if Von Planta's assumption is correct. From actual observation this was found to be true and from observation of the averages in Table III it is seen that the first appearance of disease occurs between the seventh and ninth days, varying with the conditions of the honey flow.

Referring to Dr. Miller's theories, it is hard to believe that there is not plenty of highly infectious material left in the colony after a 5 or 6 day period of queenlessness. Aside from actual observations of moist, yellow, melting larvæ present more than 6 days after the

queen has been removed, the juices of which the workers sucked up with avidity, the final eggs laid will be just at the stage where the disease first appears; that is, 3 to 4 days after hatching, at the end of a 6-day period. Furthermore, even though the nurse bees do not feed to healthy larvæ the material that is taken up in cleaning out the cells in varying stages of decomposition, infection, even from scales, may be carried on the feet, mouth parts, and tongue, particularly, as was definitely shown with colony H, since these scales are infectious. The period of queenlessness and the consequent house cleaning are absolutely dependent on the strength of the colony. A strong colony cleans up rapidly, particularly after the introduction of the new queen in a cage plugged with candy. A weak colony, on the other hand, has not sufficient bees to clean even after complete introduction of a queen, and the disease soon appears again. Under average conditions, therefore, it would appear unsafe to allow less than a 10-day period of queenlessness in treatment of European foulbrood.

MICROSCOPICAL BACTERIOLOGICAL OBSERVATIONS

A large number of microscopic examinations were made of larvæ under various conditions for the positive presence of the characteristic groups of *Bacillus pluton*. These examinations were made mainly as a check on the gross observations of the first appearance of the disease. Cover glass smears were made of crushed larvæ, stained with carbol fuchsin and mounted in Canada balsam. These examinations were made at regular intervals after the colonies were infected, larvæ of all ages being examined.

It was found in the smears of those larvæ showing the first slightly abnormal symptoms that *Bacillus pluton* was the only organism present. This substantiates White's (12) observations that before the disease could be detected by gross examination, by a histological study of sections of larvæ during the period of incubation it was demonstrated that " in the production of the disease *Bacillus pluton* was the first invader of the healthy larvæ."

As the disease advanced in the various colonies, observations were made of larvæ in various stages of decomposition. The bacterial content was found to vary with the change of appearance of the larvæ during decomposition. The presence of these secondary invaders easily explains the atypical appearance of certain types of European foulbrood that heretofore have been very confusing to the beekeeper.

For a short time after the death of the larva, the color remains a moist, creamy-grayish yellow. This is during the period when *Bacillus pluton* and such occasional secondary invaders as *Streptococcus apis* or *Bacterium eurydice* and other organisms, which do not form

spores, are predominant as described by White (12) and McCray (4). Soon the putrefactive spore-forming organisms increase in number, *Bacillus alvei*[1] being the one most commonly found. This is seen particularly in the case of the more mature larvæ, which when dying extend more or less irregularly in the cells, becoming the grayish brown slimy masses which develop into the dark brown granular rubbery scales. This fact has been observed for a long time in the many samples which have been received for diagnosis. A partial description of these scales and of the presence of *Bacillus alvei* in them is given by McCray and White (5), but the experimental observations described in this paper added to diagnostic observations show that this condition is generally much more pronounced and common than described by these writers from laboratory observations. The rapid increase and peculiar process of decomposition of *Bacillus alvei*, after the death of the larva, often to the exclusion of all other organisms, accounts for this abnormal appearance. In the case of American foulbrood, almost never is any other organism found associated with the disease but *Bacillus larvae*, the cause of the disease. This accounts for the constancy of the symptoms as compared with the variation of symptoms in European foulbrood where there may be several secondary invaders.

Furthermore, in making the smears of the diseased larvæ upon cover glasses, the peculiar whitish saclike extrusion of the larval intestines was often noticed on crushing the larvæ preparatory to smearing, which White (10) describes as a gross diagnostic character. When this sac was removed and smeared separately, it was always found to be heavily loaded with *Bacillus pluton*. Therefore it is safe to assume that the intestinal tract is the primary focus of infection, while the secondary putrefaction takes place mostly in the body tissues of the dead larva.

Coincident with the microscopic examination of larvæ, several examinations were made of the contents of the ventriculus, rectum, and in a few cases of the honey stomach and mouth parts of bees. These bees were presumably nurse bees taken from diseased combs, some in the very act of sucking up the juices of dead diseased larvæ. Although insufficient observations were made to give conclusive evidence, some interesting information was obtained.

As may be seen from Table IV, the number of cases where *Bacillus pluton* or other organisms associated with infectious material were found in the intestinal contents is not very large. However, of more

[1] *Bacillus alvei* originally was supposed to be the primary cause of European foulbrood, but has been proved by White and others to be only a common secondary invader. *Bacillus alvei* has purely putrefactive functions. From its cultural and biochemical characteristics, *Bacillus alvei* apparently belongs to the common *Bacillus subtilis* (hay bacillus) group of spore-forming organisms, all having mainly putrefactive functions.

importance probably, *Bacillus pluton* was found in a smear made from the mouth parts of a nurse bee and also in the contents of the honey stomach of another. If these observations had been carried out systematically, instead of only casually, it is expected that much more positive data might have been obtained along these lines, owing to what is known already of the habits of house-cleaning bees working on diseased material.

TABLE IV.—*The results of the microscopic bacterial examination of the contents of the intestinal tracts of nurse bees taken from diseased colonies*

Microscopic findings.	G.	F.	H.	A.	I.	J.	K.	Total.
Positive *Bacillus pluton*	1	2	2	.....	.....	.....	4	9
Negative *Bacillus pluton*	11	24	18	17	12	6	9	97
Bacillus alvei or doubtful *Bacillus pluton*	.....	3	1	.....	.....	7	.....	11

SUMMARY AND CONCLUSIONS

In arriving at the following conclusions an effort has been made to state them in a manner which will indicate the substantiation of previous observations made both in the laboratory and in the apiary. It may be noted that many of these conclusions are similar to some of the statements made in Farmers' Bulletin 975 in the summary of facts which apiary practice has brought out.

1. European foulbrood is an infectious disease. *Bacillus pluton* was found to be the primary invader, appearing in the intestinal tract of larvæ before death, contemporary with the first slightly apparent symptoms.

2. The variation in the appearance of the diseased larvæ after death is due to the presence or absence of secondary invaders.

3. The period of incubation for European foulbrood was found to be from 36 to 48 hours, although the gross symptoms usually do not become apparent in less than 3 or 4 days, varying with conditions of honey flow and strength of colony.

4. It has been noted in apiary practice that the first brood of the year usually escapes with little loss. During the first 5 to 7 days the spread of the disease in the colony after infection is slow, after which the increase is rapid under favorable conditions. The critical time, therefore, to detect the disease and start treatment is early in its course, thus making conditions unfavorable.

5. The evidence tends to confirm the theory that one of the ways the disease is spread in the colony is by the house-cleaning bees, and from colony to colony by their drifting. It is quite probable that the infective organisms are carried on the mouth parts and pedal appendages. The question of infection from intestinal contents or from

the source of larval food at various stages needs further substantiation.

6. Irrespective of strength of colony, the Italian bees were found to resist infection much better than hybrids and showed more ability to overcome the disease.

7. This apparent resistance of the Italian bees was observed to be largely due to the more vigorous house-cleaning characteristics rather than to a natural resistance or immunity to the disease. There was very little difference in the apparent period of incubation between the Italian and hybrid colonies, possibly a slight difference in favor of the Italians. Furthermore, it was noted that often there may be a slight recurrence of disease in the brood of the new Italian queen until a sufficient number of her bees have emerged to eliminate the infection by house cleaning. Apparently, infection is not always entirely removed by a period of queenlessness.

8. As a rule, requeening is necessary in the treatment of European foulbrood, except possibly in the strongest Italian colonies, which show only slight infection. Where a considerable quantity of disease is present, sufficient to require treatment, it was found unsafe to use a period of less than 10 days' queenlessness, due to the infectious condition of the diseased material remaining and the accompanying behavior of the colony.

9. The stronger the colony in Italian bees, the more rapid was the recovery.

10. A heavy honey flow tends to prevent infection from gaining a foothold. It also tends to eliminate the disease if present before the start of the heavy honey flow. This was found to be due to the effect of dilution on the infection because of the influx and direct feeding of the fresh nectar to the larvæ.

11. European foulbrood is a disease of weak colonies. It was found to be difficult effectually to infect any but the very weak colonies during the heavy honey flow. Therefore, colonies kept strong up to the time of the honey flow run very little danger of contracting European foulbrood. This and others of the facts observed are in exact harmony with facts already observed in apiary practice.

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[1] "Black brood" is an old name for European foulbrood.

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U. S. DEPARTMENT OF AGRICULTURE.

FARMERS' BULLETIN 442.

THE TREATMENT OF BEE DISEASES.

BY

E. F. PHILLIPS, PH. D.,
In Charge of Bee Culture, Bureau of Entomology.

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1911.

442

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Entomology,
Washington, D. C., February 24, 1911.

Sir: I have the honor to transmit herewith a manuscript entitled "The Treatment of Bee Diseases," by E. F. Phillips, Ph. D., in charge of bee culture in this bureau. In the preparation of this paper, which is intended to supersede Circular 79, of this bureau, the aim has been to give briefly the information needed by the beekeeper who has disease in his apiary. No discussion of the cause or distribution of these diseases has been included. I recommend the publication of this paper as a Farmers' Bulletin.

Respectfully,

L. O. Howard,
Entomologist and Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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THE TREATMENT OF BEE DISEASES.

INTRODUCTION.

The diseases which attack the honey bee may be divided into two classes, namely, those affecting the brood and those to which the adult bees are subject. The diseases of adult bees have not been investigated sufficiently to make it possible at the present time to recommend methods for their treatment. In the present bulletin, therefore, only a brief statement concerning these diseases will be made, mainly for the purpose of indicating the present state of knowledge on these subjects. Concerning the diseases of the brood more is known, and this is particularly fortunate since they are far more destructive in American apiaries than are the diseases of the adult bees.

The causes of bee diseases will not be discussed here. For information on this phase of the subject the reader is referred to other publications of the Bureau of Entomology, which are listed at the end of this bulletin. The aim of this bulletin is to give information that can be used by the practical beekeeper in combating bee diseases.

THE BROOD DISEASES OF BEES.

The brood diseases of the honey bee are already widely distributed in the United States and seem to be spreading rather rapidly. The loss to the beekeepers of the country, owing to the actual death of colonies by disease, is estimated conservatively at $1,000,000 annually. This does not include the loss of crops, resulting from the destruction of colonies, or the discouragement to the beekeeper which often causes him to give up the business. A considerable part of this loss is due to the indifference of the beekeepers to these diseases and a lack of knowledge concerning them.

It frequently happens that colonies in an apiary become infected before the owner realizes that disease is present. He may erroneously attribute the losses observed to some other cause. In this way the disease gets a start which makes eradication difficult when once the cause of the loss has been discovered. In view of the widespread distribution of these diseases, it is most desirable that all beekeepers learn to distinguish the diseases when they appear and to know how to keep them under control.

It is often a matter of surprise to beekeepers to learn that bees are subject to disease. The most frequent source of confusion is the

placing of the blame for loss of colonies on some cause other than disease. The poorer class of beekeepers attribute their losses simply to "bad luck," but even well-informed beekeepers err in this matter.

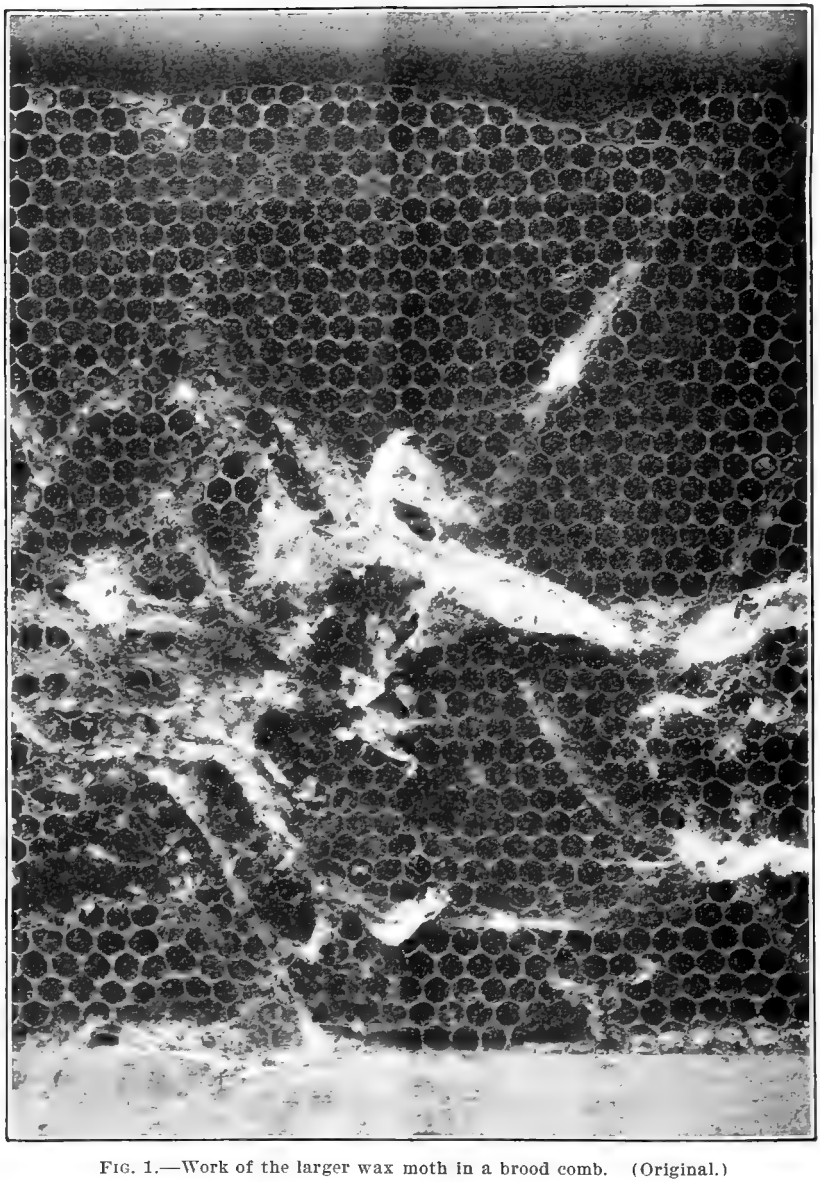


FIG. 1.—Work of the larger wax moth in a brood comb. (Original.)

The wax moths (see fig. 1) are most frequently blamed for the death of colonies, whereas they do no damage to strong, healthy colonies, properly cared for, but enter only when the colony is weakened by queenlessness, lack of stores, disease, or some other cause. In the

majority of the reports of wax-moth depredations received by this department which can be investigated it is found that the trouble is actually an outbreak of a brood disease.

The spraying of fruit trees while in bloom is possibly injurious to bees, and there exists among beekeepers a strong feeling against the practice. Since no entomologist now recommends that fruit trees be sprayed during the blooming period, this is probably rarely done by progressive fruit growers. However, it is frequently reported by beekeepers that they are losing bees by poisoning due to spraying. A number of cases of the death of colonies, reported as caused by poisoning due to spraying while trees were in bloom, have been found to be in reality outbreaks of European foul brood, which is particularly prevalent in the spring and early summer.

Other circumstances to which is often attributed the death of brood or of the colony are chilling, fumes from coke ovens, and malicious poisoning. The wise attitude on the part of the beekeeper is first to suspect diseases as being the cause of any losses which he may sustain, and to be sure that there is no infectious disease present before looking elsewhere for a cause.

NATURE OF THE DISEASES.

There are two recognized infectious diseases of the brood of bees, now known as American foul brood and European foul brood. Both diseases weaken colonies by reducing the number of emerging bees needed to replace the old adult bees which die from natural or other causes. In neither case are adult bees affected, so far as known. The means used by the beekeeper in deciding which disease is present is the difference in the appearance of the larvæ dead of the two diseases. That the diseases are entirely distinct can not now be doubted, since they show certain differences in the age of the larvæ affected, in their response to treatment, and in the appearance of the dead larvæ. This is made still more certain by a study of the bacteria present in the dead larvæ. Reports are sometimes received that a colony is infected with both diseases at the same time. While this is possible, it is not by any means the rule, and such cases are usually not authentically reported. There is no evidence that chilled or starved brood develops into an infectious disease or that dead brood favors the development of a disease.

NAMES OF THE DISEASES.

The names American foul brood and European foul brood were applied to these diseases by the Bureau of Entomology, of this department, to clear up the confusion in names which formerly existed. By retaining the words "foul brood" in each name the disease-inspection laws then in force could be interpreted as applying to

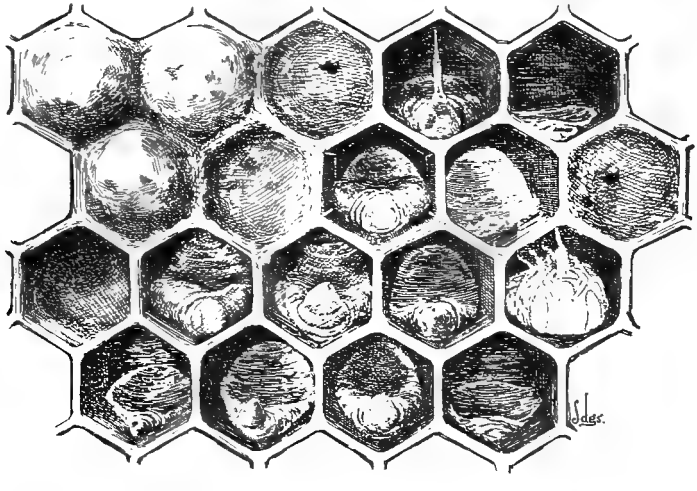
both diseases. These names were in no way intended to designate geographical distribution, since both diseases did exist and do now exist in both Europe and America, but were chosen primarily because they were convenient and easily remembered names. Their only significance is in indicating where the diseases were first seriously investigated. It was particularly desirable to change the name of the disease now known as European foul brood, since "black brood" entirely fails to be descriptive and is misleading.

SYMPTOMS.

The presence of a particular disease in a colony of bees can be ascertained most reliably by a bacteriological examination, since the symptoms are somewhat variable. It is possible, however, to describe the usual manifestations of the diseases, and the usual differences, so that the beekeeper can in most cases tell which disease is present.

American Foul Brood.

American foul brood is frequently called simply "foul brood." It usually shows itself in the larva just about the time that the larva fills the cell and after it has ceased feeding and has begun pupation.



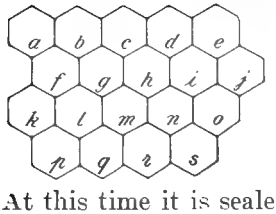


FIG. 2.—American foul brood: *a*, *b*, *f*, normal sealed cells; *c*, *j*, sunken cappings, showing perforations; *g*, sunken capping not perforated; *h*, *l*, *m*, *n*, *q*, *r*, larvæ affected by disease; *e*, *i*, *p*, *s*, scales formed from dried-down larvæ; *d*, *o*, pupæ affected by disease. Three times natural size. (Original.)

At this time it is sealed over in the comb (fig. 2, *a*, *b*, *f*). The first indication of the infection is a slight brownish discoloration and the loss of the well-rounded appearance of the normal larva (fig. 2, *l*). At this stage the disease is not usually recognized by the bee-

keeper. The larva gradually sinks down in the cell and becomes darker in color (fig. 2, *h*, *m*), and the posterior end lies against the bottom of the cell. Frequently the segmentation of the larva is clearly marked. By the time it has partially dried down and has became quite dark brown (coffee colored) the most typical characteristic of this disease manifests itself. If a match stick or tooth-pick is inserted into the decaying mass and withdrawn the larval remains adhere to it and are drawn out in a thread (fig. 3), which sometimes extends for several inches before breaking. This ropiness is the chief characteristic used by the beekeeper in diagnosing this disease. The larva continues to dry down and gradually loses its ropiness until it finally becomes merely a

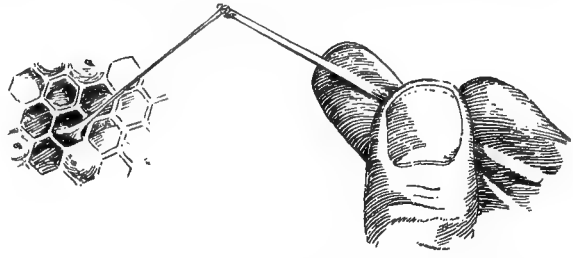


FIG. 3.—The ropiness of American foul brood. (Original.)

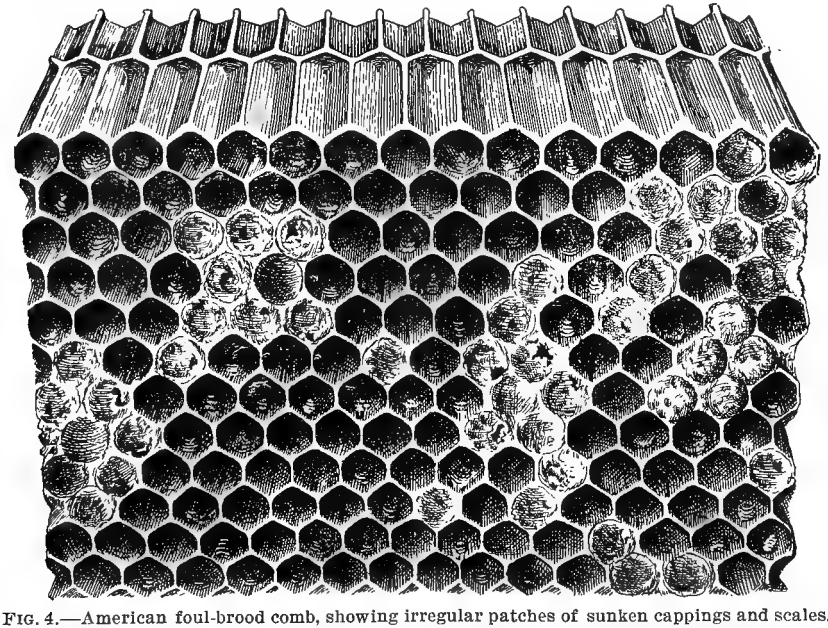


FIG. 4.—American foul-brood comb, showing irregular patches of sunken cappings and scales. The position of the comb indicates the best way to view the scales. (Original.)

scale on the lower side wall and base of the cell (fig. 2, *e*, *p*, *s*). The scale formed by the dried-down larva adheres tightly to the cell and can be removed with difficulty from the cell wall. The scales can best be observed when the comb is held with the top inclined toward the observer so that a bright light strikes the lower side wall (fig. 4).

A very characteristic and usually penetrating odor is often noticeable in the decaying larvæ. This can perhaps best be likened to the odor of heated glue.

The majority of the larvæ which die of this disease are attacked after being sealed in the cells. The cappings are often entirely removed by the bees, but when they are left they usually become sunken (fig. 2, *g*, *c*, *j*) and frequently perforated (fig. 2, *c*, *j*). As the healthy brood emerges the comb shows the scattered sunken cappings covering dead larvæ (fig. 4), giving it a characteristic appearance.

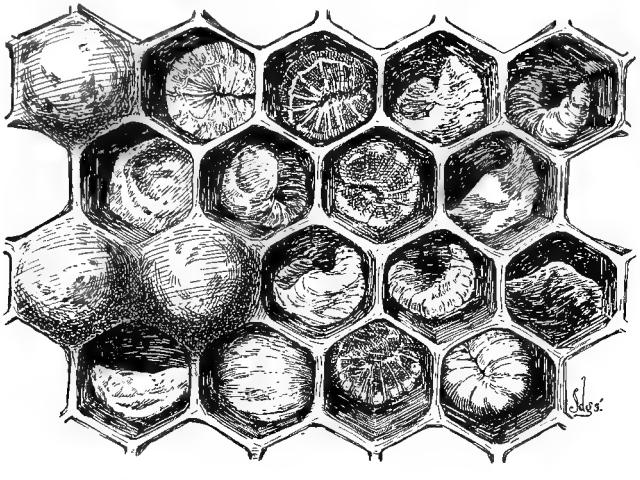
Pupæ also may die of this disease, in which case they, too, dry down (fig. 2, *o*, *d*), become ropy, and have the characteristic odor and color. The tongue frequently adheres to the upper side wall and often remains there even after the pupa has dried down to a scale. Younger unsealed larvæ are sometimes affected. Usually the disease attacks only worker brood, but occasional cases are found in which queen and drone brood are diseased. It is not certain that race of bees, season, or climate have any effect on the virulence of this disease, except that in warmer climates, where the breeding season is prolonged, the rapidity of devastation is more marked.

European Foul Brood.

European foul brood was formerly called "black brood" or "New York bee disease." The name "black brood" was a poor one, for the color of the dead brood is rarely black or even very dark brown. European foul brood usually attacks the larva at an earlier stage of its development than American foul brood and while it is still curled up at the base of the cell (fig. 5, *r*). A small percentage of larvæ dies after capping, but sometimes quite young larvæ are attacked (fig. 5, *e*, *m*). Sunken and perforated cappings are sometimes observed just as in American foul brood (fig. 2, *c*, *g*, *j*). The earliest indication of the disease is a slight yellow or gray discoloration and uneasy movement of the larva in the cell. The larva loses its well-rounded, opaque appearance and becomes slightly translucent, so that the tracheæ may become prominent (fig. 5, *b*), giving the larvæ a clearly segmented appearance. The larva is usually flattened against the base of the cell, but may turn so that the ends of the larva are to the rear of the cell (fig. 5, *p*), or may fall away from the base (fig. 5, *e*, *g*, *l*). Later the color changes to a decided yellow or gray and the translucency is lost (fig. 5, *q*, *h*). The yellow color may be taken as the chief characteristic of this disease. The dead larva appears as a moist, somewhat collapsed mass, giving the appearance of being melted. When the remains have become almost dry (fig. 5, *c*) the tracheæ sometimes become conspicuous again, this time by retaining their shape, while the rest of the body content dries around them. Finally all that is left of the larva is a grayish-brown scale against

the base of the cell (fig. 5, *f*, *h*), or a shapeless mass on the lower side wall if the larva did not retain its normal position (fig. 5, *n*, *o*). Very few scales are black. The scales are not adhesive, but are easily removed, and the bees carry out a great many in their efforts to clean house.

Decaying larvæ which have died of this disease are usually not ropy as in American foul brood, but a slight ropiness is sometimes observed. There is usually little odor in European foul brood, but sometimes a sour odor is present, which reminds one of yeast fermentation. This disease attacks drone and queen larvæ[1] almost as quickly as those of the workers.



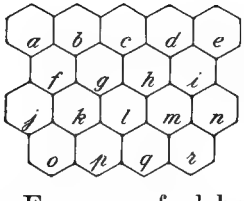


FIG. 5.—European foul brood: *a*, *j*, *k*, normal sealed cells; *b*, *c*, *d*, *e*, *g*, *i*, *l*, *m*, *p*, *q*, larvæ affected by disease; *r*, normal larva at age attacked by disease; *f*, *h*, *n*, *o*, dried-down larvæ or scales. Three times natural size. (Original.)

European foul brood is more destructive during the spring and early summer than at other times, often entirely disappearing during late summer and autumn, or during a heavy honey flow. Italian bees seem to be better able to resist the ravages of this disease than any other race. The disease at times spreads with startling rapidity and is most destructive. Where it is prevalent a considerably larger percentage of colonies is affected than is usual for American foul brood. This disease is very variable in its symptoms and other manifestations and is often a puzzle to the beekeeper.

[1] The tendency of this disease to attack queen larvæ is a serious drawback in treatment. Frequently the bees of a diseased colony attempt to supersede their queen, but the larvæ in the queen cells often die, leaving the colony hopelessly queenless. The colony is thus depleted very rapidly.

The So-Called "Pickle Brood."

In addition to the two infectious diseases just described, brood dead from other causes is often observed. The most common disease of this kind is what is known among beekeepers as "pickle brood." This name is seemingly applied to a great many different appearances and nothing is known of the cause or methods of spread. The most typical form kills the larva when it has extended itself in the cell. The larva usually lies on its back with the head turned upward. The color varies, but is frequently light yellow or brown, and the head is often almost black. The body is swollen and the contents watery, and the head may be quite hard. There is no ropiness. In case the larvæ are sealed before dying the cappings are usually normal. The name usually applied to this condition was unwisely chosen, and for the present and until more is known concerning the disease it is spoken of as the "so-called pickle brood."

This trouble does not appear to be infectious and is usually not serious, except that in the aggregate it may cause loss by weakening colonies. No treatment is necessary, as the trouble usually soon disappears. The most serious aspect of this disease is that it is often mistaken for one of the infectious diseases, and the colony is needlessly treated.

Brood dead of other causes.

Many different external factors may cause brood to die. If brood is killed by chilling in the spring or fall, or by overheating in extremely hot weather, or in shipping colonies of bees, or by starvation, the loss is often mistakenly attributed to an infectious disease. Such dead brood is soon removed by the bees. When the cause is removed the trouble then soon disappears. When a considerable quantity of brood is killed a disagreeable odor is usually present.

"Bald-headed brood."

It sometimes happens that unsealed or only partially sealed pupæ, known as "bald-headed brood," are observed in the hive, and frequently beginners mistake such a condition for disease. The partially built capping is often mistaken for the punctured capping of American foul brood. If, on examination, the pupæ are normal no fear need be entertained.

METHODS OF SPREAD.

Both American foul brood and European foul brood spread from colony to colony and from apiary to apiary in much the same way. The common means of carrying the virus is in honey which has become contaminated. The disease may be carried when bees rob a hive in which a colony has died of disease or may be transmitted by

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	AMERICAN.	EUROPEAN.	SAC.
Cause	B. Larvae	B. Pluton	A filterable virus
Age of	Sealed larvae largely	Unsealed largely	Sealed largely
Symptoms	Cappings sunken perforated	No cappings	Cappings normal
Larvae	Outstretched, gluey, viscous.	Sunken, slumped, pastey	Boat shaped and distended, watery
Colour	Dark brown	Creamy to gray or black	Gray to dark brown when punctured hangs like a sack
Scale	Thin, extended, adhesive 		 black head
Ropey	Ropey	Irregular, free sometimes	Similar to American

Oder	Glue pot, strong	Sour	No oder
Speed	Slow two seasons	Rapid a few weeks here today gone tomorrow	Spring seldom serious
Treatment	Shaking	Dequeen and requeen in ten to twenty days	Requeen

the use of honey from diseased colonies for feeding bees. It is not always necessary that bees be intentionally fed for them to get disease from contaminated honey. Discarded honey receptacles which have contained honey from a contaminated colony, if not thoroughly cleaned, may contain enough honey to carry disease to a healthy apiary. This may occur in the vicinity of bakeries or confectionery shops, or may even occur when empty honey bottles are thrown out from private houses. It is also possible to introduce disease into a colony in introducing queen bees purchased from a distance, probably due to the use of contaminated honey in making the candy to supply the queen cages.

Precautionary Measures.

In combating diseases it is much better to prevent disease from getting a foothold than it is to eradicate it after it has begun its work. All beekeepers, wherever located, should practice the following precautionary measures:

(1) If a colony becomes weak from any cause, or if disease is suspected, contract the entrance to prevent robbing, and if robbing is imminent close the entrance entirely.

(2) *Never feed honey purchased on the open market.* In case of doubt as to the source of honey feed sugar sirup.

(3) If within the range of possibility, see that no honey that comes from diseased apiaries is sold in the neighborhood. This may sometimes be accomplished by cultivating the home market so that there will be no incentive for bringing in other honey.

(4) In introducing purchased queens, transfer them to clean cages provided with candy known to be free from contamination, and destroy the old cage, candy, and accompanying workers. Of course, if it is certain that the queen comes from a healthy apiary this is not necessary.

(5) Colonies of bees should never be purchased unless it is certain that they are free from disease.

(6) The purchase of old combs or second-hand supplies is dangerous, unless it is certain that they came from healthy apiaries.

TREATMENT FOR BOTH INFECTIOUS DISEASES.

The treatment of an infectious bee disease consists primarily in the elimination or removal of the cause of the disease. It is definitely known that American foul brood is caused by a bacillus named *Bacillus larvæ.* In treating this disease, therefore, the aim of the manipulation is to remove or destroy all of the bacteria of this species. It should be remembered that the effort is not to save the larvæ that are already dead or dying, but to stop the further de-

vastation of the disease by removing all material capable of transmitting the cause of the trouble.

The cause of European foul brood is not definitely known, but the same principles of treatment doubtless apply in this disease also. In all of the operations great pains should be taken not to spread the disease through carelessness. After handling a diseased colony the hands of the operator should be washed with water to remove any honey that may be on them. It does not pay to treat colonies that are considerably weakened by disease. In case there are several such colonies they should be united to form strong, vigorous colonies before or during treatment.

In discussing treatment it is assumed that hives with movable frames are in use. Box hives are a menace in regions where disease is present. These may be treated for disease by drumming the colony into another box and then hiving it like a swarm in a hive, but box hives are not profitable and are especially to be condemned where disease is present on account of the difficulty in inspecting and treating.

Shaking Treatment.

The shaking treatment consists essentially in the removal of all infected material from the colony, and in compelling the colony to take a fresh start by building new combs and gathering fresh stores. This is done by shaking the bees from the old combs into a clean hive on clean frames.

Time of treatment.—The shaking treatment should be given during a flow of honey, so that other bees in the apiary will not be inclined to rob. If this is not possible the operation may be performed under a tent made of mosquito netting. The best time is during the middle of a clear day when a large number of bees are in the field. It is sometimes recommended that shaking be done in the evening, but this is impossible if many colonies are to be treated. The colony can be handled more quickly when the field force is out of the hive.

Preparation.—All implements that will be needed, such as queen and drone trap, hive tool, and lighted smoker, should be in readiness before the operation is begun. A complete clean hive with frames is provided, as well as a tightly closed hive body in which to put the contaminated combs after shaking. An extra hive cover or some similar apparatus should be provided to serve as a runway for the bees as they enter the new hive. The new frames should contain strips of comb foundation from one-fourth to 1 inch wide. Full sheets are not desirable, and if combs built on full sheets of foundation are desired they may be built later.

Operation.—The old hive containing the diseased colony (fig. 6, *A*) is now lifted to one side out of the flight of returning field bees and the clean hive (*B*) set exactly in its place. The cover (*G*) is

now taken off and a few frames (*E*) removed from the center of the hive. If unspaced frames are used, those remaining in the hive should be pushed tightly to either side of the hive, thus making a barrier beyond which the bees can not crawl as they move to the top of the hive after shaking. This largely prevents them from getting on the outside of the hive. If self-spacing frames are used, a couple of thin boards laid on the top bars on either side will accomplish the same result. The runway (*D*) is put in place in front of the entrance. The old hive is now opened for the first time. The frames are removed one at a time, lowered part way into the new hive, and with a quick downward shake the bees are dislodged. The frames are then put into the extra hive body (*C*) and immediately covered to prevent robbing. After all the frames are shaken the bees remaining on the sides of the old hive (*A*) are shaken out.

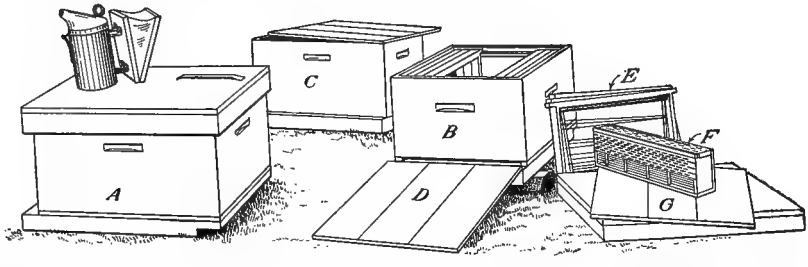

FIG. 6.—Apparatus for the shaking treatment: *A*, Hive containing diseased colony (formerly in position of *B*); *B*, clean hive; *C*, empty hive to receive combs after shaking; *D*, hive cover used as runway; *E*, frames removed from *B* to give room for shaking; *F*, queen and drone trap; *G*, cover for clean hive, *B*. (Original.)

If honey is coming in freely, so that thin honey is shaken out of the combs, cover the runway (*D*) with newspapers and shake the bees in front of the new hive (*B*), leaving all frames in place and the cover on. After the operation the soiled newspapers should be destroyed. In shaking in front of the entrance the first one or two frames should be so shaken that the bees are thrown against the entrance, where they can locate the hive quickly. They then fan their wings and the others follow them into the hive. If this is not done the bees may wander about and get under the hive or in some other undesirable place.

After the bees are mostly in the new hive a queen and drone trap (*F*) or a strip of perforated zinc is placed over the entrance to prevent the colony from deserting the hive. The queen can not pass through the openings in the perforated zinc and the workers will not leave without her. By the time that new combs are built and new brood is ready to be fed, any contaminated honey carried by the bees into their new hive will have been consumed and the

disease will rarely reappear. If it should, a repetition of the treatment will be necessary.

Saving the healthy brood.—The old combs are now quickly removed. If several colonies are being treated at one time it may pay to stack several hive bodies containing contaminated combs over a weak diseased colony to allow most of the healthy brood to emerge, thereby strengthening the weak colony. After 10 or 12 days this colony is treated in turn and all the combs rendered into wax. If only one or two colonies in a large apiary are being treated it will not pay to do this.

Saving the wax.—Any but a very small apiary should have included in its equipment a wax press for removing wax from old combs. After the contaminated frames are taken to the honey house the combs should be kept carefully covered, so that no bees can reach them until the wax can be rendered. This should not be delayed very long or the combs may be ruined by wax moths. The slumgum or refuse remaining after the wax is removed should be burned. Contaminated combs should not be put into a solar wax extractor for fear of spreading the disease. The wax from contaminated combs may safely be used for the manufacture of comb foundation.

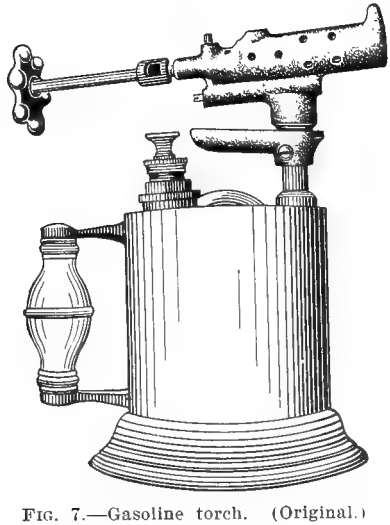

FIG. 7.—Gasoline torch. (Original.)

Cleaning the hive.—The hive which has contained the diseased colony should be thoroughly cleaned of all wax and honey, and it is desirable that it be carefully disinfected by burning out the inside with a gasoline blue-flame torch (fig. 7). If this piece of apparatus is not available several hive bodies may be piled together on a hive bottom and some gasoline or kerosene poured on the sides and on some straw or excelsior at the bottom. This is then ignited and after burning for a few seconds a close-fitting hive cover is placed on top of the pile to extinguish the flames. The inside of the hive bodies should be charred to a light brown. The careful cleaning and disinfection of frames always costs considerably more in labor than new frames would cost, but these also may be carefully cleaned and used again. Frames may be cleaned by boiling in water for about half an hour, but this frequently causes them to warp badly. The disinfection of hives and frames with chemicals is not recommended, as the ordinary strengths used are valueless for the purpose.

Disposal of the honey.—If there is a considerable quantity of honey in the contaminated combs it may be extracted. This honey is not safe to feed to bees without boiling, but it is absolutely safe for human consumption. If there is a comparatively small quantity it may be consumed in the beekeeper's family, care being taken that none of it is placed so that the bees can ever get it.

To put such honey on the market is contrary to law in some States. There is always danger that an emptied receptacle will be thrown out where bees can have access to it, thus causing a new outbreak of disease. It can be safely used for feeding to bees, provided it is diluted with at least an equal volume of water to prevent burning, and boiled in a closed vessel for not less than one-half hour, counting from the time that the diluted honey first boils vigorously. The honey will not be sterilized if it is heated in a vessel set inside of another containing boiling water. Boiled honey can not be sold as honey. It is good only as a food for bees, and even then should never be used for winter stores, as it will probably cause dysentery.

The second shake.—Some beekeepers prefer to shake the bees first onto frames containing strips of foundation as above described, and in four days to shake the colony a second time onto full sheets of foundation, destroying all comb built after the first treatment. This insures better combs than the use of strips of foundation, but is a severe drain on the strength of the colony. Since it is desirable to have combs built on full sheets, the best policy is to replace any irregular combs with full sheets of foundation or good combs later in the season.

The cost of shaking.—If the treatment just described is given at the beginning of a good honey flow, it is practically equivalent to artificial swarming and results in an actual increase in the surplus honey, especially in the case of comb-honey production. The wax rendered from the combs will sell for enough to pay for the foundation used if full sheets of foundation are employed. Since a colony so treated actually appears to work with greater vigor than a colony not so manipulated, the cost of treatment is small. If treatment must be given at some other time, so that the colony must be fed, the cost is materially increased. In feeding, it is best to use sugar sirup, or honey that is known to have come from healthy colonies.

Treatment with Bee Escape.

As a substitute for the shaking treatment just described, the bees may be removed from their old combs by means of a bee escape. The old hive is moved to one side and in its place is set a clean hive with clean frames and foundation. The queen is at once transferred to the new hive and the field bees fly there on their return from the

field. The infected hive is now placed on top of or close beside the clean hive and a bee escape placed over the entrance, so that the younger bees and those which later emerge from the cells may leave the contaminated hive but can not return. They therefore join the colony in the new hive. If desired, the infected hive may be placed above the clean hive and a tin tube about 1 inch in diameter placed from the old entrance so that the lower end is just above the open entrance of the new hive. The bees follow down this tube and on their return enter the new hive. When all of the healthy brood has emerged from the infected combs the old hive is removed. This treatment induces less excitement in the apiary and is preferred by many experienced beekeepers. Care should be taken that the old hive is absolutely tight to prevent robbing. The old hive and its contents of honey and wax are treated as indicated under the shaking treatment.

Fall Treatment.

If it is necessary to treat a colony so late in the fall that it would be impossible for the bees to prepare for winter, the treatment may be modified by shaking the bees onto combs entirely full of honey so that there is no place for any brood to be reared. This will usually be satisfactory only after brood rearing has entirely ceased. Unless a colony is quite strong it does not pay to treat in the fall, but it should be destroyed or united to another colony. In case a diseased colony dies outdoors in the winter there is danger that other bees may have opportunity to rob the hive before the beekeepers can close the entrance. In case bees are wintered in the cellar it is more advisable to risk wintering before treatment, for if the colony does die the hive will not be robbed.

Drugs.

Many European writers have in the past advocated the use of various drugs for feeding, in sugar sirup, to diseased colonies, or the fumigation of contaminated combs. In the case of American foul brood, of which the cause is known, it has been found that the drugs recommended are not of the slightest value and no time should be wasted in their use.

TREATMENT FOR EUROPEAN FOUL BROOD.

European foul brood is a very peculiar disease and its cause has not yet been satisfactorily determined. It is, therefore, impossible to discuss the treatment of this disease as definitely as that of American foul brood. From the experience of many careful beekeepers it is, however, possible to suggest some additional manipulations which may be tried by experienced beekeepers. The treatments given previously are strongly recommended for this disease.

Introduction of Italian Stock.

Since, as stated previously (p. 11), Italian bees seem to be better able to withstand European foul brood than are other races, it is recommended that apiaries in regions where this disease is prevalent be requeened with young, vigorous Italian queens of good stock. This should be done whether or not the shaking treatment is given.

Dequeening.

It has been found that the removal of the queen and the keeping of the colony queenless for a period often results in the disappearance of European foul brood. The length of time that this should be done is in dispute. Mr. E. W Alexander, who advocated this method,[1] recommended that the colony be kept queenless (by cutting out all queen cells at the end of 9 days) for a period of 20 days, at which time a cell containing a queen of Italian stock ready to emerge is to be given the colony. The young queen will thus begin to lay in about 27 days after the old queen has been removed, or in at least 3 days after the last of the drone brood has emerged. Other writers have advocated a shorter time.

The dequeening treatment is not always successful, and it is therefore recommended that care be exercised in trying it. Since there is a considerable percentage of successful results, this would indicate that there is an important principle involved. It should not be forgotten, however, that European foul brood often disappears in the late summer of its own accord if the case is not severe (p. 11), and it is probable that in many of the cases of dequeening reported as successful the disease would have disappeared without the treatment. This treatment is suggested only for the experienced beekeeper.

INSPECTION OF APIARIES.

Several States have passed laws providing for the inspection of apiaries for contagious disease and creating the office of apiary inspector. The men holding these offices are usually practical beekeepers, capable of giving excellent advice regarding disease, and it is desirable, when disease exists in a community, that the owners of apiaries take steps to learn who the inspector is and to notify him of the existence of disease. The Bureau of Entomology of this department can usually give information concerning the inspector and is always glad to be of service in bringing the beekeepers and inspectors in touch with one another.

Apiary inspection has proved beneficial to the beekeeping industry in spreading information concerning the nature, symptoms, and

[1] Alexander, E. W.—How to rid your apiary of black brood. Gleanings in Bee Culture, vol. 33, pp. 1125–1127, 1905.

treatment of the contagious diseases and particularly in compelling negligent and careless beekeepers to treat their diseased colonies. It is quite possible for the individual beekeeper to clean up his own apiary by following the directions given in this bulletin, but unless all of the beekeepers in the neighborhood do the same thing there will probably be a recurrence of the trouble due to infection from outside apiaries. It is therefore manifestly to the advantage of the beekeepers that they cooperate with the inspectors in the fight against diseases.

EXAMINATION OF SAMPLES OF DISEASED BROOD.

The Bureau of Entomology of this department is prepared to assist in the diagnosis of disease in cases where the beekeeper is unable to tell whether or not disease is present, or to determine which disease is in his apiary. Samples of brood comb about 5 inches square containing diseased or dead larvæ should be sent by mail in a strong wooden or tin box. The comb should not be wrapped in paper or cotton, but should be cut to fit the box closely. It is not possible to diagnose from empty combs, and no honey should be included in the sample, as it is valueless in diagnosis and will probably spoil the sample as well as other mail matter. The name of the sender must always appear on the package, and any available data should be sent in a separate letter. Never inclose a letter in the box with the sample.

THE DISEASES OF ADULT BEES.

The diseases affecting adult bees are but imperfectly known. At present four are known to beekeepers by name. Whether these are entirely distinct or whether under each name one or more diseases are included is not known. As stated in the introduction, these diseases have not been sufficiently investigated to give much help to the practical beekeeper.

DYSENTERY.

Dysentery affects bees only in the winter and is manifested by a distension of the abdomen, due to an accumulation of fecal matter in the intestine. When a day warm enough for flight occurs the bees fly from the hive to cleanse themselves, and the hive and surroundings are spotted with yellow excreta. After a good cleansing flight the trouble usually disappears, but if the bees are unable to fly they often die in great numbers. It is generally believed that dysentery is due to improper winter stores, the honey containing too high a percentage of indigestible matter. Honeydew honey almost always produces dysentery, while bees wintered on high-class honey or sugar sirup are not affected. From the wide experience of many bee-

keepers in this matter it is safe to assume that this explanation of the disease is the correct one, and consequently great care should be exercised that the colonies are provided with good stores for winter.

Recently it has been claimed that there are two types of dysentery, one form as above described and another form which is infectious. American beekeepers are not familiar with an infectious dysentery, and in practical manipulations it is necessary to consider only the type above described.

THE SO-CALLED PARALYSIS.

It is quite possible that under the name "paralysis" are included several distinct diseases. This is indicated by the variety of symptoms reported by beekeepers and the number of different seasons and conditions under which the disease is supposed to occur. The usual manifestation described is that the worker bees are seen crawling in front of the hive with their abdomens trembling. The abdomens are also frequently distended. The bees often climb grass blades and on attempting to fly from the top fall again to the ground. Frequently the bees so affected are almost hairless. The same trembling motion may often be observed on opening the hive. The colony is often depleted very rapidly. There is no evidence that the disease is infectious.

The cause of this peculiar trouble is unknown, and no remedy can be recommended. It is claimed by some writers that a salt-water spray applied to the combs or salt or sulphur sprinkled on the top bars or entrance is sometimes an effective remedy.

ISLE OF WIGHT DISEASE.

Recently a supposedly infectious disease of adult bees has decimated the bees on the Isle of Wight and is said to be spreading in England. It resembles somewhat the so-called paralysis. No treatment other than destruction to prevent the spread of the disease has been recommended. So far as is known no trouble of this kind has been experienced in America.

SPRING DWINDLING.

It sometimes happens that the adult bees in a colony die off in the spring more rapidly than they are replaced by emerging brood. This dwindling may be diminished somewhat by keeping the colony warm and by stimulative feeding, so that all of the energy of the old bees may be used to the best advantage. This condition is probably due to the fact that the colony goes into winter with too large a percentage of old worn-out bees. To prevent this, brood rearing should be continued as late as possible in the fall; if necessary, by stimulative feeding.

PUBLICATIONS OF THE DEPARTMENT OF AGRICULTURE ON BEE DISEASES.

There are several other publications of the Bureau of Entomology of this department which deal with bee diseases. They may be obtained on request to the Editor and Chief of the Division of Publications, Department of Agriculture, and are the following:

Circular No. 94, "The Cause of American Foul Brood." By G. F. White, Ph. D. 1907. 4 pp.

This publication contains a brief account of the investigations which demonstrated for the first time the cause of one of the brood diseases of bees, American foul brood.

Bulletin No. 70, "Report of the Meeting of Inspectors of Apiaries, San Antonio, Tex., November 12, 1906." 1907. 79 pp., 1 pl.

Contains an account of the history of bee-disease investigations, the relationship of bacteria to bee diseases, and a discussion of treatment by various inspectors of apiaries and other practical beekeepers who are familiar with diseases of bees.

Bulletin No. 75, Part II, "Wax Moths and American Foul Brood." By E. F. Phillips, Ph. D. 1907. Pp. 19–22, 3 pls.

An account of the behavior of the two species of wax moths on combs containing American foul brood, showing that moths do not clean up the disease-carrying scales.

Bulletin No. 75, Part III, "Bee Diseases in Massachusetts." By Burton N. Gates. 1908. Pp. 23–32, map.

An account of the distribution of the brood diseases of bees in the State, with brief directions for controlling them.

Bulletin No. 75, Part IV, "The Relation of the Etiology (Cause) of Bee Diseases to the Treatment." By G. F. White, Ph. D. 1908. Pp. 33–42.

The necessity for a knowledge of the causes of bee diseases before rational treatment is possible is pointed out. The present state of knowledge of the causes of disease is summarized.

Technical Series, No. 14, "The Bacteria of the Apiary, with Special Reference to Bee Diseases." By G. F. White, Ph. D. 1906. 50 pp.

A study of the bacteria present in both the healthy and the diseased colony, with special reference to the diseases of bees.

FARMERS' BULLETINS.

Bulletins in this list will be sent free, so long as the supply lasts, to any resident of the United States, on application to his **Senator, Representative, or Delegate in Congress,** or to the Secretary of Agriculture, Washington, D. C. *Because of the limited supply, applicants are urged to select only a few numbers, choosing those which are of special interest to them.* Residents of foreign countries should apply to the Superintendent of Documents, Government Printing Office, Washington, D. C., who has these bulletins for sale. Price 5 cents each to Canada, Cuba, and Mexico; 6 cents to other foreign countries. The bulletins entitled "Experiment Station Work" give briefly the results of experiments performed by the State experiment stations.

22. The Feeding of Farm Animals.
27. Flax for Seed and Fiber.
28. Weeds: And How to Kill Them.
30. Grape Diseases on the Pacific Coast.
32. Silos and Silage.
34. Meats: Composition and Cooking.
35. Potato Culture.
36. Cotton Seed and Its Products.
44. Commercial Fertilizers.
48. The Manuring of Cotton.
49. Sheep Feeding.
51. Standard Varieties of Chickens.
52. The Sugar Beet.
54. Some Common Birds.
55. The Dairy Herd.
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60. Methods of Curing Tobacco.
61. Asparagus Culture.
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64. Ducks and Geese.
65. Experiment Station Work—II.
69. Experiment Station Work—III.
73. Experiment Station Work—IV.
77. The Liming of Soils.
78. Experiment Station Work—V.
79. Experiment Station Work—VI.
81. Corn Culture in the South.
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83. Tobacco Soils.
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85. Fish as Food.
86. Thirty Poisonous Plants.
87. Experiment Station Work—VIII.
88. Alkali Lands.
91. Potato Diseases and Treatment.
92. Experiment Station Work—IX.
93. Sugar as Food.
96. Raising Sheep for Mutton.
97. Experiment Station Work—X.
99. Insect Enemies of Shade Trees.
101. Millets.
103. Experiment Station Work—XI.
104. Notes on Frost.
105. Experiment Station Work—XII.
106. Breeds of Dairy Cattle.
113. The Apple and How to Grow It.
114. Experiment Station Work—XIV.
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121. Beans, Peas, and Other Legumes as Food.
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128. Eggs and Their Uses as Food.
131. Household Tests for Detection of Oleomargarine and Renovated Butter.
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135. Sorghum Sirup Manufacture.
137. The Angora Goat.
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139. Emmer: A Grain for the Semiarid Regions.
140. Pineapple Growing.
142. Nutrition and Nutritive Value of Food.
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145. Carbon Bisulphid as an Insecticide.
149. Experiment Station Work—XX.
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152. Scabies of Cattle.
154. Home Fruit Garden: Preparation and Care.
156. How Insects Affect Health in Rural Districts.
156. The Home Vineyard.
157. The Propagation of Plants.
158. How to Build Small Irrigation Ditches.
162. Experiment Station Work—XXI.
164. Rape as a Forage Crop.
166. Cheese Making on the Farm.
167. Cassava.
169. Experiment Station Work—XXII.
170. Principles of Horse Feeding.
172. Scale Insects and Mites on Citrus Trees.
178. Primer of Forestry. Part I: The Forest.
174. Broom Corn.
175. Home Manufacture and Use of Unfermented Grape Juice.
176. Cranberry Culture.
177. Squab Raising.
178. Insects Injurious in Cranberry Culture.
179. Horseshoeing.
181. Pruning.
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225. Experiment Station Work—XXIX.
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260. Seed of Red Clover and Its Impurities.
262. Experiment Station Work—XXXVI.
263. Practical Information for Beginners in Irrigation.
264. The Brown-tail Moth and How to Control It.
266. Management of Soils to Conserve Moisture.
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269. Industrial Alcohol: Uses and Statistics.
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272. A Successful Hog and Seed-corn Farm.
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FARMERS' BULLETIN 975

THE CONTROL OF EUROPEAN FOULBROOD

E. F. PHILLIPS
Apiculturist

UNITED STATES
DEPARTMENT OF AGRICULTURE

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EUROPEAN FOULBROOD is a disease of the brood of bees which has caused great losses to American beekeepers. It was first recognized as a distinct disease in the United States by New York beekeepers in 1894, but it has probably been present in the United States for a long time.

It is important that the beekeeper know whether European or American foulbrood is in his apiary, for the two do not respond to the same treatment. In European foulbrood control the most important step is to prevent the entrance of the disease by keeping all colonies strong and by having all stock resistant to the disease. This can be done successfully even though the disease is in the neighborhood.

In case, through failure to take all precautions, the disease does enter, there are certain practices by which the disease can be readily eliminated, but all of these must be used with care.

The facts about the disease on which the preventive and remedial measures are based are discussed in this bulletin.

Contribution from the Bureau of Entomology

L. O. HOWARD, Chief

Washington, D. C.

Issued July, 1918
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THE CONTROL OF EUROPEAN FOULBROOD.

CONTENTS.

DIFFICULTIES OF CONTROL.

EUROPEAN FOULBROOD has caused much trouble in treatment and causes more anxiety among beekeepers than does American foulbrood. It is recognized generally that European foulbrood requires less drastic methods than does American foulbrood, but seemingly one cannot always be so sure of the efficacy of the treatment, and it is often said by beekeepers that European foulbrood "does not fight fair." The difficulty seems to lie in the fact that the course of the disease in the colony has not been sufficiently studied and the features of treatment have not been adequately analyzed. It is not enough simply to know the name of the organism which causes the disease, but it is essential to know the habits of the germ in the colony.

European foulbrood was first recognized in New York State in 1894, and previous to that time no adequate differentiation had been made between this disease and American foulbrood. Various writers, especially those in Europe, had recorded two types of brood diseases and had differentiated them sufficiently to call one mild and the other virulent. Careful observations of beekeepers, as well as bacteriological investigations, have shown that the two diseases are entirely distinct, that one does not change to the other, and that in treatment they behave differently.

Now that the symptoms of the two diseases have been carefully studied, one can examine the earlier literature and find indications that European foulbrood was rather widespread in the United States before it was recognized as a distinct disease. At any rate it appears certain that all the European foulbrood in the country did not spread from the first recognized outbreak in New York State. New York beekeepers with justice objected to the name "New York bee disease" which was at one time applied to the disease.

NAME OF THE DISEASE.

When American beekeepers first differentiated this disease the name "black brood" was generally applied to it. When the investi-

gation of bee-disease control was inaugurated by the Bureau of Entomology it was recognized that this name was not well chosen, for black is not the predominating color of the dead larvæ. If any color designation were to be used, yellow would be best, but color is not a safe guide, as this is a variable symptom. Any descriptive name seemed unsafe for a disease with such variable manifestations, and the author therefore proposed that the name be changed. After consultation with beekeepers and apiary inspectors it was decided to adopt the name European foulbrood. This was first used in a circular [1] of the Bureau of Entomology and the name has been generally accepted by beekeepers throughout the country. The adjective "European" was chosen because it appeared that this disease had first been subjected to bacteriological investigation by European investigators, while the other disease, American foulbrood, had not been investigated carefully until such work was undertaken in America. The names obviously are not intended to convey the idea that the diseases originated one in America and the other in Europe, for the honeybee is not native to America. The names were chosen simply that beekeepers might have names which could be used with safety, and which would not lead to confusion by being descriptive.

SYMPTOMS.

The beekeeper should know whether he has to deal with American or European foulbrood, for they do not respond to the same treatment. The symptoms of European foulbrood are simply the outward manifestations of the disease, being chiefly the appearance of the larvæ after death. The symptoms are therefore variable. The most accurate method of diagnosis is by bacteriological examination, but this is, of course, not possible in apiary practice. In cases of doubt samples should be sent to the Bureau of Entomology for diagnosis.[2]

In regions where both diseases occur, beekeepers at times experience difficulty in differentiating them, due chiefly to insufficient observation of the symptoms. If European foulbrood appears in an apiary in the spring, and if American foulbrood is then observed later, the beekeeper may erroneously conclude that both types are

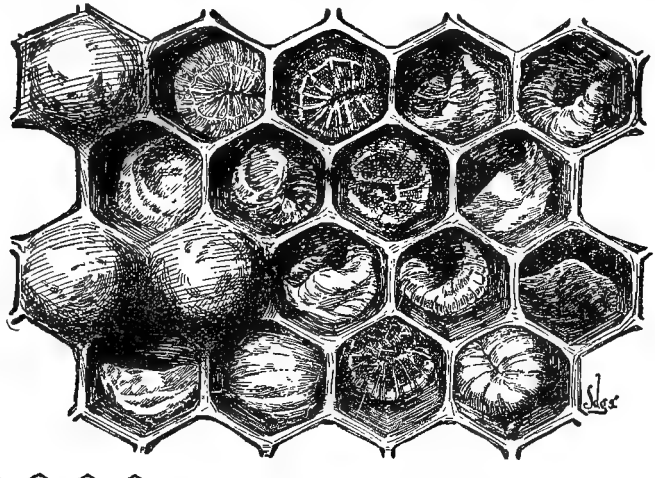
[1] Phillips, E. F. The brood diseases of bees. U. S. Dept. Agr. Bur. Ent. Circ. 79. 5 p. 1906.

[2] If dead brood is observed and the beekeeper is not able to diagnose it with accuracy, samples may be sent the Bureau of Entomology for examination. A piece of comb containing dead larvæ about 4 by 5 inches should be cut out and mailed in a heavy pasteboard or wooden box. Tin boxes should never be used, as the brood usually molds in transit, making examination impossible. The sample should not be wrapped before being placed in the box. A suitable box for sending samples will be mailed on request.

It is not possible to diagnose from empty combs, and no honey should be included in the sample, as it is valueless in diagnosis and will probably spoil the sample as well as other mail matter. The name of the sender must always appear on the package, and any available data should be sent in a letter. Never inclose a letter in the box with the sample.

manifestations of one disease, or that European foulbrood changes to American foulbrood. Such is not the case. It is therefore essential that the symptoms be studied with great care, since to treat American foulbrood by methods applicable only to European foulbrood will result in the spread rather than in the eradication of the disease.

(1) *Age of larvæ affected.*—European foulbrood usually attacks the larva at an early stage of its development, while it is still curled up at the base of the cell (fig. 1, R). At the time of the first manifestation of disease the larva is about three days old, from the hatching of the egg. A very small percentage of larvæ die after



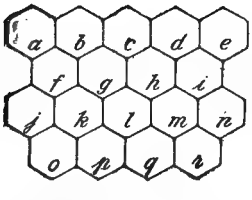


FIG. 1.—Portion of comb showing the effect of European foulbrood upon the larvæ: *a, j, k,* Normal sealed cells; *b, c, d, e, g, i, l, m, p, q,* larvæ affected by disease; *r,* normal larva at age attacked by disease; *f, h, n, o,* dried-down larvæ or scales. Three times natural size.

capping, but sometimes quite young larvæ are attacked (fig. 1, E, M). Sunken and perforated cappings, which are such common symptoms of American foulbrood, are sometimes seen in colonies suffering with European foulbrood.

(2) *Early symptoms.*—The earliest indications of the disease are a slight yellow or gray discoloration and the uneasy movement of the larva in the cell. The larva loses its well-rounded, opaque appearance and becomes slightly translucent, so that the tracheæ may become prominent (fig. 1, B), giving the larva a clearly segmented appearance.

(3) *Position of larvæ.*—The larva may be flattened against the base of the cell, may turn so that the two ends are to the rear of the cell (fig. 1, P), or may fall away from the base (fig. 1, E, G, L). The

position of the larva is one of the best means of differentiating American foulbrood and European foulbrood. In American foulbrood the larvæ almost without exception are found on the lower side wall, while in European foulbrood they may be there, or at the base of the cell, or on any of the side walls, even the upper one.

(4) *Color.*—As the decay proceeds the color changes to a decided yellow or gray and the translucency is lost (fig. 1, Q, H). When the disease first appears in a region the yellow color of the decaying larvæ seems more constant than later, due probably to the fact that as the disease spreads the germ causing the disease is accompanied by other organisms. The yellow color may be taken as the chief characteristic of the disease. The dead larva appears as a moist, somewhat collapsed mass, giving the appearance of being melted.

(5) *Scale.*—When the remains have become almost dry (fig. 1, C), the tracheæ sometimes become conspicuous again, this time by retaining their shape, while the rest of the body content dries around them. Finally all that is left of the larva is a yellow or grayish-brown scale against the base of the cell (fig. 1, F, H), or a shapeless mass on one of the side walls if the larva did not retain its normal position before death (fig. 1, N, O). Very few scales are black.

(6) *Adhesion to cell.*—At no time during the decay does the larva adhere to the wax closely, but is easily removed, and the bees carry out a great many of them in their efforts to clean house.

(7) *Usual lack of ropiness.*—A slight ropiness is sometimes observed in the decaying larvæ. This is not, however, at all like the fine ropiness observed in larvæ dead of American foulbrood, but the decaying mass behaves more like an old rubber band which has lost its elasticity and which breaks when stretched.

(8) *Odor.*—There is usually little odor in European foulbrood, but sometimes a sour odor is present which reminds one of yeast fermentation. This odor is quite constant in some regions and seems to come from the decay due to organisms other than the one which causes European foulbrood.

(9) *Sex.*—A symptom of the greatest importance is the fact that the disease attacks drone and queen larvæ[1] nearly as quickly as those of the workers.

(10) *Epidemic character.*—In regions where the disease occurs a considerably larger percentage of colonies is affected than is usual for American foulbrood. However, not many colonies die of European foulbrood, but the chief trouble is that weakened colonies succumb during winter unless well cared for. The disease spreads at

[1] The tendency of this disease to attack queen larvæ is a serious drawback in treatment. Frequently the bees of a diseased colony attempt to supersede their queen, but the larvæ in the queen cells often die, leaving the colony hopelessly queenless. The colony is thus depleted rapidly.

times with startling rapidity, much more rapidly than American foulbrood.

(11) *Variability.*—In all its symptoms European foulbrood is more variable than is American foulbrood. Color is perhaps the most constant symptom.

BASIS OF TREATMENT.

The confusion in the treatment of the disease is due to a failure to analyze the factors forming the basis of treatment. Various treatments have been described in the beekeeping journals as distinct when they were simply modifications of the same treatment.

(1) European foulbrood is a disease of weak colonies. While at times one may observe larvæ dead of this disease in strong colonies, usually they are removed before the disease can do much harm. It should be pointed out, further, that it is the colony which is failing to increase in strength in the spring which is most seriously affected, for a small colony which is rich in young and vigorous bees and which is increasing in strength is often able to overcome the disease. It is therefore a disease of weak rather than small colonies.

(2) The disease is prevalent in the spring and early summer. While at times it is observed at other periods of the year, this is not usual. Samples of European foulbrood have been received by the Bureau of Entomology in every month of the year, but, as will be seen from Table I, they are far more commonly received in the early part of the active season. These samples are listed according to the date of receipt at the bureau laboratory. The highest number is received in June and the average date for the removal of these samples from the hives is probably a few days previous to June 15, perhaps June 10. The earliest samples received are regularly those from California, where the season opens early. There is a sudden increase in May and June and almost as rapid a decline later. The few samples received from October to April may be largely disregarded, as they are almost without exception dried material of unknown age.

TABLE I.—*Distribution of European foulbrood by months, including all positively diagnosed samples received by the Bureau of Entomology from 1906 to December, 1917.*

Month.	Total number.	California.	New York.
January	3	0	0
February	4	3	0
March	17	10	0
April	33	17	2
May	180	24	23
June	334	30	50
July	240	20	41
August	164	9	20
September	98	8	8
October	17	1	3
November	7	3	1
December	2	1	0

(3) The disease disappears later in the summer unless the colony has become so badly weakened that it can not remove the dead larvæ. Such weakened colonies usually die in winter or in a time of dearth. Colonies do not as a rule die as a direct result of European foulbrood. There may still remain some dead larvæ in the combs, showing that the bees have not been able to remove all of them, but in any but the worst cases even these disappear. If conditions which commonly prevail in early summer again appear there may be a recurrence of the disease the same season.

(4) This disappearance of the disease usually accompanies the beginning of the honey flow. At this time, unless the colony has already reached maximum strength, there is a rapid increase in brood rearing and the colony increases in strength, bringing about conditions unfavorable for the development of the disease. If the honey flow fails, the disease may continue and under such conditions is at its worst. It should be noted that in regions where the early honey flows are uncertain or usually lacking European foulbrood has done the most damage, for in years of failure the disease spreads with such rapidity that the entire region becomes badly infected. European foulbrood is rarely observed in regions where an early honey flow is certain.

(5) The earliest brood of the year usually escapes with little loss. This important fact has been overlooked in previous discussions of this disease, but it is evident from Table I. The scarcity of European foulbrood in the early spring was mentioned in the earliest accounts of its prevalence in New York. This in all probability is due to the fact that the colonies have been able to remove most of the disease during the previous summer and there has been left only a little of the infecting material.

(6) Some bees resist the disease more successfully than others. It has been found through the experience of beekeepers generally that the three-banded Italian bees are best for this purpose. These bees have a further advantage in that they give excellent results in all lines of beekeeping activity, and it is therefore safe to recommend them as the best. This does not at all indicate that other races of bees would not give as good results, as far as European foulbrood control is concerned, but that it is easier to get good three-banded Italian than good bees of any other race. The resistance appears to be either a form of immunity or a greater ability to remove the dead larvæ completely.

(7) European foulbrood is an infectious disease. This was clearly shown by the experience of beekeepers before the disease was investigated from a bacteriological standpoint, and these investigations have supported the observations of the beekeeper. The bacteriological

work has shown, further, that the disease is caused by an organism[1] which has never been found in any other brood disease of bees. The cause of the disease is, therefore, a specific organism, and the disease is entirely distinct from American foulbrood. This is an important point, for there has in the past been considerable confusion in that a few beekeepers have claimed that one disease changes to the other. It should be made clear that this supposition is not supported by any careful observation in the apiary, and that it was recognized generally by beekeepers before the bacteriological investigations were made that the diseases were distinct.

(8) The organism causing European foulbrood does not seem from observations in the apiary to be so difficult to eradicate as does the one causing American foulbrood. This is partially confirmed by the bacteriological observations also.

(9) When a bee larva dies of European foulbrood the decaying mass does not adhere closely to the cell wall at any time in the decay or when it has dried down to a scale in the back or on the side walls of the cell. Dead larvæ may therefore be removed easily by the bees if conditions are favorable for this cleaning.

(10) The bees are able under suitable conditions of colony strength and resistance to clean the cells so thoroughly that when future larvæ are reared in these cells the disease is not contracted.

(11) The method of spread of the disease is not well known, although there is some evidence that the infection is carried chiefly by nurse bees. It has been observed that under some circurstances it may be transmitted through feeding, but the experience of beekeepers indicates that contaminated honey is not the common means of carrying the disease. It is well known that honey from infected colonies may be given to healthy colonies with entire safety provided the healthy colonies are in such condition that they are able to resist the disease. It is therefore not necessary to disinfect the honey from colonies having European foulbrood, as is the case with that from colonies suffering from American foulbrood.

(12) It has not been found necessary to disinfect hives, combs, or frames from diseased colonies. This does not indicate that the germ causing the disease is absent from such material, but that if present it does not do any damage.

(13) While the disease spreads with great rapidity at times, it does not seem to be so malignant as is American foulbrood, since many colonies exposed to infection fail to contract the disease.

These facts concerning the disease have been discovered in the apiary rather than in the laboratory. The facts are supported by repeated observations, and while the records of observation are not as accurately made as are those of the laboratory the correctness of most

[1] *Bacillus pluton.*

of the facts is attested by the experience of hundreds of beekeepers. In certain cases the findings have been corroborated by bacteriological investigation.[1] The methods of treatment have also all been devised in the apiary.

The difficulty in drawing conclusions from practical observations is that too often beekeepers fail to show the ways in which their experience differs from that of others or in what manner the same principles have been applied in a slightly different manner.

PREVENTIVE MEASURES.

In keeping European foulbrood under control it is far more important to prevent the disease from getting a foothold in a colony than it is to eradicate the disease afterward.

This is not true of American foulbrood, for reliable and practicable preventive measures have not been found for that disease.

(1) The use of resistant stock is of the greatest importance, otherwise there is no hope of warding off the disease when it enters a region or of eradicating it from the apiary after it is once introduced. The use of strong, vigorous Italian stock is best from the standpoint of honey-production, and every beekeeper should therefore see that his apiary is provided with such queens even before European foulbrood appears in the immediate neighborhood or in the apiary. When the disease is absent it is quite permissible for the beekeeper to save any mismated queens which show themselves to be good, but when European foulbrood is near by this course is unsafe, and in no case should a mismated queen be used as breeding stock. The purity of mating of queens then becomes a matter of first importance and this entails more work than is necessary in the ordinary practices of the apiary.

It is not enough simply that queens be pure bred and purely mated, however, for it often occurs that a queen will be poor from other causes. Whenever a queen shows signs of failing it is good beekeeping to replace her with a good queen. When European foulbrood is present this becomes far more important.

Not all Italian stock is equally resistant to European foulbrood, and when the disease is nearby it becomes important that the beekeeper find out which stock is best. Not all queens sold as Italians are pure bred. By far the best plan is to buy a few untested Italian queens from each of several queen breeders and after these have been under observation for a short time the beekeeper will be able to choose from the lot those best suited for breeding purposes. It is not so good a practice to buy a breeding queen, for such queens do not ship

[1] Bacteriological studies of bee diseases have been useful to practical beekeepers in explaining the reasons for success or failure with various treatments attempted. These studies have been especially important, however, because through them methods of laboratory diagnosis of the different diseases have been worked out.

so well in the mails, and even a breeding queen of the most resistant stock might allow her colony to become infected simply because she had been so injured in the mails that she could not keep up egg-laying properly. The buying of untested queens is to be advised at all times, for until more accurate work in breeding is done the individual beekeeper can choose breeding stock as well as most breeders.

It would be possible to recommend certain stock as the best were it not for the fact that the stock of the various queen breeders is not constant. The stock which in one year makes the best showing possibly can not be duplicated by the queen breeders the next year. The best course therefore is for each beekeeper, or possibly a group of beekeepers, to try out several strains of Italian bees to find which is best. Having done this, they can continue to breed from the best stock obtained, and they can do as well by that means as they can if they continue to buy queens from the queen breeders.

(2) Strength of colony is fully as important as resistant stock. Unfortunately too many beekeepers fail to provide conditions necessary to the bees in order that the colonies may be at the proper strength in time to combat European foulbrood successfully. It is good beekeeping to have all colonies strong, and nothing leads to large honey crops as does this factor, yet throughout the country there are thousands of beekeepers who annually fail to get half the crop through failure to have strong colonies at the right time. When the honey-flow comes early in the season, as is the case throughout most of the United States, it is important that every colony be at maximum strength early in the spring. Since European foulbrood appears in the spring and early summer, good beekeeping practice again coincides with the requirements for preventing the ravages of this disease.

One difficulty arises from the fact that there is no standard for strength of colony and what one beekeeper considers a strong colony may be considered weak by another and better beekeeper. At the opening of the honey-flow every colony from which a full crop is to be expected should be strong enough to have 10 full combs of Langstroth size filled with brood. Of course this brood may be in a larger number of combs, since the bees usually store some honey at the top of each comb, but it is easy to estimate the brood in terms of full combs. If now we accept the same standard for the desired strength of colony for the purpose of resisting European foulbrood, we will have a condition under which (assuming resistant stock) this disease will never get a start in any colony in the apiary. It is of course recognized that such a standard is seldom realized before or at the beginning of the honey-flow, and this fact is the reason for the loss of so much honey as well as the full explanation of the ravages of European foulbrood in so many places. It is suggested that each beekeeper in a region where European foulbrood exists ask

himself whether his colonies are actually in as good condition at the opening of the year as he has supposed and that he find out how strong the colonies may be made by providing the best of conditions for the development of the colony population. A beekeeper whose colonies do not measure up to this standard should not condemn the standard until he assures himself that it is entirely impossible, under his conditions, to reach it.

Obviously the proper wintering of bees becomes a matter of the highest importance in regions where European foulbrood is found. Those who fail to practice good wintering are the ones who first lose so many colonies that they become discouraged and give up beekeeping, while those whose wintering has been better are able to treat the disease although their standard of colony strength may not be high enough entirely to ward it off.

As was pointed out earlier, the first brood of the year usually escapes with little loss. If proper conditions are provided for winter, either in the cellar or outdoors, brood-rearing is delayed, whereas in poor wintering brood-rearing may begin during the coldest period of the winter.[1] If then brood-rearing is delayed by protection, it will begin as a reaction to incoming nectar and pollen. The vitality of the bees has not been destroyed by unseasonable brood-rearing and the colony can rear large quantities of brood from the very beginning. This can, of course, occur only when the colony has proper spring protection. The earliest brood will emerge without appreciable loss from disease, the colony is increased in strength at once, and its capacity for brood-rearing is great. Provided the stock is resistant, the colony is then able to ward off the disease. To bring about all the proper conditions with the least labor on the part of the beekeeper and the least waste of effort on the part of the bees, it is desirable to winter outdoor colonies in two hive-bodies, which has been recommended by this department for other reasons also.

Good beekeeping, in so far as handling the bees is concerned, consists of providing conditions in the fall so that the colony is full of young, vigorous bees for winter; of providing conditions of protection and good stores such that the bees are not depleted in numbers and vitality during the winter by excessive heat-production; of providing plenty of stores, adequate room for breeding, and abundant protection during the period of heavy brood-rearing in spring; and of preventing reduction in the strength of the colony by swarming. All of these things, and there are no others of importance, pertain to keeping colonies strong. The beekeeper who provides conditions such that the bees can keep up their own strength will not only reap the honey-crop but he will escape the ravages of European foulbrood.

To a large degree the failure of American beekeepers to get their colonies strong enough is due to the use of small hives that are in-

[1] The explanation is given in the publications of the Bureau of Entomology on wintering.

sufficiently protected during the winter and spring. The single-walled hive was first made as a means of reducing the cost. Such a hive is a good tool for the beekeeper but it is a poor home for the bees. When the 10-frame hive was found too large to be filled with bees in time for them to go into the supers as soon as the honey-flow opened, instead of protecting the hive the use of the 8-frame hive was commonly adopted. This hive is in rather general use throughout the United States, although fortunately it is now being replaced by the 10-frame hive in many localities. In order that the beekeeper may reduce his labor, it would be well to raise the standard of colony strength by providing better protection and more room for the bees. This will to a large degree eliminate the spring manipulations so often practiced, will get better crops, and will make European foulbrood a minor trouble of the apiary.

REMEDIAL MEASURES.

When strong colonies headed by vigorous queens of resistant stock are present, European foulbrood will usually make little if any headway, yet from time to time there may appear cases which require treatment. The shaking treatment used for American foulbrood[1] is often advocated for European foulbrood and is recommended by many inspectors of apiaries. It was recommended in previous publications of this department, but later observations show that other methods are more reliable. If colonies are given young Italian queens at the time of shaking, results will usually be good, but unless this is done shaking is of little or no value. Some beekeepers practice heavy feeding of either honey or sugar sirup when European foulbrood appears. This often gives good results, for it brings about the conditions which are advocated as preventive measures, although as applied it constitutes a remedial measure. The same amount of stores left with the colony the previous fall will usually do more good than heavy spring feeding as a means of disease control.

The remedial measures here described should be used only to remove the disease if it enters the apiary. Preventive measures should then be employed to avoid a recurrence of the disease.

(1) The dead larvæ are easily removed from the cells, and the remedial treatment serves to provide conditions such that these may be removed by the bees during a period when no new diseased material is appearing in the combs. Usually the queen is removed from the colony, and, since a queen whose colony becomes badly infected is rarely of any value, she is killed. In five or six days all queen cells are removed, so that the colony is hopelessly queenless. The workers do not clean out the diseased cells so rapidly unless they have a queen

[1] For a description of this treatment the reader is referred to Farmers' Bulletin 442, "The Treatment of Bee Diseases."

or a queen cell. As soon as the dead larvæ are removed, which may be easily determined by examinations, the colony is given a young vigorous Italian queen of resistant stock. If only a few diseased cells are observed and if the colony is fairly populous the queen may simply be caged and released later when the dead brood is removed.

The length of time necessary for the cleaning out of the dead larvæ varies with the strength of the colony, and for weak colonies it may be necessary to wait until all brood has emerged before giving a young queen.[1] This method should not be employed unless each colony has enough bees to sustain at least five combs full of brood. Some colonies seem to clean out dead brood more rapidly than others of the same strength. If the honey-flow comes early it will usually be possible to reduce the period of queenlessness to a few days. A beekeeper may use the time necessary for cleaning up as an indication of the strength of his colonies, for if he finds a long time needed he may be sure that his colonies, for some reason, are not as prosperous as they should be. If it is certain that there will be no honey-flow until midsummer or later it is not so necessary, from the standpoint of good beekeeping, to have all colonies strong so early in the year, but it is surely an exceptional locality where there is nothing for the bees to get in early summer.

Where the beekeeper is dependent on a late honey-flow it is often desirable to move the bees during the early part of the season to some place where nectar may be obtained. This will often be easier and less expensive than treating the colonies. For example, the author was shown a location in the west where European foulbrood caused great annoyance during the spring, while apiaries not many miles away were able to get enough nectar to ward off the disease and at the same time to give the beekeeper enough profit to justify the expense and time of moving. In such a case preventive measures are cheaper and better than the remedial measures here described. Apiary inspectors should exercise judgment in such cases and permit the moving of colonies to such places, provided they are sure that due precautions will be taken. No precautions need be demanded if the new location is already infected.

[1] This method of treatment was described in its essentials in 1905, in an article published in a periodical devoted to beekeeping. The writer of that article advised that the colony be left queenless for three days after all drone-brood has emerged, thus making a queenless period of 27 days. Later other beekeepers tried shorter periods with success. It should be remembered that the apiaries belonging to the writer of the article referred to were located in the buckwheat region of New York, and that he used a small hive, and on account of these conditions it may be safely assumed that at the time when European foulbrood attacks colonies his colonies were unusually weak. Those who have found a shorter time sufficient have been located in regions where the colony strength may be developed earlier because of earlier honey-flows, or perhaps in some cases these beekeepers wintered better, so that in the spring their colonies were in better condition to resist the ravages of the disease. It would be quite possible to refer to apiaries where the wintering is good and where the spring care is sufficient to eliminate entirely the period of queenlessness.

The methods of requeening and rearing the queens are matters aside from the treatment of European foulbrood, but in many cases the directions have been obscured by including all such details. Usually it is easier to introduce a queencell of the proper age for the queen to emerge and mate by the time egg laying may again proceed safely in the colony.

(2) A substitute for the treatment just described introduces no new principle. The colonies found to have European foulbrood are graded according to strength, and half or more of the stronger ones are shaken to dry extracting combs (not comb foundation) at the same time that the old queens are killed and replaced by young, vigorous stock. No colony too weak to have five frames of brood should be so treated. If there is no honey coming in, the combs may contain some honey, and it is immaterial whether or not it comes from a colony having European foulbrood. The removed brood is now stacked on the weaker diseased colonies so that they may be increased in strength. Just as soon as these have reached the degree of strength possessed by the first colonies shaken, they, too, may be shaken to drawn combs containing no brood, and the diseased brood is given to the remaining few diseased colonies. Usually by the time that the last colonies are ready for treatment it will be found that treatment is not necessary, for in many cases the dead brood will have been removed. If necessary, of course, every diseased colony may be treated.

This substitution for the more usual method of treatment has certain advantages. No colony is left queenless and, as a result, the total brood reared in the apiary is increased. No brood is wasted, and the colonies which receive the most of the combs containing diseased brood are usually made sufficiently strong to gather a good crop.

(3) Another method which is much used is to place all the brood combs of the infected colony except one in the second hive body over a queen-excluder and to place the queen below with the one frame of brood and frames containing foundation or even drawn combs. Others prefer to put the queen and one frame of brood above. Of course only good Italian queens should be used.

It is interesting to note that the methods used in the control of European foulbrood are exactly the same as are used in remedial methods for swarm control.[1] Either the queen or the brood is removed or the queen and brood are separated within the hive. Such a similarity is probably of significance, but this at present is merely a matter of speculation.

GOOD BEEKEEPING WILL ERADICATE THE DISEASE.

It can not be emphasized too strongly that the practices of good beekeeping are those which result in the eradication of European

[1] See Farmers' Bulletin 503, "Comb Honey."

foulbrood. It does not follow that because a beekeeper is troubled with European foulbrood he is a poor beekeeper, for he may have had good results before the disease appeared. With the entrance of the disease, however, he can change his system so as to overcome the trouble and he may do this with assurance that the changes are such as to result in good beekeeping. Unlike American foulbrood, the disease does not make it necessary that anything of value be destroyed by the beekeeper, and if the proper system of management for the particular locality can be found it will result, in most circumstances, in larger crops than are usually obtained.

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STUDIES ON THE BACTERIA ASSOCIATED WITH EUROPEAN FOULBROOD

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The etiology of European foulbrood of bees is an unsettled problem, several theories having been advanced regarding the cause of this disease. In 1885 Cheshire and Cheyne *(2)* described *Bacillus alvei,* which they claimed was the cause of the brood disease now known as European foulbrood. In 1907 Maassen *(9)* stated his belief that the etiology of the mild form of foulbrood (European foulbrood) is not uniform but that the disease is caused principally by *Streptococcus apis* and *B. alvei.* White *(11, 12, 13)* was unsuccessful in attempts to produce typical European foulbrood with cultures of *B. alvei, S. apis,* or *Bacterium eurydice* and concluded that this disease is caused by a new species, *Bacillus pluton* White, which failed to grow on artificial media. Borchert (*1 p. 12*) and Lehmann and Newman (*4 p. 236*), of Germany, have pointed out that uncertainty still exists concerning the etiology of European foulbrood. Wharton *(14)* reported having cultured *B. pluton* and producing infection in a colony of black bees by inoculation with cultures derived from primary colonies. Lochhead *(5)* says that this organism cultured by Wharton "appears to be closely related if not identical with *Streptococcus apis* described by Maassen." Wharton *(14)* also says that "cultures of *B. pluton* have been observed to change to *B. alvei* form resembling biologically the *B. alvei* isolated from infected larvae." Lochhead *(5, 6)* reported the origin of a coccoid bacillus in cultures of *B. alvei.* The coccoid was isolated and stabilized and is said to have "all the appearance of what White calls *Bacillus pluton.*" Both Lochhead and Wharton question the secondary-organism theory of White as regards European foulbrood.

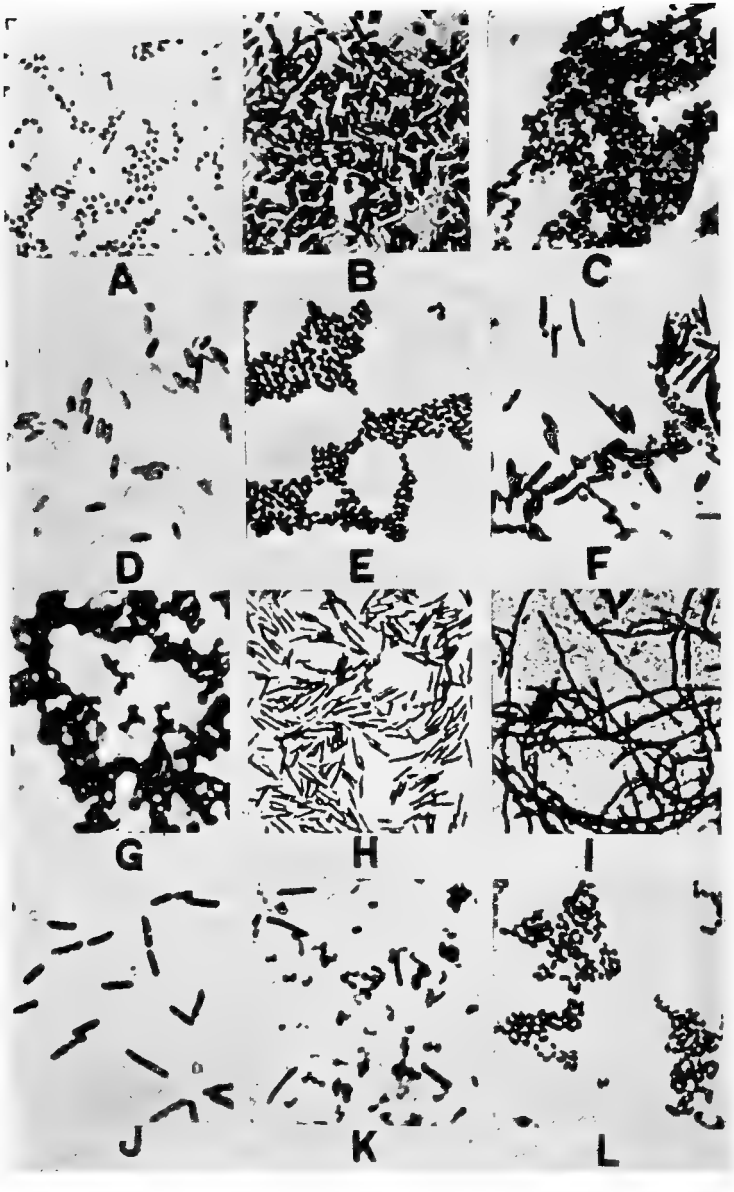
At the time White conducted his studies on European foulbrood it was generally believed that bacterial species remain constant in morphological and cultural characteristics. In recent years evidence has been constantly increasing that bacteria are capable of morphological,

cultural, and biological transformation, and the old doctrine of fixity of bacterial species is gradually giving way before this evidence. Chief among the investigators in this field is Mellon, whose extensive works have demonstrated that many species of bacteria, when cultured under different environments, produce mutants and variants with greater frequency than is commonly supposed. Works of Mellon, Hadly (*3*), Löhnis and Smith (*7, 8*), and others strongly indicate the existence of life cycles among bacteria similar to life cycles among the fungi. Mellon (*10*) has aptly stated what seems to be the situation in the following quotation: "Thus the analogy is complete, constituting rather formidable evidence for our contention that biologically bacteria may be properly regarded as fungi which have been telescoped down into a state of existence where their life cycles, although much compressed and often abbreviated, are still not obliterated."

In 1928 the writer started observations and experiments on European foulbrood to obtain evidence in support of one or another of the theories regarding the cause of this disease. He repeated experiments of others but sometimes interpreted them differently, and he also performed new experiments. Experimental results were not always so conclusive as might be desired and the significance of observations was not always apparent. Observations and experimental results which may aid, directly or indirectly, in arriving at a true conception of the etiology of European foulbrood are reported in this paper.

Morphology of Bacteria in Affected Brood.—Wide variation was observed in the morphology of bacteria present in sick or dead brood. (Plate 6, *A, B,* and *C*.) In recently infected larvae the bacteria were mostly very short rods occurring singly, in pairs, or in short chains. Medium-long rods were sometimes present, but no distinctly pointed cells were found during early infection. As the disease progressed and bacteria increased in number, variability in their morphology increased. Most frequently coccoid cells predominated, but at times moderately long rods were equally numerous. Cells of the *B. pluton* type (Plate 6, *C*) originated from the coccoid cells at about the time multiplication of bacteria was checked by overcrowding. The pointed condition appeared to be an expression of dormancy, since these cells usually occurred singly in coherent masses with rarely any indication of active division. In very late infection pointed cells usually predominated, but among different larvae and different colonies the proportion ranged from 10 per cent or even less to nearly 100 per cent. In some larvae coccoid cells predominated (Plate 6, *A*), in others moderately long rods were most numerous (Plate 6, *B*), and occasionally long, slender, faintly staining

Plate 6



BACTERIAL FORMS FROM LARVAE INFECTED WITH EUROPEAN FOULBROOD
(x 1,500)

A, *B*, and *C*, Smears from the stomach of different larvae in an advanced stage of infection, showing difference in morphology of the bacteria. In *A* only a few

rods were present in small numbers (Plate 6, *C*). Thus it is apparent that the morphological forms encountered in sick larvae present a complex and variable picture.

When bacterial growth occurred after death of larvae, it usually consisted almost entirely of moderate-sized rods, of which a variable percentage formed spores of *B. alvei.* (Plate 6, *D.*) Occasionally coccoid bacilli indistinguishable morphologically from bacilli that grow in the digestive tract of sick larvae caused decay of the body tissues after death. In still other larvae decay was caused by both the rod and the coccoid form.

CULTURES FROM SICK OR DEAD BROOD YIELDED DIFFERENT MORPHOLOGICAL FORMS.—Rough inoculation of bouillon agar slants from sick or dead brood most frequently yielded cultures of *B. alvei* which sporulated promptly. Many cultures, particularly those prepared from the digestive tract of larvae in an early stage of infection, yielded a coccoid organism in apparently pure culture which morphologically and culturally closely resembled *S. apis,* and there seems to be little doubt that it is identical with the form described by Maassen (*9*) in 1908 and later studied by White (*11, 12, 13*), Wharton (*14*), and Lochhead (*5*). On egg-yolk agar many cells of this form become lancet-shaped and were

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of the cells are still dividing; the majority are coccoid with rounded ends, while some are more or less pointed. In *B* the coccoid cells, short rods, and medium long rods are about equally numerous. In *C* most of the cells have pointed ends and are typical of the type known as *Bacillus pluton;* two long, slender rods, such as occur in small numbers in infected larvae, are also seen.

D, Spores of *Bacillus alvei* from the decayed remains of a larva dead of European foulbrood.

E, Pure culture of *Streptococcus apis* from agar culture containing unheated egg yolk. In some cultures 50 per cent or more of the cells become more or less pointed and are indistinguishable morphologically from *Bacillus pluton.*

F, Bacillus alvei and *Streptococcus apis* from an agar culture prepared directly from a sick larva. (2 days at 36° C.)

G, Streptococcus apis from a culture prepared directly from a sick larva in brood filtrate. (2 days at 36° C.)

H, Asporogenic agar culture of *Bacillus alvei,* which morphologically closely resembles *Bacterium eurydice.* (5 days at 20° C.)

I, Threadlike rods from an asporogenic agar culture of *Bacillus alvei.* (17 days at 20° C.)

J, Rods from an asporogenic bouillon-agar culture of *Bacillus alvei* with beaded and granular protoplasm. (5 days at 36° C.)

K, Culture of *Streptococcus apis* in bouillon broth, showing rods of *B. alvei* which appeared after 6 days at 36° C.

L, Pure culture of *Streptococcus apis* from bouillon agar to which 10 per cent honey was added. (36° C.)

indistinguishable morphologically from *B. pluton.* (Plate 6, *E.*) Many cultures yielded both *B. alvei* and *S. apis.* (Plate 6, *F*) In some cultures the cells of the coccoid form were observed to be dissociated. Occasional cultures of *B. alvei* prepared from sick or dead brood—the relative number varied in different samples of infected brood comb—grew slowly and sporulation was delayed and incomplete. In a few instances rough inoculation from affected brood yielded cultures of rods which did not form spores at all when cultivated at room temperature. Cultures of asporogenic rods were also obtained, some of which closely resembled *B. eurydice,* by plating directly from sick larvae at room temperature.

In a few cultures on bouillon agar or egg-yolk agar prepared with bacteria from the digestive tract of sick larvae no growth was detected. From the same larvae, however, prompt and abundant growth was obtained in dilute sterile filtrate prepared from macerated honeybee larvae. In filtrate medium a coccoid organism resembling *S. apis* (Plate 6, *G*) was usually obtained, but some cultures yielded also small or moderate-sized rods. It is evident that failure to obtain growth on ordinary nutrient agar does not prove the absence of culturable bacteria.

Cultures from healthy-appearing larvae from different infected colonies yielded in a variable percentage of the tubes apparently one or another of the same forms obtained in cultures from sick or dead larvae (*B. alvei* or *S. apis*). When combs of brood were removed from colonies shortly after infection had subsided and were kept either at room temperature or at 36° C., none of the larvae dying of starvation or chilling were noticeably decayed by *B. alvei,* even though this organism was found by cultural tests to be present in the digestive tract of more than 90 per cent of them.

Bacteria Present in Honey from Infected Colonies.—*Bacillus alvei* was found to be abundant in honey and pollen from the brood chamber of infected colonies. In advanced cases inoculations of nutrient agar with a single loopful of honey (about 0.001 cc) practically always yielded *B. alvei,* while a few also yielded *S. apis.* In early or mild cases part of the cultures prepared with honey or pollen yielded *B. alvei.*

Bacteria Found in Colonies with European Foulbrood Not Present in Healthy Colonies.—In striking contracts to the prevalence of bacteria in larvae from infected colonies is the complete absence of these forms in healthy colonies. The writer has made microscopical examinations of and prepared cultures numbering well into the thousands from larvae dead of American foulbrood, sacbrood, fungus diseases, plant poisoning, and other brood disorders, as well as from healthy larvae,

without having found or obtained *B. alvei* or *S. apis* in culture, except on rare occasions when mixed infection was suspected. Likewise cultures prepared with honey and pollen from healthy colonies in which European foulbrood never existed have never yielded *B. alvei.*

The writer's observations on this point differ from those of Maassen (*9*), who claims to have found *B. alvei* present in some cases in larvae dead of "virulent foulbrood" (American foulbrood). In a few cases the writer obtained *B. alvei* in cultures from combs infected with American foulbrood, but a thorough inspection of the brood comb and of the scales used in preparing the cultures generally revealed mixed infection and occasionally a scale of European foulbrood which resembled that of American foulbrood. It seems possible that Maassen may likewise have been dealing with cases of mixed infection.

TRANSMISSION OF EUROPEAN FOULBROOD WITH CULTURES.—When conditions are favorable, typical European foulbrood is readily transmitted by inoculation with bacteria taken from the digestive tract of sick or dead brood. On the other hand, typical European foulbrood has only rarely been produced by inoculation with cultures, although several investigators, in inoculation experiments with cultures of *B. alvei* (rods and spores), have obtained an atypical infection. In the writer's experiments an occasional larva or pupa was attacked by *B. alvei* when a water suspension of sporulating cultures recently isolated from infected brood was sprayed over developing brood. It appears that *B. alvei* in the usual sporogenic state may, under favorable circumstances, produce disease in larvae or pupae, but this disease is not typical European foulbrood.

Likewise, attempts to produce European foulbrood by inoculation with pure cultures of *S. apis* have usually been unsuccessful. Maassen (*9*) failed to demonstrate pathogenesis for *S. apis* by feeding pure cultures, and White (*12*) states that "No disease results when the brood of bees is fed cultures of *Streptococcus apis* either by the direct or indirect method." In speaking of the coccoid form of *B. alvei,* Lochhead (*6*) states, "Our attempts to produce the disease in a colony of black bees through feeding cultures of the coccus have so far been inconclusive." On the other hand, Wharton (*14*), in inoculation experiments with cultures of a coccoid bacillus which Lochhead (*5*) says "appeared to be closely related to, if not identical with, *Streptococcus apis,*" claims to have produced typical European foulbrood. Concerning this experiment Wharton says, "The writer has obtained infection in a healthy colony of black bees in four days, using as inoculum cultures of the organism derived from isolated colonies. The symptoms of the diseased larvae

accorded with those observed in naturally infected larvae and the microscopical picture was typical—*B. alvei* forms being also present, though only in small numbers." If Wharton's cultures were pure, as he assumes, to him belongs the credit of first producing typical European foulbrood by inoculation with pure cultures.

The writer's inoculation experiments with *S. apis* and with non-spore-forming rod cultures (resembling *B. eurydice*) isolated from sick or dead brood gave results that were largely negative or inconclusive. On one occasion typical European foulbrood was produced by inoculation with cultures of *S. apis* freshly isolated from sick larvae. In isolating pure cultures plating was ordinarily done two or more times. Occasionally larvae inoculated with such cultures appeared to become infected and were removed by the bees, but the symptoms were not typical of European foulbrood and the infection disappeared promptly.

In an experiment performed in 1933, bacteria from the digestive tract of a naturally infected larva were streaked on egg-yolk-agar plates. After 24 hours at 34° C. isolated colonies of *S. apis* were touched with a platinum loop and cultures were prepared on egg-yolk-agar slants. With the abundant growth obtained on these slants after 44 hours at 36° C., a colony of black bees was inoculated by spraying the bacteria, in water suspension, over two combs of young and hatching larvae. Two days later numerous coccoid bacteria were found within the digestive tract of some of the larvae. On the following day coccoid bacteria had greatly increased in number in many of the inoculated larvae and larvae were being removed rapidly by the bees. On the fourth day more than 90 per cent of the inoculated larvae had been removed. None of those remaining showed outward symptoms, but upon microscopical examination coccoid bacteria morphologically identical with the bacteria in the inoculum were so abundant within the digestive tract that infection could be definitely ascertained. All the unsealed brood in the inoculated combs was finally removed by the bees and no dead larvae were found in the cells.

A water suspension of bacteria from the artificially infected larvae was next sprayed over another comb of young brood in the same colony. After 3 days fully 25 per cent of the inoculated larvae in this comb were dead or dying from infection, of which the gross symptoms and the bacteriological picture were typical of European foulbrood. Pointed or lancet-shaped cells (*B. pluton*) were at first absent or present only in small numbers, but later they became numerous. The coccoid bacillus was reisolated, but out of about 100 cultures *B. alvei* was obtained in only one. With cultures prepared by rough transfer from those with

which infection was obtained three succeeding experiments gave negative results. The results of this experiment and the comparable experiment performed by Wharton (*14*) seem to point to retention of virulence by *S. apis* during only about two generations on artificial culture media. It is recognized, however, that the purity of such recently isolated cultures may be questioned.

PLEOMORPHISM AND VARIABILITY IN *Bacillus alvei.* Several investigators have observed variation in size and shape of individual cells in cultures of *B. alvei.* Maassen (*9*) says that cultures of *B. alvei* degenerate on the usual artificial medium and that nuclei or granules develop in the plasma while the ability to form spores disappears. Lochhead (*5, 6*), using a special nutrient agar, observed the origin of coccoid cells from rods of *B. alvei,* which he reported (6) to be indistinguishable morphologically from *B. pluton.*

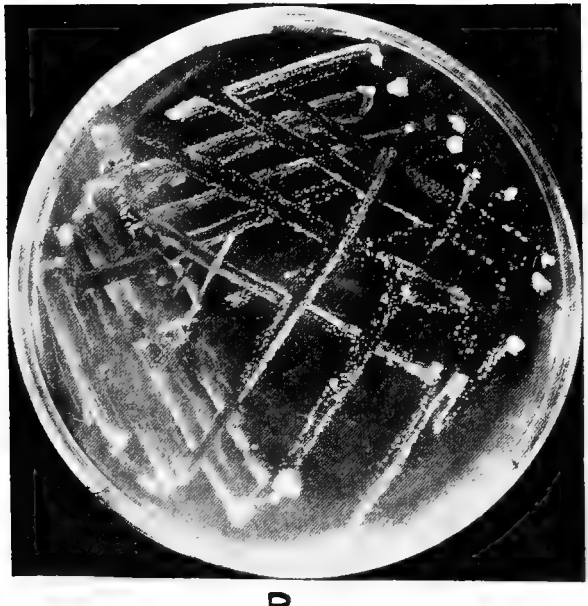
In the writer's experiments *B. alvei,* in the form in which it is usually isolated from dead brood, grew luxuriantly, spread rapidly over the agar, and formed spores promptly and abundantly on bouillon agar and on egg-yolk agar at 36° C. (Plate 7, *A.*) In repeated transfers at 36° C. on these agars no morphological or cultural changes were observed. In bouillon broth, potato broth, and milk, and in media containing sterile filtrate prepared from honeybee larvae, the luxuriance of growth and the tendency to form spores gradually decreased in repeated transfers. After about 10 generations in potato broth, cultures prepared by rough transfers to bouillon agar and egg-yolk agar grew slowly while sporulation was incomplete and delayed or lacking. Growth either spread slowly or was confined to small colonies. (Plate 7, *B.*) By planting and culturing from isolated colonies, strictly asporogenic cultures were obtained which in repeated transfers remained asporogenic. When cultured at room temperature the transformation in potato broth from a sporogenic to an asporogenic condition was more rapid. Bouillon broth seemed less effective in producing the change, and results with filtrates from honeybee larvae were irregular.

These asporogenic cultures of *B. alvei* varied in morphology and cultural characteristics (Plate 6, *H, I, J*), but in some cases the resemblance to cultures of asporogenic rods isolated by plating from sick larvae was marked. It seems probable, therefore, that *B. alvei* may exist in infected larvae in either sporogenic or asporogenic condition.

Morphologically and culturally the characteristics of some of the cultures were indistinguishable from the characteristics given by White (*11*) for *B. eurydice.* (Plate 7, *B;* plate 6, *H.*) Concerning this form White (*13*) says: "In studying this species cultures were isolated which

Plate 7

A



B

TYPES OF GROWTH OF BACILLUS ALVEI

A, Two spreading colonies of *Bacillus alvei* on a bouillon-agar plate, showing difference in type of growth.

B, *Bacillus alvei* on glucose-agar plate growing in small colonies after transformation from a sporogenic to an asporogenic condition.

in some respects differed from it. Whether these are different species or belong to a group of which *B. eurydice* is a representative has not been definitely determined." Concerning methods of culture White further says: "Incubation must be carried out at room temperature. Growth of the species is always slow and never luxuriant." In view of the writer's observations it seems probable that the culture described by White as *B. eurydice* and cultures which "in some respects differed from it" may have been asporogenic variants of *B. alvei*.

The variability of *B. alvei* in morphology and cultural characteristics appeared to depend upon the physiological condition of the organism as well as upon the culture medium. To retain viability of cultures frequent transfers were necessary. The description of the organism given below is of cultures produced as follows: Agar slant cultures of sporogenic *B. alvei* were prepared from isolated colonies. A water suspension of spores was boiled for 3 to 5 minutes, after which the organism was cultured by transferring for 10 generations in potato broth. Cultures prepared from isolated colonies on agar plate by transfer to nutrient agar on which sporulation is ordinarily prompt were then asporogenic at room temperature.

Glucose-agar plate.—Colonies slightly convex and rounded with uniform outline, 1 to 2 mm in diameter, grayish by reflected light, bluish gray by transmitted light; under a binocular appearing very light brown and finely granular.

Morphology.—Variable; rods nonmotile and asporogenic, occurring singly, in pairs, or in chains, ends rounded; protoplasm homogeneous or granular or broken; smaller and more slender than sporogenic *B. alvei* in some cultures, of equal dimensions in others.

Staining properties.—Stained readily with the usual dyes and Gram-negative; granules sometimes darkly staining and Gram-positive.

Oxygen requirements.—Growth occurring under anaerobic conditions but more luxuriant in the presence of air.

Bouillon.—Medium slightly clouded after 48 hours, a slightly viscid sediment forming slowly at bottom of tubes.

Sugars.—With the usual sugars acid but no gas produced; both arms of tube clouded, but growth most luxuriant in open arm; litmus discharged.

Brood filtrate.—In some cultures brood filtrate added to the medium increased growth, but in other cultures no effect observed; growth also variable in water solution of filtrate.

Milk.—Slight growth with little or no change apparent in either litmus milk or plain milk.

Potato broth.—Growth slow, with slight uniform clouding and slight sediment.

Potato.—Feeble, grayish growth.

Gelatine stab.—No liquefaction.

In asporogenic cultures of *B. alvei* coccoid bodies were observed which morphologically resembled the coccoid bodies observed by Lochhead (*6*), but attempts to isolate this form have thus far been unsuccessful.

In recently formed asporogenic cultures the protoplasm (from bouillon or glucose agar) was usually homogeneous. After several transfers, especially on egg-yolk agar, the protoplasm often became granular or broken. At times the rods assumed a beaded appearance resembling chains of coccoid cells. One culture in brood-filtrate medium assumed a decided coccoid appearance with many forms indistinguishable morphologically from chains of coccoids observed in cultures of *S. apis*. Rods were frequently observed in a state of dissociation, and in some cultures few rods remained undissociated after 4 or 5 days' incubation.

Pleomorphism in *Streptococcus apis.* — On ordinary bouillon agar *S. apis*, when freshly isolated, appears in diplococcoid form with occasional single cells and short chains. The cells are only rarely spherical, their length being usually aproximately 1½ times their thickness. In bouillon broth the tendency to grow in chains is accentuated, while on nutrient agar containing egg yolk the cells are smaller than in bouillon agar and appear singly or in pairs. The ends are sharply rounded and frequently pointed, many forms being morphologically indistinguishable from *B. pluton*. In some of the cultures on egg-yolk agar approximately 50 per cent of the cells became more or less pointed after multiplication ceased. (Plate 6, *E.*) After prolonged cultivation further changes in morphology have been observed from time to time.

Wharton (*14*) reported that his morphological studies suggest the identity of *B. pluton* and *B. alvei* and stated that "Cultures of *B. pluton* have been observed to change to *B. alvei* form, resembling biologically the *B. alvei* isolated from infected larvae." In a few instances the writer's cultures of *S. apis* derived originally from isolated colonies have yielded rods (Plate 6, *K*) and eventually spores of *B. alvei*. This has been observed only in broth cultures prepared by transfer from old cultures on nutrient agar. After incubation for 7 to 12 days at 27° C., rods of *B. alvei* appeared in small numbers, but nothing was determined concerning their origin. Spores were produced in the original broth cultures and in transfers on nutrient agar. On other occasions rods that failed either to grow or to produce spores in transfers originated in broth cultures. Occasionally rods with length equal to about five times

their thickness, shorter rods, coccoid cells, and lancet-shaped cells were observed in the same chains in broth cultures of *S. apis.* In cultures on bouillon agar to which 10 per cent honey was added, some of the cells were increased in size, many were distinctly rod-shaped, while others assumed lancet shapes indistinguishable from *B. alvei* (Plate 6, *L*). In broth cultures of *S. apis* containing both honey and unheated egg yolk, several variants were observed after 2 days, including large, irregular, barrel-shaped, and spherical cells, occurring usually in pairs or in chains.

CONCLUSIONS.—Several morphologically different bacteria forms are more or less constantly present in honeybee larvae sick or dead of European foulbrood. These forms are absent in larvae sick or dead of other causes.

No evidence has yet been obtained which satisfactorily explains the etiology of European foulbrood or why these different bacterial forms are constantly associated with this disease.

It has been found that *Bacillus alvei* is capable of morphological, cultural, and biological transformation and is also capable of stabilization, at least temporarily, as a sporogenic rod, an asporogenic rod resembling *Bacterium eurydice,* or a coccoid resembling *Bacillus pluton.*

There seems to be insufficient reason for assuming that the lancet-shaped bacterial cell, *B. pluton,* found in late stages of infection in European foulbrood, is of different genus and species from the similar form *Streptococcus apis,* which is readily obtained in culture from sick larvae.

The identity of *Streptococcus apis* and *Bacillus pluton* is suggested by morphological similarity, by the fact that the pointed or lancet shape is a variable character in both forms and appears to be only an expression of restricted growth or dormancy accentuated in infected larvae, and also by the usual, if not invariable, occurrence of *Streptococcus apis* in recently infected larvae, and by the fact that typical European foulbrood was produced in Wharton's and in the writer's experiments when young brood was inoculated with cultures of *S. apis* prepared with isolated colonies.

That *Bacillus pluton* and *Streptococcus apis* are variants, or stages in the life history, of *Bacillus alvei* is suggested by the occurrence of variants resembling *B. pluton* in pure cultures of *B. alvei* and by the apparent origin on rare occasions of sporogenic *B. alvei* in cultures *S. apis.*

The transformation at room temperature of sporogenic *B. alvei* into an asporogenic nonmotile rod which morphologically, culturally, and bio-

logically is closely allied to *Bacterium eurydice* likewise suggests the identity of these forms.

Regarding the etiology of European foulbrood and the variety of bacterial forms present in sick and dead larvae much remains to be determined. The writer is of the opinion that the evidence now available points more strongly to a pleomorphic organism as the etiological factor in this disease than to the secondary organism theory advanced by White.

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MIXED INFECTION IN THE BROOD DISEASES OF BEES

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The two principal brood diseases of bees, European foulbrood and American foulbrood, heretofore have not been found associated together commonly in the same colony. The generally accepted belief has been that it is indeed a rare occurrence to find both diseases under these conditions. Sacbrood, on the other hand, is much more often found in greater or less quantity associated with either European foulbrood or American foulbrood, but seldom assuming dangerous proportions, either alone or in conjunction with the others. Statistics for the past few years, however, show that these cases of what may be called mixed infection are probably more common than was previously supposed and may account for some of the puzzling instances where colonies have not responded to treatment in the customary manner, thereby causing beekeepers to believe they have some new form of brood disease, or that the disease is showing some new unheard of characteristics.

Cases of so-called mixed infections are not at all uncommon among human diseases. Where this condition occurs, such as when a person affected with typhoid fever develops pneumonia at the same time, it is always the individual to whom the term mixed infection is applied. It is a somewhat different matter in the case of the brood diseases of bees. In the first place, so far as is known, the organisms causing these two diseases, *Bacillus larvae* of American foulbrood and *Bacillus pluton* of European foulbrood, have never been found together in the same individual larva. It is, therefore, the colony as whole which is to

be considered as the individual unit, as is the case in the majority of the manipulations of beekeeping practice. This fact makes the problem slightly different from a case of mixed infection as considered from the point of view of human medicine. However, since different individuals are involved in the mixed infections there is no "a priori" reason for considering such cases as impossible.

The first published report of an authentic instance where both American and European foulbrood were found together in the same comb from a diseased colony was reported by McCray.[1] This report was concerning a sample (4982) received at the laboratory for diagnosis May 4, 1916, from Stanislaus County, California. Previous to this case only one other such sample (2598 from Brown County, Wisconsin in 1911) had been received for diagnosis, showing the presence of both diseases, but no report concerning it was published. These two samples were the only known authentic cases on record either in the Bee-Culture Laboratory among practically 5000 samples received up to 1916, or in the beekeeping literature. These two cases were considered to be interesting in that they demonstrated that the presence of both diseases at the same time in a colony was possible, but not much importance was given the matter because of their rare occurrence. White[2] states that "such a double infection has been encountered in the writer's experience very rarely. In such diagnoses, therefore, after European foulbrood had been found in the sample, American foulbrood is seldom looked for." This practice has been the custom generally as well when American foulbrood was found present in a sample, no further search for European foulbrood being made unless there were present strikingly prominent symptoms abnormal for American foulbrood. As a result the diagnostic records of the Office of Bee-Culture show but six cases of mixed infection up to December 31, 1918, among the approximately 6000 sample records.

Developments during the year 1919, however, showed that mixed or double infection is more probable than had been previously supposed. These facts were particularly impressed upon the writer during the spring of 1919 while on a trip investigating the bee disease conditions in the State of California. While in the field during a period of less than one month, and in three different counties of the State of California, six cases were found showing both American foulbrood and European foulbrood in the same colonies. Each case was diagnosed posivitely at once in the field by means of microscopic examination of dead larvae showing characteristic symptoms of the two diseases and found to contain the specific causative organisms. It is interesting to note that three

[1]McCray, A. H. 1916. Report of the finding of American Foulbrood and European foulbrood in the same comb. JOUR. OF ECO. ENT. Vol. IX, p. 379.

[2]White, G. F., 1920. European foulbrood. U. S. Dept. of Agric. Bul. 810.

of the six samples were found in Stanislaus County in the same locality as the sample reported by McCray in 1916. These cases were all found in regions where both diseases are exceedingly prevalent and of long standing. A few of the samples were fairly self evident from gross appearances, but the majority required a more minute examination.

From that time on, particularly after returning to the laboratory in Washington, more careful examination was made, both gross and microscopic of all samples received because of suspicions aroused by the unusual prevalence of the obvious cases found in California. This was done in order to eliminate the danger of overlooking cases where one disease might be predominant over the other, whether both diseases were suspected or not, causing the less prominent to be overlooked.

As a result, during the remainder of the year 1919 from June until December, twelve more such samples were received in the laboratory from various parts of the country, (18 in all for that year, total 24) all of which proved upon careful diagnosis to contain both American foulbrood and European foulbrood in the same sample of comb. Furthermore, during the year 1920, up until November 15th, fourteen more such samples were received, making a total in all of 38. Tables 1 and 2 give the data from sample records.

TABLE I.—CASES OF MIXED INFECTION FROM LABORATORY RECORDS

Date	Lab. No.	State	County	Apparent primary invader from gross appearance	Remarks
9–20–11	2598	Wisconsin	Brown	?	Diagnosed by G. F. White
5– 4–16	4982	California	Stanislaus	American fb.	Diagnosed by A. H. McCray
6– 3–16	5061	California	Stanislaus	American fb.	Diagnosed by A. H. McCray
5–16–17	5392	Missouri	Jasper	Probably Afb.	
5– 9–18	5836	Mississippi	Washington	?	Apparently about equa
10– 9–18	6122	Wisconsin	Barron	?	More Efb than Afb
4–19–19	6437	California	Santa Barbara	Probably Efb.	One cell Afb.
4–26–19	6441	California	Sacramento	American fb.	From history of case
4–26–19	6442	California	Sacramento	American fb.	
4–28–19	6445	California	Stanislaus	European fb.	Few cells Afb.
4–30–19	6449	California	Stanislaus	American fb.	Few cells Efb.
5– 1–19	6452	California	Stanislaus	European fb.	From history of case
5–20–19	6304	Missouri	Lewis	?	
6–11–19	6401	Ohio	Ashtabula	?	
6–27–19	6498	Iowa	Johnson	American fb.	Efb early stages, also Sacbrood
8– 1–19	6629	Ohio	Trumbull	?	
8–15–19	6672	Connecticut	Tolland	Probably Efb.	Afb slight amount
8–25–19	6698	Kansas	Cherokee	?	
8–29–19	6716	New York	Cayuga	American fb.	Efb active Afb scales
9– 2–19	6721	Washington	Pacific	?	
9– 2–19	6722	Washington	Pacific	?	Efb more prominent
9–19–19	6768	California	Santa Barbara	?	Afb 1st disease reported for county
9–26–19	6778	California	Santa Barbara	?	
10– 5–19	6834	California	Santa Cruz	?	
5–12–20	6985	California	Butte	European fb.	Afb one or two cells
5–29–20	7023	Michigan	Calhoun	?	
5–29–20	7025	Michigan	Calhoun	?	
5–29–20	7026	Wisconsin	Fond du Lac	European fb.	Few cells Afb
6–17–20	7119	Washington	Lewis	?	
6–17–20	7120	Washington	Lewis	?	Also Sacbrood
6–22–20	7143	New York	Allegany	European fb.	Few cells Afb.
6–24–20	7158	Pennsylvania	Crawford	?	
6–26–20	7172	New York	Cayuga	?	
6–26–20	7174	New York	Cayuga	?	
6–26–20	7177	Pennsylvania	Crawford	?	
7–21–20	7335	New York	Seneca	Probably Afb.	
8– 5–20	7386	Indiana	Blackford	?	
8– 5–20	7387	Indiana	Blackford	?	

TABLE II.—SAMPLES OF MIXED INFECTION BY YEARS

Year	Samples of mixed infection	Total Samples received
1911	1	1042
1916	2	374
1917	1	449
1918	2	429
1919	18	693
1920	14	698
1905–1920	38	7568

This marked apparent increase in cases of mixed infection carries the subject over from one of scientific interest to one of practical importance. As is shown in Table III, the 38 samples of mixed infection have come from 24 counties in thirteen states, most of these located in prominent beekeeping regions. In eleven of these thirteen states both European foulbrood and American foulbrood as shown by samples of disease received in the laboratory for diagnosis are prevalent and of long standing. There are only about three or four other states where both diseases have been found in quantity from which samples of mixed infection have not been received, while only from two states of the many where the diseases are only occasionally bad have such samples been received.

TABLE III.—SAMPLES OF MIXED INFECTION BY STATES AND COUNTIES

State	Counties	Samples
California	5	12
Connecticut	1	1
Indiana	1	2
Iowa	1	1
Kansas	1	1
Michigan	1	2
Mississippi	1	1
Missouri	2	2
New York	3	5
Ohio	2	2
Pennsylvania	1	2
Wisconsin	2	3
Washington	2	4

Statistics obtained from the sample records, however, are not entirely conclusive since a majority of the samples come to the laboratory unsolicited. If a careful survey could be made of the regions where the brood diseases are bad and widespread, probably many more such cases would come to light.

TABLE IV.—DISTRIBUTION OF SAMPLES OF MIXED INFECTION BY MONTHS

April	5
May	9
June	10
July	1
August	6
September	5
October	1
November	1

These samples of mixed infection have been examined in eight out of the twelve months of the year, April to November inclusive, as shown in Table IV. Twenty-four of the total 38 samples, nearly 65 per cent., were examined during the months of April, May and June, the months during which European foulbrood is most prevalent.[3] In contrast to the spring months, eleven samples of mixed infection were examined during August and September, and only one each in July, October and November, a total of fourteen.

The question, however, of which diesase is most often the primary invader in a colony is difficult to answer, particularly without a history of the colony and locality. (Table I). If only dried adhesive American foulbrood scales are found, accompanied by numerous coiled fresh moist melting larvae of European foulbrood, it is not difficult to say that American foulbrood was the primary invader, perhaps during the previous season, as was the case of the sample reported by McCray. But often there is no such demarkation. Because the presence of American foulbrood depletes the strength of the colony this increases the probability of European foulbrood infection.

Since the requirements of the treatment of the two diseases are so entirely different, the necessity for correct diagnosis becomes of importance, particularly in regions where both diseases have been prevalent for some time. The presence of both diseases in the same colonies or even in the same apiary is a complicating factor in the diagnosis and treatment. Furthermore there is danger from the possibility of continued and confusing losses due to the ignorance of the presence of mixed infection in colonies under such circumstances and resulting therefrom, improper treatment which would only continue the losses.

Several samples have been received for diagnosis which beekeepers have thought contained both diseases and which indeed seemed to have some of the characteristics of each. Upon careful examination, however, both gross and microscopic, these have mostly proven to be definitely not mixed infections. The recognition of cases of mixed infection in

[3]Phillips, E. F., 1918. The control of European foulbrood. U. S. Dept. of Agric. Farmers' Bulletin 975, 16 pp.

colonies is often difficult because of the fact, as is particularly the case with European foulbrood, there are many irregularities and variations in symptoms that often add to the confusion of the beekeeper in making gross diagnosis hurriedly in the field. In order to more easily differentiate some of these confusing symptoms to assist in gross diagnosis, they may be divided into three classes. Occasionally in an unusually virulent case of American foulbrood or in one where the bees have deserted the brood because of its foul condition allowing what healthy brood there is to starve, larvae will be found which have died while still coiled in the cell, among the typical American foulbrood larvae.[4] These coiled larvae often have much the same appearance as typical European foulbrood coiled larvae. However, the consistency is generally quite different from European foulbrood, more like the typical slimy glue-like consistency of American foulbrood material. As a rule, however, the symptoms of American foulbrood are uniformly constant because of the fact that *Bacillus larvae* is almost always the only invader of the larvae causing death and a type of decomposition which prevents growth of other organisms. Several such cases were found in California.

A second class of confusing symptoms are found in samples which come particularly from regions where European foulbrood has been allowed to run unchecked for a long time. Such samples were found in certain sections of California and have been received from various other sections of the country. These samples show along with more or less of the typically coiled European foulbrood larvae, large numbers of larvae which have died after extending and even being sealed in the cell, showing a consistency somewhat like that of American foulbrood but more lumpy or like an old partly rotten rubber band.[5] Sometimes scales are found extended in the cells in such large numbers as to appear on casual examination like an old comb of American foulbrood. Close examination, however, shows the consistency, irregular shape and position with lack of adherence to the cell wall to be different from that in American foulbrood. This type was found to be quite prevalent in California.

The third class is composed of cases of actual mixed infection where typical American foulbrood, ropy larvae or scales, are associated in the same comb with typical European foulbrood, coiled moist melting larvae, or possibly occasionally the abnormal rubbery irregular larvae mentioned above. The active stage of the two diseases often seems to be localized more or less in different parts of the comb. This is probably due to

[4]White, G. F. 1920. American foulbrood. U. S. Dept. of Agric. Bul. No. 809.

[5]Sturtevant, A. P., 1920. A study of the behavior of colonies affected by European foulbrood of bees. U. S. Dept. of Agric. Bul. No. 804.

the fact that the queen would tend to desert that section of the comb containing the American foulbrood, particularly where this disease was the primary invader. In many cases one or the other of the diseases will be more prominent, at least in the active stages. This fact may be one of the causes for cases of mixed infection having been overlooked, the beekeeper seeing only the prominent outstanding symptoms. Therefore in cases where there is doubt or suspicion that both diseases may be present in the same colony, a positive laboratory diagnosis often appears to be desirable.

As is well known, the shaking method of treatment in its essentials is so far the only successful way of treating American foulbrood.[6] The nature of *Bacillus larvae* has prevented success along any other line, because of its ability to form exceedingly resistant spores and especially to decompose the dead larva in such a way as to cause the mass containing large numbers of these spores to adhere to the cell wall as if glued. It has been learned furthermore, often by sad experience, that the shaking treatment is practically never successful in the treatment of European foulbrood; in fact, often when used causes the disease to be spread all the more because of the weakening effect the shaking has on the colonies.[7] The requirements for the successful treatment of European foulbrood have been found to be fundamentally dependent upon adequately strengthening the colonies with young bees sufficiently to throw off the disease,[7] at the same time combined with the requeening of the diseased colonies with vigorous young Italian queens, permitting the bees themselves to remove the infected material.

The apparent logical solution of the problem of the treatment for a known case of mixed infection, therefore, is to combine the treatments for both American foulbrood and European foulbrood as a single treatment. In other words, the one or more colonies known or strongly suspected to have mixed infection should be shaken as for American foulbrood, requeening them with vigorous young Italian queens and later strengthening them by the addition of young bees or hatching brood from a healthy colony, or by uniting later. Strength of colony is the important factor combined with the shaking and requeening with vigorous Italian stock.

The problem of the control of mixed infections of American foulbrood and European foulbrood is primarily associated with the control of European foulbrood. In localities where both diseases are prevalent

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[6]Phillips, E. F. 1920. The control of American foulbrood. U. S. Dept. of Agric., Farmers' Bulletin No. 1084.

[7]Phillips, E. F. 1918. The control of European foulbrood. U. S. Dept. of Agric., Farmers' Bulletin No. 975.

and there is suspicion of both being present in the same apiary, and possibly even some as mixed infection in the same colony, control of the two diseases will depend upon the elimination of European foulbrood first. This should be done by treating the entire apiary for European foulbrood, by strengthening and requeening all the colonies with young and vigorous Italian queens, which is after all only good beekeeping. After the elimination of European foulbrood it will be a simple matter to determine those colonies that have not responded to this treatment, as being American foulbrood. This method is possible because of the fact that American foulbrood seldom spreads with the rapidity of European foulbrood, particularly if care is taken to prevent robbing and mixing up of combs. Those colonies which continue to show American foulbrood remaining may now be given the usual shaking treatment.

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RELATION OF COMMERCIAL HONEY TO THE SPREAD OF AMERICAN FOULBROOD

BY

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(Contribution from Bureau of Entomology)

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RELATION OF COMMERCIAL HONEY TO THE SPREAD OF AMERICAN FOULBROOD[1]

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INTRODUCTION

The relation and importance of commercial honey to the spread of American foulbrood of bees has occupied the attention of the beekeeping industry more or less prominently for many years. The theory has been promulgated that honey which has not come from disease-free apiaries is dangerous because of the possibility of its disseminating American foulbrood. A few States and at least one foreign country require that honey intended for interstate shipment be accompanied by a certificate from the bee inspector of the State in which the honey originated to the effect that such honey was produced in apiaries free from American foulbrood.

It is a well-established fact that honey taken directly from the combs of the brood chambers of colonies affected by American foulbrood is capable of producing the disease if fed to healthy colonies. Since commercial beekeeping practice bans the extracting of honey from the brood nest, it is difficult to understand how heavily infected honey, in large quantities, could get on the market. Whether honey from supers that have been on colonies affected with American foulbrood is of serious importance in transmitting the disease is still open to question. White (*30, p. 35*)[3] says: "The likelihood that the disease will be transmitted by combs from diseased colonies, which contain honey but no brood, probably is frequently overestimated." On the other hand, Millen (*23*) found that combs built from foundation and completely filled above an excluder with honey from colonies that had been destroyed by American foulbrood produced disease in all of 10 colonies made from package bees to which one comb each of the honey had been given. Corkins (*8*) expressed the belief, as a result of preliminary studies, that "Extracted honey produced above an excluder in a colony in the early stages of American foulbrood is insignificant in the spread of this disease through commercial honey." The conflicting nature of these observations emphasizes the need for further research before the certification of honey is required as a means of alleviating the foulbrood situation.

In both animal and plant disease bacteriology it is known that pathogenic microorganisms may vary considerably, even within indi-

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[3] Reference is made by number (italics) to Literature Cited, p. 284.

vidual species, in virulence and in ability to produce disease. Furthermore, as stated by Zinsser (*31, p. 188–189*)—

> Whether or not infection occurs depends also upon the *number of bacteria* which gain entrance to the animal tissues. A small number of bacteria, even though of proper species and of sufficient virulence, may easily be overcome by the first onslaught of the defensive forces of the body. Bacteria, therefore, must be in sufficient number to overcome local defenses and to gain a definite foothold and carry on their life processes, before they can give rise to an infection. The more virulent the germ, other conditions being equal, the smaller the number necessary for the production of disease. The introduction of a single individual of the anthrax species, it is claimed, is often sufficient to cause fatal infection; while forms less well adapted to the parasitic mode of life will gain a foothold in the animal body only after the introduction of large numbers.

In the case of American foulbrood the quantity of infectious material that honey must carry in order to produce disease in a colony has never been determined. White (*30, p. 20, footnote 1*) states, in connection with inoculating healthy colonies experimentally with *Bacillus larvae:*

> It was found that less than one scale is sufficient disease material to produce a considerable amount of disease in the colony. In some experiments one scale, therefore, might supply all the spores needed although the use of a somewhat greater quantity of material is advisable in most instances.

While infected honey no doubt does become mixed with disease-free honey, it is probable in many cases that, because of the practice of using large settling and storage tanks, infected honey would be so diluted with spore-free honey as to make the spore content insufficient to produce infection even if fed to healthy bee larvae. Therefore, one object of these investigations was to determine the minimum number of spores of *Bacillus larvae* in honey necessary to produce American foulbrood in healthy colonies of bees as correlated with the infectivity or spore content of the average commercial honey.

In order to obtain information relative to this subject, experiments were conducted in the apiary over a period of five years. In these experiments honey or sugar sirup with a known content of spores of *Bacillus larvae* was fed to healthy colonies and the minimum number of spores that would produce infection was determined. At the same time laboratory studies were carried on with cultures of spores of *B. larvae*, concerning certain growth phases of the organism, particularly the minimum number of spores that would produce vegetative growth on artificial culture media. Methods for demonstrating the presence or absence of spores of *B. larvae* in samples of commercial honeys were also investigated, and these honeys were studied in relation to their infectiousness as correlated with the spore-feeding experiments. These three phases of the investigation will be discussed in the order mentioned.

MINIMUM NUMBER OF SPORES OF BACILLUS LARVAE NECESSARY TO PRODUCE DISEASE IN HEALTHY COLONIES OF BEES

METHODS OF PROCEDURE

Location of Experiments

These investigations were started during the summer of 1926 in a small experimental apiary located about half a mile from the bee culture laboratory of the Bureau of Entomology at Somerset, Md. The location at Somerset was undesirable, however, because of its

close proximity to the apiary connected with the laboratory and to other privately owned colonies of bees, necessitating extreme precautions to prevent spread of the disease. In 1927 the experimental work was transferred to the Intermountain States bee culture field laboratory at Laramie, Wyo.[4] In Wyoming an ideal isolated location was found about 14 miles east of Laramie in the Medicine Bow National Forest, the nearest colonies of bees being at least 14 miles away and probably farther. Since this location is more than 8,000 feet above sea level, there is only a slight nectar flow from wild flowers, which assures the immediate use of any inoculated sirup fed to colonies of bees. In fact, after the middle of the summer it was found necessary in most cases to feed the experimental colonies with uninoculated sugar sirup in order to prevent starvation.

In 1927 and 1928 the colonies used for experimentation were located in two yards between a quarter and a half mile apart. The arrangement of the colonies in the two yards was such as to prevent drifting as much as possible. In 1929 and 1930, in order to limit still further the danger of transmission of disease because of drifting or robbing, 20 colonies were stationed in pairs, so arranged as to minimize the danger from drifting, in 10 isolated locations at least a quarter of a mile apart.

Make-up of Colonies

Five-frame nucleus hives were used for the spore-feeding experiments. The colonies were prepared either with two or three frames of brood, honey, and adhering bees taken from healthy colonies, together with a young laying queen, or, as in 1927, 1928, and 1929, by placing a 2-pound package of bees containing a laying queen on foundation or on combs containing honey from healthy colonies and feeding them sugar sirup. During a good honey flow these small colonies were allowed to build up in the apiary connected with the laboratory until they consisted of three or four frames of brood before they were moved to the isolated locations. The bees making up the colonies used for the feeding experiments from 1927 to 1930 at Laramie, Wyo., were all from the same general strain.

Material Used for Inoculation

Spores of *Bacillus larvae* were obtained from American foulbrood scales in combs taken from diseased colonies located in the States of Maryland, Iowa, and Wyoming. The strain used at Somerset, Md., was obtained from a sample sent to that laboratory for diagnosis. Two different strains were used at Laramie during 1927, 1928, and 1929, one obtained from a diseased colony in the experimental apiary belonging to the University of Wyoming and one obtained from a beekeeper at Lander, Wyo. In 1930 three other strains were used in the feeding experiments, one from Iowa and two from apiaries in Wyoming.

Preparation of Spore Suspensions

In preparing the spores for feeding to the healthy colonies, scales were removed from the combs by means of sterile forceps (the necessary precautions being taken against contamination) and placed in

[4] This laboratory is maintained cooperatively by the University of Wyoming and the U. S. Department of Agriculture.

a flask containing 50 c c of sterile water and glass beads. After the scales had softened in the water, the flask was shaken for one-half hour to insure complete maceration of the scales. The suspension was then filtered through two thin layers of sterile absorbent cotton into another sterile flask in order to remove any lumps or débris.

In preparing the stock suspensions of spores, at first 75 to 100 scales were taken by counting. Later it was found that the average American foulbrood scale weighs 0.0223 g. Therefore, the 100 scales for the stock suspensions were obtained by weight, the scales being weighed in a sterile covered glass dish before they were deposited in the flask of sterile water.

After the suspension had been filtered and tested for contamination and was ready for use, the number of spores per cubic centimeter was determined by the following method: By means of a blood-diluting pipette giving a dilution of 1 to 20, the spore suspension was diluted with a weak solution of carbol fuchsin and a drop placed in the counting chamber of a Helber bacteria-counting cell 0.02 mm deep and ruled in squares of 0.0025 mm^2 each.[5] With the use of two 15× eyepieces in a binocular microscope and a 1.8-mm oil-immersion objective, the spores in 25 squares of the Helber chamber were counted. Then by means of the formula

$$\frac{\text{Total spores counted} \times \text{dilution} \times 20{,}000 \times 1{,}000}{\text{Number of squares counted}}$$

the approximate number of spores per cubic centimeter in the suspension was determined.

Later this method was checked by the method of Breed and Brew (*2*) for counting bacteria in milk. With the aid of a binocular microscope having two 15× eyepieces and a 1.8 mm oil-immersion objective, the area of a circle etched on an ocular micrometer disk was determined by means of a stage micrometer. One one-hundredth cubic centimeter of a 1 to 100 dilution of the stock suspension of spores was placed on a glass slide on which 1 cm^2 had been ruled with a diamond pencil. This was mixed with a small loopful of carbol fuchsin stain and the whole spread over the 1 cm^2 of surface[5] and allowed to dry uniformly. The number of spores per cubic centimeter of the stock suspension was determined according to the formula

$$\frac{\text{Area 1 cm}^2}{\text{Area of circular field}} \times \frac{\text{total number of spores counted} \times \text{dilution} \times 100.}{\text{number of circular fields counted}}$$

These two methods were found to check fairly closely within the limits of the precision of the methods used in counting. Furthermore, by both methods it was found that in the majority of cases 100 scales in 50 c c of water give approximately 5,000,000,000 spores per cubic centimeter for each suspension made up in this way. Therefore, this number was used as a standard for making all dilutions.

[5] Mm^2 and cm^2 are the abbreviations for square millimeter and square centimeter, respectively, recently adopted by the Style Manual for United States Government printing.

After a considerable number of counts had been taken in making up several stock suspensions of spores, counting was eliminated and the spore content of the stock suspensions was standardized according to the method described by Gates (*11*, p. 114), as follows: "The opacity of a bacterial suspension is measured by the length of a column of the suspension required to cause the disappearance of a wire loop." An instrument known as a suspensiometer was used for this purpose. The use of this method saved considerable time and labor without appreciably affecting the precision of the counts. One liter of a 50 per cent solution of sugar in water was used as the standard quantity of inoculated sirup fed to each experimental colony. A series of dilutions of the original stock suspension containing 5,000,000,000 spores was made by adding different quantities of the spore suspension to 1 liter of sugar sirup. In this way the approximate total number of spores in each liter of sugar sirup to be fed to colonies of bees was known.

Method of Inoculating Colonies

In 1926 at Somerset, Md., the sugar sirup containing the various dilutions of spores was fed to the colonies by means of galvanized-iron troughs that were hung inside the hives after two combs had been removed. In these troughs sterile excelsior was placed for the bees to walk on in order to prevent them from drowning. This method was found unsatisfactory, however. At Laramie, Wyo., the sugar sirup containing the spores was first placed in Boardman feeders, but owing to the danger of robbing at the entrance of the hives, the method finally used was to invert the jars in holes bored in the hive covers. In this way any leakage into the hives was cleaned up by the bees without danger of causing robbing. To prevent the jars from being broken or knocked over, box covers were placed over them and fastened to the hive covers. Each colony was usually inoculated only once with an individual dilution of spores. Duplicate colonies were inoculated with each dilution of spores. Uninoculated check colonies were placed among those that were inoculated.

PRIMARY OBSERVATIONS

Observations of the condition of the brood were made at least once a week, and sometimes oftener, after the colony was given the liter of inoculated sirup. In 1926 at Somerset, Md., as soon as diseased larvae appeared in a colony, the colony was killed and at once removed from the apiary. Because of the isolated location near Laramie, Wyo., the colonies were left until the end of the brood-rearing season, when final observations were made.

The results of the spore-feeding experiments are shown in Table 1.

TABLE 1.—*Results of spore-feeding experiments* [a]

[Duplicate colonies of bees (A and B) were used in the first 4 years, and triplicate colonies (A, B, and C) in 1930]

Total number of spores fed	Extent of foulbrood in—																		
	1926		1927				1928, repeat		1928				1929				1930, final		
			During season		Final				During season		Final		During season		Final				
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	C
5,000,000,000	+	?+																	
2,500,000,000	+	+							+	+	+	+							
1,000,000,000	+	+							+	+	−*	+							
750,000,000	+	+																	
500,000,000	+	+	+		−*				+	+	−*	+	+	+					
350,000,000	+	0	+		+				+		+								
200,000,000	+	?+	+	+	−*	−*	+												
175,000,000			+	?+	+	−*		+											
150,000,000			?+	+	−*	−*	−	−											
125,000,000			0	?+	0	−*	0	−											
100,000,000	0	0	0	?+	0	−*	0	−	+	+	−*	+							
75,000,000			+	?+	−*	−*		+	0	+	0	+	+	0	+	0			
50,000,000	0	0	0	0	0	0			0	+	0	−*	0	+	0	−*	0	0	0
25,000,000													0	0	0	0	0	0	0
10,000,000	0	0											0	0	0	0			
5,000,000													0	0	0	0			
2,500,000													0	0	0	0			
1,500,000													0	0	0	0			
500,000													0	0	0	0			
100,000													0	0	0	0			
Controls	1+,12−0		1+,2−0		1+,2−0		1−0		1+,3−0		1+,3−0		8−0		8−0		2−0		

[a] +, Positive American foulbrood; ?+, probable American foulbrood, very slight and unconfirmed and disappearing by end of brood-rearing season; 0, no disease found during season; −*, disease cleaned out by end of brood rearing; −, no recurrence in second season.

In 1926 a total of 200,000,000 spores fed to a colony was the smallest number that produced disease; in 1927, on the other hand, 75,000,000 was the smallest number. However, in the latter year the spores were obtained from another locality in which environmental conditions were quite different. In an effort to obtain check results, the feeding experiments were repeated in 1928. Through an error in making up the spore dilutions, which was not discovered until too late for rectification, no colony received less than 50,000,000 spores. This season one colony of the pair receiving an inoculation of 50,000,000 spores became infected. The feeding experiments were repeated again in 1929, with dilutions of spores from 75,000,000 down to 100,000—considerably less than the minimum number in 1928. Again only one colony of the pair receiving a total of 50,000,000 became infected. As a result of two years' experiments this was found to be the apparent minimum number of spores of *Bacillus larvae* capable of producing infection when fed in 1 liter of sugar sirup. In 1930 spores from three different localities were fed in duplicate to six healthy colonies in dilutions of 50,000,000 and 25,000,000 without producing disease.

It is therefore apparent that a certain minimum number or mass of spores is required to start the initial action capable of producing American foulbrood in healthy larvae. Under the conditions of these experiments this minimum number was approximately 50,000,000 spores of inoculum per liter of sirup.

SECONDARY OBSERVATIONS

During the first three years of the experiments, or previous to 1929, at which time the experimental colonies were isolated in pairs, certain of the uninoculated control colonies developed disease, 1 out of 13 in

1926, 1 out of 3 in 1927, and 1 out of 4 in 1928. It was assumed that the disease was probably not spread by robbing, since no active robbing was observed at any time. In practically every case where a control colony became infected, it was so located in relation to the inoculated colonies that drifting of young nurse bees during play flights could account for the spread of the disease, in one or two cases quite definitely so. In 1929 all eight uninoculated colonies, although they were not located with the inoculated colonies but were within robbing range of all, remained free from disease. The prevention of drifting apparently eliminated the casual spread of disease.

Occasionally a colony of bees affected with American foulbrood will try to clean out the diseased remains, often removing parts of the scales and sometimes actually tearing a comb down to the midrib in order to do this. White (*30, p. 34–35*) states:

> There is considerable evidence to support the belief that occasionally in cases of light infection the disease may disappear unaided by treatment. * * * It should be emphasized that such a course for the disease, if it occurs at all, is unusual. Although American foulbrood spreads more or less rapidly within an infected colony, the fact remains that it frequently does not.

Lineburg (*16*) in 1925 reported that in two colonies which were diseased in the spring the disease apparently disappeared later in the season. Three colonies were divided and used for making increase in June and July, but all remained free from disease, at least until the end of that season. Further observations were not reported. Corkins (*8*) in 1928 reported five colonies which were given combs containing scales of American foulbrood at the beginning of the honey flow of 1927 and developed no disease up to July 10, 1928. Two other colonies were observed to have cleaned out the disease and remained healthy for an entire season. However, during the several years of his experimental work on American foulbrood, the writer never observed a colony in which the disease was permanently cleaned out until 1927. In that year, of 16 colonies inoculated with various dilutions of spores, 4 colonies, 2 of which received more than the probable minimum dose causing infection, showed no disease during the season. The disease completely disappeared by the end of brood rearing in 10 of the 12 other colonies that had showed either positive or probable disease some time during the summer. In 1928 package bees were placed on the combs of seven of these colonies that had apparently cleaned out the disease during the previous summer and on two that had been inoculated with presumably a sufficient number of spores but which had remained healthy. Three of the seven developed disease again the second season, while four remained healthy during the entire season. Neither of the two inoculated colonies that had remained free from disease in 1927 developed it in 1928. Of the 11 colonies inoculated in 1928 that developed disease, 4 cleaned up the disease by the end of the brood-rearing season and 2 inoculated colonies showed no disease. In 1929, 1 of the 2 colonies developing disease cleaned up by the end of the brood-rearing season, making a total of 15 cases in which the disease was cleaned up by the end of brood rearing. Two of the colonies inoculated with the minimum infectious dose or more showed no disease during that summer.

It is possible that, in the high altitude of Laramie, and in similar places where the air is very dry, the scales of American foulbrood

become dried without adhering so tenaciously to the cell walls as they do in more humid climates at lower altitudes. These observations indicate the necessity of further work on the resistance of bees to the disease and variation in virulence of different strains of the organism.

INOCULATION OF INDIVIDUAL BEE LARVAE WITH DEFINITE NUMBERS OF SPORES OF BACILLUS LARVAE

In the light of the results of the foregoing experiments, in which colonies were inoculated with presumably a quantity of spores sufficient to produce infection but in which no disease developed, the question arises as to what became of the spores in the sugar sirup, some of which presumably were fed to healthy larvae. In those colonies developing disease that received a minimum number of spores, how many spores did each larva developing the disease receive? In order to obtain information on these points, a preliminary series of experiments was planned in which individual larvae were inoculated with known numbers of spores.

Toumanoff (*29*) reports that he was unable to cause infection by giving individual larvae a drop of a rich emulsion of a culture of *Bacillus larvae* in salt solution. He found that many of the larvae so treated were removed from the cells by the bees, and those remaining failed to develop disease. He further found that larvae given only uninoculated salt solution were also removed in the same way. Therefore, in the present experiments sugar sirup was used instead of salt solution. In a comb from a healthy colony containing numerous coiled larvae, a drop of an uninoculated 50 per cent solution of sugar in water was placed in each cell containing a larva, as near the mouth parts of the larva as possible. The rim of each cell so treated was marked with a paint consisting of 1 part of liquid white shellac, 1 part of a paint pigment, and 4 parts of ethyl alcohol. The sugar sirup was slightly colored with water-soluble eosin in order to aid in determining the effect. Frequent observations showed that practically all larvae that were fed this colored sugar sirup developed normally and were sealed over, the pigment markings still being present on the edges of the cappings. In most of the cells a residue of colored sirup could be observed for several hours after the larvae had fed.

A series of 5-frame nuclei was prepared, each containing one or two combs having a large number of unsealed larvae. A set of dilutions of spores was made from a stock suspension with a sterilized 50 per cent sugar sirup in such a way that each 0.01 c c of the dilution would contain an approximate known number of spores, as indicated in Table 2. Sterilized 2 c c Luer tuberculin hypodermic syringes graduated in 0.01 c c, the needles of which had been blunted, were used in inoculating the cells containing coiled larvae. Fifty or more coiled larvae at least 4 days old were each given 0.01 c c of a dilution of spores, each dilution being given to larvae in one comb in a separate colony, and the cells so inoculated were distinctively marked. A few larvae that had just been sealed also were inoculated by puncturing the capping with the inoculating needle and depositing the 0.01 c c in the cell. Observations were taken at the end of 24 hours and at frequent intervals thereafter until the end of the brood-rearing season.

TABLE 2.—*Results of inoculation of individual healthy bee larvae with known numbers of spores of bacillus larvae, 1930*

Spores fed each larva in 0.01 cubic centimeter of dilution	First inoculation, July 14		Second inoculation, Aug. 1			Third inoculation, Aug. 19							Fourth inoculation, Aug. 21							
	Colony No.	Larvae developing disease Aug. 1	Colony No.	Larvae developing disease		Colony No.	Length of time isolated from nurse bees	Effect on larvae of being isolated from nurse bees	Larvae developing disease				Colony No.	Length of time isolated from nurse bees	Effect on larvae of being isolated from nurse bees	Larvae developing disease			
				Aug. 19	Oct. 7				Aug. 26	Sept. 9	Sept. 19	Oct. 7				Aug. 26	Sept. 9	Sept. 19	Oct. 7
Number		*Number*		*Number*	*Number*		*Hours*		*Number*	*Number*	*Number*	*Number*		*Minutes*		*Number*	*Number*	*Number*	*Number*
50,000,000	------	------	------	------	------	12	1	Larvae removed	0	0	0	0	12	5	Larvae removed	0	0	0	0
25,000,000	------	------	------	------	------	13	1	----do	0	0	0	0	13	10	----do	0	0	0	0
10,000,000	------	------	------	------	------	20	¾	Larvae not removed	0	[1] 13	------	------	20	15	Larvae not removed	0	[a] 9	------	------
7,500,000	------	------	------	------	------	15	1	Three-fourths of larvae removed.	0	0	0	0	15	20	----do	0	0	0	0
5,000,000	------	------	14	0	0	4	¾	One-half of larvae removed.	0	0	0	0	4	30	One-half of larvae removed.	0	0	0	0
1,000,000	------	------	34	0	0	16	1	Larvae not removed	0	0	0	0	------	------	------	------	------	------	------
500,000	------	------	16	0	0	22	1	----do	0	0	0	0	------	------	------	------	------	------	------
250,000	------	------	18	0	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------
100,000	------	------	22	0	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------
75,000	------	------	26	0	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------
50,000	------	------	28	0	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------
25,000	------	------	4	0	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------
10,000	------	------	15	0	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------
7,500	------	------	20	0	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------
5,000	12	0	12	0	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------
1,000	13	0	13	0	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------
750	14	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
500	15	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
250	18	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
100	22	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
75	26	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
50	28	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
25	4	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
10	15	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
5	20	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
1	34	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------
0	32	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------

[a] Number of cells showing American foul-brood remains.

In the first series of inoculations the number of spores fed each larva ranged from approximately 5,000 down to 1. None of the larvae inoculated developed disease. (Table 2.) Later a second series of inoculations was made. The same colonies were used because of the limited number available, but the larvae inoculated were in a different comb in each colony and a different color was used to mark the cells. In these inoculations the number of spores fed ranged from 5,000,000 down to 1,000 per larva. No disease developed from this set of inoculations.

It was thought possible that the nurse bees might be removing most, if not all, of the inoculated sugar sirup before the larvae had had time to ingest a sufficient number of spores to bring about infection. Therefore, in a third series of experiments each inoculated comb was placed in a screen-wire queen-nucleus introducing cage, and this cage was put back in the colony for periods ranging from one-half to one hour before the unprotected comb was replaced in the colony, thus theoretically giving the larvae time to ingest some of the sugar sirup before the nurse bees had access to the inoculated cells. In these tests the larvae were kept from the bees so long that many of them, becoming hungry, were starting to crawl from the cells. The number of spores fed ranged from 50,000,000 down to 500,000 per larva. Twenty-four hours after the larvae were fed it was found that all receiving 50,000,000 and 25,000,000 spores had been removed from the cells, while those receiving a smaller number of spores were either partly removed or remained in the cells, according to the strength of the dilution and the length of time that the larvae were kept away from the nurse bees. (Table 2.)

Two days later another set of larvae was inoculated with the same dilutions as were previously used for these colonies but on the other side of the same combs. In this series the combs were kept away from the bees for periods ranging from 5 minutes for the heaviest dilution to 30 minutes for the weakest. Again all the larvae receiving the 50,000,000 and 25,000,000 spores were removed, while those receiving the 5,000,000, which were kept from the bees for half an hour, were partly removed, and those receiving 7,500,000 or 10,000,000 were not removed. Apparently there are two factors concerned in the removal of the larvae—the length of time they are kept away from the bees and the amount of foreign matter in the sirup, as indicated by the spore content, that is given to the larvae.

The results of the last two series of inoculations showed that in the colonies in which the larvae were not removed, or were not entirely removed, several larvae in the colony receiving 10,000,000 spores per larva developed disease, while those in the colonies receiving a smaller number remained healthy. (Table 2.) This work should be repeated with a different colony for each set of inoculations, although apparently the disease did not spread in the colonies used. Only one colony of the entire number developed disease. Although a certain degree of success was obtained, these results seem to bear out Toumanoff's (*29*) conclusion that the artificial infection of individual larvae is not brought about so easily as one had been in the habit of believing. Apparently, also, a considerable number of spores are necessary to establish an infection under these conditions.

MINIMUM NUMBER OF SPORES OF BACILLUS LARVAE PRODUCING VEGETATIVE GROWTH ON ARTIFICIAL CULTURE MEDIA

Bacteria are known to pass through a definite cycle of growth, particularly when cells from an old culture are transferred to fresh culture media. The growth stages have been described by Buchanan (*3; 13, Ch. V*), Henrici (*12*), and Winslow (*13, Ch. VI*) somewhat as follows: The initial stationary phase during which no growth takes place; the logarithmic phase when the organisms begin to divide, slowly at first but gradually accelerating; and so on through the complete cycle of growth. Henrici (*12, p. 21, 24*) has observed that—

> Various factors, as temperature; the size, the age, and previous history of the inoculum; and the composition and nutrient value of the medium, influence the growth curves of bacteria. * * * Of the various factors which influence the rate of growth and form of the growth curve, the initial number of cells introduced into a unit volume of medium seems to be one of the most important.

Robertson (*25*), in studies of cultures of certain protozoa, has shown that growth seems to be stimulated by the presence of other cells of the same type. This characteristic has been described at various times as mass action or communal activity.

Early in 1929, in conjunction with the spore-feeding experiments in the apiary, an investigation was started to determine whether there is a similar manifestation of mass action in the vegetative growth of spores of *Bacillus larvae* on artificial culture media. In a preliminary paper on this subject the writer (*27, p. 456*) made the following observations: Starting with a seeding of 5,000,000,000 spores of *B. larvae* on a suitable slanted solid culture medium, it was found at the end of 48 hours' incubation at 37° C. that growth had occurred in the original and in a diluted seeding containing 60,000,000 spores, but not in one containing 50,000,000 spores. Growth occurred in a diluted seeding containing only 5,000,000 spores after six days' incubation, and in one containing 700,000 spores after 10 days' incubation. (Table 4, Group 1.) These observations indicated that a certain initial mass of spores is necessary to start vegetative growth. Furthermore, although the growth results were rather irregular owing to the comparatively small number of cultures made, they seemed to show that, within certain limits, the smaller the seeding the longer the incubation period necessary to obtain germination of the spores and vegetative growth. From this preliminary work it was assumed that the lower limits of dilution of the stock suspension that would give growth on longer incubation had not been reached.

Ahrens (*1*) has observed, in cultural studies of scales treated with formalin solution for different lengths of time, that growth may occur in cultures from such scales after varying periods of incubation up to 30 days, depending on the length of treatment and the percentage of formalin in the solution. Burnside (*7*) states, in connection with studies of disinfection of American foulbrood combs by fumigation with formaldehyde gas, that "it is probable that if scales had been washed and the incubation period increased, growth of *Bacillus larvae* would have been obtained in some instances when negative results were recorded."

Therefore, a single trial series of cultures was run (No. 7, Table 4), the total incubation period being 30 days. Results from this set of cultures showed that in some cases growth was obtained after 30

days' incubation where no growth was observed after 10 days' incubation. Work on this phase of the problem was continued during the summer and fall of 1930. Several sets of cultures were made in which *Bacillus larvae* from eight different localities were used in a series of seedings with a decreasing number of spores for each lot of the organism and all incubated for 30 days. (Table 4, Group 2.)

METHODS OF PROCEDURE

Culture Media

A culture medium was used similar to that employed by the writer in the preliminary experiments (*27*) and also in earlier cultural work with *Bacillus larvae* (*26*)—that is, a combination of the medium made of yeast-extract and egg-yolk suspension and the carrot-extract medium of Lochhead (*18*). The yeast-carrot extract medium was prepared as follows:

(A)	Dried yeast	grams	10
	Peptone	do	10
	Buffer (sodium glycerophosphate)	do	2. 5
	Water (distilled)	cubic centimeters	500

This solution was heated in flowing steam for one-half hour and, after a tablespoonful of siliceous earth had been added to assist in the filtration and clarification, it was filtered through filter paper on a perforated porcelain funnel with suction.

(B) Two hundred grams of cleaned carrots was macerated in a meat grinder, added to 500 c c of distilled water, and allowed to stand for at least 30 minutes, preferably longer. The macerated carrot was removed by filtration through fine muslin, as much liquid as possible being squeezed from the mass. The filtrate was then clarified by the addition of siliceous earth and filtration in the same manner as the yeast-extract medium.

(C) The final base medium was prepared by mixing 500 c c of A with 200 c c of B and adding 700 c c of a 3 per cent solution of washed agar.

The reaction of the medium was so adjusted that when 2 c c of sterile egg-yolk suspension, prepared as described in a previous paper (*26*), was added to 10 c c of the yeast-carrot extract base medium by means of the apparatus shown in Figure 1, and described previously (*26*), the pH value was 6.8. The medium was then sterilized in the autoclave at 15 pounds' pressure (sea level) for 15 minutes. After it had cooled to 45° C., 20 drops, or about 2 c c, of the sterile egg-yolk suspension was added to each tube of medium, mixed by shaking, and the medium was then allowed to solidify in a slanting position.

The Lochhead yeast-extract medium was tried without the addition of egg-yolk suspension, but although it gave good growth with the heavier seedings of spores, the combination medium was found to give more uniform germination and heavier vegetative growth with the more dilute seedings. The addition of the carrot extract, while possibly adding somewhat to the growth-producing qualities of the medium, served in these experiments as an indicator for vegetative growth because of the ability of *Bacillus larvae* to produce nitrite in the carrot-extract medium without the addition of potassium nitrate (*18*).

Preparation of Dilutions of Spores

The stock suspensions of spores of *Bacillus larvae* were made up as described earlier in this paper. A series of primary dilutions, each one-tenth of the preceding dilution, was then made up in sterile 125

c c flasks by adding 4 c c of a dilution to 36 c c of sterile water. The series of dilutions containing gradually decreasing numbers of spores per cubic centimeter to be used in inoculating the culture medium were then prepared as indicated in Table 4. Sterile burettes were used in adding the proper proportions of spore suspension or spore-suspension dilutions to the proper quantities of sterile water in sterile test tubes, in order to make up the desired series of dilutions containing approximately known numbers of spores.

Inoculation of Culture Medium

Swann has observed that in old cultures of anthrax a considerable percentage of spores are dead and therefore never germinate. Because of the possibility that some of the spores in the stock suspensions of *Bacillus larvae* might not be viable, an effort was made to determine the approximate proportions of viable and dead spores in the stock suspensions. Since the determination of viable spores of *B. larvae* by means of plate cultures is difficult because of the opaqueness of the special culture medium that is required, an attempt was made to determine the percentage of viable spores by the differential staining method of Burke (*4*) as modified by Koser and Mills (*15*). The procedure is as follows: A small quantity of the spore suspension is spread in a thin film on a slide and allowed to dry without heating. The slide, after immersion in a solution of carbol fuchsin at room temperature for two minutes, is washed in water and decolorized with absolute acetone for a few seconds, washed again, and immersed in Loeffler's alkaline methylene blue for two minutes, washed, dried, and examined. Very few solid-staining forms were observed in any of the suspensions examined, possibly one or two spores in several fields. It was therefore assumed that the number of nonviable spores could be considered as negligible and probably within the limits of the precision of the measurements as indicated by this procedure.

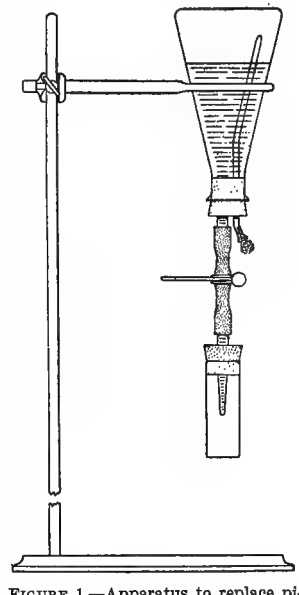

Figure 1.—Apparatus to replace pipetting of egg-yolk suspension

One cubic centimeter of each dilution was added to duplicate tubes of the slanted solid medium by means of sterile 1 c c pipettes, each cubic centimeter of inoculum containing an approximately known number of spores of *Bacillus larvae*. After inoculation the cultures were incubated at 37° C. In order to prevent the liquid in the tubes from drying out on long incubation, from time to time, as the water of condensation evaporated, 2 or 3 c c of sterile broth similar in composition to that of the base medium, without the egg, was added to each tube by means of the apparatus shown in Figure 1. A total of 556 cultures was made during this series of experiments.

Method of Making Observations

The culture tubes were incubated for 30 days at 37° C. Each tube was examined usually every 24 hours during this period. The presence or absence of vegetative growth was noted at each observation, and in cases of slight or doubtful growth the vegetative growth was checked both by microscopic examination of a stained smear and by testing for nitrite production in the culture medium by the sulphanilic acid and alpha-naphthylamine acetate test. After a large number of such observations had been made, it was found that vegetative germination of spores of *Bacillus larvae*, almost too slight to be seen, would give a definite pink color on the addition of the reagents.

Lochhead (*17, p. 14*) states:

> It was found, however, that ordinary nitrate-reducing species, such as *B. cereus* or *Es. coli*, which are able to form nitrites readily in nitrate media, were unable to produce nitrites in recognizable amount in the peptone-carrot media, though capable of doing so upon the addition of nitrates. *Bacillus larvæ* under the same condition readily forms nitrites without the addition of nitrate to the medium.

Despite this statement, a series of miscellaneous organisms was tested in standard nitrate broth, in carrot-extract broth, and on carrot-extract agar. Several organisms that commonly reduce nitrates and a few that do not were used. (Table 3.) Observations were made at short intervals during the first 24 hours. Most of these organisms gave positive nitrite tests within a few hours after inoculation in all the media used, but in the carrot-extract medium the nitrate had apparently disappeared in most cases after 24 hours' incubation, and in all cases after 48 hours. The same organisms on standard nitrate medium still gave positive tests after 48 hours' incubation. A positive nitrite test was obtained in cultures of *Bacillus larvae* that were incubated for 5 days and in one culture that was incubated for 4 days and then allowed to stand at room temperature for 16 days more before testing. Therefore, it appears probable—at least the results in Table 3 indicate—that in the case of many contaminating organisms having the power to reduce nitrite that might get into the culture tubes inoculated with spores of *B. larvae* the nitrite, if produced by the contaminating organism, would have disappeared after 48 hours' incubation, leaving contamination to be determined by gross appearence of the culture and microscopic examination. Nevertheless, in order to be sure that contaminating growth of any kind was not giving erroneous results with the nitrite test when this was used alone, any suspicious-looking growth in the culture tubes was examined under the microscope before it was tested with the reagents for nitrite production. Even though a positive nitrite test might be observed in some cases, the contaminations were recorded only as such.

Observations and Results

In no instance was positive growth obtained in cultures inoculated with less than 50,000 spores, even after 30 days' incubation, and growth with 50,000 spores was obtained from only two of the eight lots of spores used, namely, Nos. 19 and 23. (Table 4.) In the other six strains the minimum number of spores that produced positive growth ranged from 5,000,000 to 70,000.

TABLE 3.—*Nitrate reduction by various miscellaneous organisms in standard nitrate broth and carrot-extract media during different periods of time* [a]

Organism	Standard nitrate broth					Carrot-extract broth							Carrot-extract agar					
	3 hours	6 hours	8 hours	24 hours	48 hours	3 hours	6 hours	8 hours	10 hours	14 hours	16 hours	24 hours	3 hours	6 hours	24 hours	48 hours	5 days	20 days
Escherichia communior	3+	4+	4+	4+	4+	2+	−	−	−	−	−	−	+	+	−	−		
Escherichia coli	−	3+	4+	4+	4+	−	+	−	−	−	−	−	+	+	−	−		
Eberthella typhi	−	3+	4+	4+	4+	−	−	−	−	−	−	−	+	±	−	−		
Alcaligines fecalis	+	3+	4+	±	−	Tr.	2+	2+	+	+	−	−	2+	+	+	−		
Aerobacter aerogenes	3+	4+	4+	4+	4+	+	−	−	−	−	−	−	Tr.	Tr.	±	−		
Pseudomonas aeruginosa	−	3+	4+	−	−	+	2+	2+	+	±	−	−	+	+	+	−		
Serratia marcescens	−	3+	4+	4+	4+	−	+	−	−	−	−	−	Tr.	Tr.	+	−		
Staphylococcus albus	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−		
Bacillus subtilis				−	−							−			−	−		
Bacillus mesentericus				−	−							−			−	−		
Bacillus larvae				2+	4+							2+			2+	4+	4+	4+

[a] +, 2+, 3+, and 4+ indicate relative degrees of reaction; Tr. indicates trace; − indicates no reaction; ± indicates that the reaction was doubtful.

TABLE 4.—*Vegetative growth in cultures of Bacillus larvae with varying dilutions of a stock suspension*

Method of making dilutions			Spores per cubic centimeter inoculated ($\times 10^3$)	Strains of bacillus larvae [a]													
				Group 1 (preliminary experiments)						Trial series	Group 2						
Primary dilution taken	Quantity of primary dilution taken	Water added		No. 1	No. 4	No. 5	No. 6A	No. 6A2	No. 6D	No. 7	No. 12	No. 13	No. 14	No. 15	No. 19	No. 20	No. 21	No. 23
	Cubic centimeters	*Cubic centimeters*																
	Original (A)	0	*Number* 5,000,000	+2 +2	+2 +2	+2	+2	+6	+10	+5	+2 +2	+2 =2	+2 +2	+2 +2	+2 +2	+2 +2	+2 +1	+2 +2
	8	2	4,000,000			+2												
A	6	4	3,000,000			+2												
	4	6	2,000,000			+2												
	2	8	1,000,000			+2												
	1 (B)	9	500,000	+2 +2	+2 +2	+2	+2	+6	+10	+5	+2 +2	+2 +2	+2 +2	+2 +2	+2 +2	+2 +2	+2 +2	+2 +2
	8	2	400,000		+2	+2	+2											
	6	4	300,000		+2	+2	+2											
	4	6	200,000		+2	+2	+2											
B	2	8	100,000		±2?	+2	+2											
(1A+9H$_2$O)	1.8	8.2	90,000			+2	+2											
	1.6	8.4	80,000			+2	+6											
	1.4	8.6	70,000			−2	+6											
	1.2	8.8	60,000			−2	+2											
	1.0 (C)	9.0	50,000	±2±2	±2?−2	−2	−6	+6	+10	+5+30	+3 +3	+3 +3	+3 +3	+2 +2	+2 +2	+4 +4	+5 +5	+4 +4
	8	2	40,000			−2	−6	−6	+10	+10+10	+3 +3	+3 +5	+4 +4	+2 +2	+2 +2	+4 +4	+5 +5	+4 +4
	6	4	30,000			−2	+6	−6	+10	+10+15	+3 +3	+5 +5	+4 +4	+2 +2	+3 +3	+4 +6	+5 +5	+4 +4
	4	6	20,000			−2	−6	+6	+10	+10+30	+3 +3	+5 +5	+4 +4	+3 +3	+3 +3	+4 +6	+7 +7	+4 +4
C	2	8	10,000			−2	−6	+6	+10	−30−30	+7 +8	+5 +7	+6 ×	+3 +3	+6 ×	+6 +6	+7+14	+4 +4
(1B+9H$_2$O)	1.8	8.2	9,000			−2	+6	+6	−10	−10+30				+4 +4	+6 +6	+6 +6	+9+27	+4 +4
	1.6	8.4	8,000			−2	−6	+6	+10	−30−30				+4 +4	+6+16	+6+11	−27−30	+4 +4
	1.4	8.6	7,000			−2	−6	+6	−10	+10−10				+4 +4	−30−30	+6 +6	−30−30	+4 ×
	1.2	8.8	6,000			−2	−6	−6	+10	−30−30				+5 +6	+6−30	+6 +6	+7−30	+4 +4
	1.0 (D)	9.0	5,000	−2−2	−2 −2	−2	−6	+6	+10	−10+30	+7−30	+14−30	+12−30	+6 ×	−30−30	+11+14	−30−30	+4 +4
	8	2	4,000						+10	−10−30	+7+12	× ×	+6+10	−30 ×	+8−30	−30×	+17−30	+4 +4
	6	4	3,000						+10	−10+30	+6+14	+ 7×	+10+10	−30−30	+8×	+6+12	+27+27	+6 ×
	4	6	2,000						−10	−10+30	+6−30	+17−30	+20−30	−30−30	+8−30	+27−30	−30−30	+6 +6
D	2	8	1,000						−10	−10−30	+10+14	+21×	−30−30	−30−30	−30−30	−30−30	−30−30	+6 ×
(1C+9H$_2$O)	1.8	8.2	900						−10	−10+30	+6×	−30×	+10+16	−30−30	−30−30	+25−30	−30−30	−30−30
	1.6	8.4	800						−10	−10+30	+14+22	+21−30	−30×	−30−30	−30−30	+20−30	+27−30	−30−30
	1.4	8.6	700						+10	−10−30	−30−30	+30×	−30×	−30−30	+8+10	+30−30	+27−30	+8×
	1.2	8.8	600						−10	−10+30	+27−27	−30−30	−30×	−30−30	+13−30	−30−30	−30−30	+6−30
	1.0 (E)	9.0	500	−2−2	−2 −2	−2	−6	−6	−10	−10−30	−30−30	−30−30	−30×	−30−30	−30−30	−30−30	+27+27	−30−30

E (1D+9H₂O)	8		2	400						−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30×	−30−30
	6		4	300						−10+30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30	+27−30	−30−30
	4		6	200						−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	+14−30	−30−30	−30−30
	2		8	100						−10−30	−30−30	+30−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30
	1.8		8.2	90						−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30	+8−30
	1.6		8.4	80						−10−30	−30−30	+30−30	−30−30	−30−30	−30−30	−30−30	−30−30	+8−30
	1.4		8.6	70						−10−30	−30−30	−30−30	−30−30	−30−30	× −30	−30−30	+27+27	+6−30
	1.2		8.8	60						−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30	+8−30
	1.0	(F)	9.0	50	−2−2	−2 −2	−2 −6	−6	−10	−10−30	−30−30	−30−30	−30−30	−30−30	+13−30	−30−30	−30−30	+11−30
F (1E+9H₂O)	8		2	40						−10−30	−30−30	−30−30	×−30	−30−30	−30−30	−30−30		
	6		4	30						−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30		
	4		6	20						−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30		
	2		8	10						−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30		
	1	(G)	9	5	−2−2	−2 −2	−2 −6	−6	−10	−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30
G (1F+9H₂O)	8		2	4						−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30		
	6		4	3						−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30		
	4		6	2						−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30		
	2		8	1						−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30		
	1	(H)	9	0.5	−2−2	−2 −2	−2 −6	−6	−10	−10−30	−30−30	−30−30	−30−30	−30−30	−30−30	−30−30		

[a] Numbers in these columns refer to number of days of incubation before observations were made; + indicates vegetative growth; − indicates no vegetative growth; × indicates contamination; ± indicates that it was impossible to tell whether or not there was growth; ±? indicates that the positive was more doubtful than the negative. Where two numbers are given for a dilution, two cultures of this dilution was carried.

The length of the incubation period in relation to the decreasing number of spores used varied greatly with the different lots of spores, even with the duplicate inoculations of each lot. Table 5 gives the results of positive cultures obtained in relation to the period of incubation and the dilution of the spores. The coefficient of correlation (*14, p. 179*) for the positive cultures only, in relation to length of incubation and dilution of spores, was found to be 0.3558 ± 0.0440. While this does not show a strong correlation, it indicates that with the smaller numbers of spores there is a tendency for growth to take place with longer periods of incubation. However, when the cases of positive growth were correlated with the dilution and incubation time on the basis of the percentage of positive cultures to negative cultures for each observation period of incubation time, an insignificant negative correlation was obtained. Apparently there is a variable uncontrollable factor present, more obvious when spores are used from different lots of the organism, which makes it impossible to correlate the other factors closely. However, the data summarized in Table 6 indicate that, of the 120 cultures made with seedings of between 5,000,000,000 and 9,000,000 spores per seeding, 98.33 per cent showed growth at the end of 10 days' incubation, while 100 per cent (120 cultures) showed growth after 30 days' incubation. This is 56.87 per cent of the 211 total cultures showing growth after 30 days.

TABLE 5.—*Summary of the positive vegetative cultures of Bacillus larvae in relation to length of incubation period and with varying dilutions of the stock suspension*

Number of spores per cubic centimeter inoculated	Number of positive cultures after days of incubation																												Number of cultures		
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Positive	Negative	Total
5,000,000,000	16																													16	0	16
500,000,000	16																													16	0	16
50,000,000	4	6	4	2																										16	0	16
40,000,000	4	3	6	3																										16	0	16
30,000,000	2	4	5	4	1																									16	0	16
20,000,000		6	5	2	1	2																								16	0	16
10,000,000		2	2	1	4	3	1						1																	14	0	14
9,000,000			4		4			1																		1				10	0	10
8,000,000			4		2					1					1															8	2	10
7,000,000			3		2																									5	4	9
6,000,000			2	1	4	1																								8	2	10
5,000,000			2		1	1				1	1		2																	8	7	15
4,000,000			2		1	1	1		1		1					1														8	4	12
3,000,000					3	1	1		2		1		1													2				11	2	13
2,000,000					3		1									1			1							1				7	9	16
1,000,000					1				1				1							1										4	10	14
900,000					1				1						1									1						4	10	14
800,000													1						1	1	1					1				5	10	15
700,000							2		1																	1			2	6	7	13
600,000					1							1														1				3	12	15
500,000																										2				2	13	15
400,000																														0	15	15
300,000																										1				1	15	16
200,000													1																	1	15	16
100,000																													1	1	15	16
90,000							1																							1	15	16
80,000							1																						1	2	14	16
70,000					1																					2				3	12	15
60,000							1																							1	15	16
50,000										1		1																		2	14	16
0–40,000																														0	123	123
Total positive cultures	42	21	39	13	30	9	9	1	6	3	3	2	7	0	2	2	0	0	2	2	1	0	0	1	0	12	0	0	4	211		
Total negative cultures	514	493	454	441	411	402	393	392	386	383	380	378	371	371	369	367	367	367	365	363	362	362	362	361	361	349	349	349	345		345	
Total cultures																																556

TABLE 6.—*Summary of positive vegetative cultures of Bacillus larvae grouped in relation to size of seedings and length of incubation period*

Number of spores per seeding	10 days' incubation				11 to 30 days' incubation				Final observations after 30 days' incubation								
	Number of positive cultures	Number of negative cultures	Percentage positive	Percentage negative	Number of positive cultures	Number of negative cultures	Percentage positive	Percentage negative	Total number of positive cultures	Total number of negative cultures	Total number of all cultures	Percentage positive	Percentage negative	Percentage positive of total of all positive cultures	Percentage negative of total of all negative cultures	Percentage positive of total of all cultures	Percentage negative of total of all cultures
5,000,000,000–9,000,000	118	2	98.33	1.66	2	0	1.67	0	120	0	120	100	0	56.87	0	21.58	0
8,000,000–500,000	48	123	28.07	71.93	31	92	18.12	53.80	79	92	171	46.20	53.80	37.44	26.66	14.21	16.55
400,000–50,000	4	138	2.82	97.18	8	130	5.63	91.55	12	130	142	8.45	91.55	5.69	37.68	2.16	23.38
40,000–0	0	123	0	100	0	123	0	100	0	123	123	0	100	0	35.65	0	22.12
Total	170	386	30.58	69.42	41	345	7.37	62.05	211	345	556					37.95	62.05

Of the 171 cultures made with seedings between 8,000,000 and 500,000 spores per seeding, 48, or 28.07 per cent, showed growth at the end of 10 days' incubation, while 79, or 46.20 per cent, showed growth after 30 days' incubation. The latter number is 37.44 per cent of the 211 total cultures showing growth after 30 days' incubation.

Of the 142 cultures made with seedings between 400,000 and 50,000 spores per seeding, only 4, or 2.82 per cent, showed growth at the end of 10 days' incubation, while 12, or 8.45 per cent, showed growth after 30 days' incubation. The latter figure is 5.69 per cent of the 211 cultures showing growth after 30 days' incubation.

Of the 123 cultures made with seedings of 40,000 or fewer spores per seeding, no growth was obtained after 30 days' incubation.

Of the 556 cultures made with all seedings, 30.58 per cent showed growth at the end of 10 days' incubation and 69.42 per cent showed no growth. The 170 positive cultures after 10 days' incubation is 80.57 per cent (not shown in Table 6) of the 211 total positive cultures obtained. In the interval between the 10 and 30 day incubation periods, 19.43 per cent (not shown in Table 6) of the 211 total positive cultures, or another 7.37 per cent of all cultures made, showed growth, making a total of only 37.95 per cent of all cultures which showed growth at the end of 30 days' incubation, with 62.05 per cent still showing no growth.

The initial growth phases as described by Buchanan (*3; 13, Ch. V*) are clearly more marked with spores than with simple vegetative organisms, since there is a varying length of time necessary for spores to germinate and start growing after implantation in a suitable medium. In the light of observations on other spore-forming organisms, it is probable that this factor, which seems to cause variations in the germination time of *Bacillus larvae* even within a lot from a single source, is what has been termed "dormancy." Burke (*5, p. 283*), working with *Clostridium botulinum*, found:

> The individual (unheated) spores in a given culture of *Cl. botulinum* vary greatly in the time required for germination under optimum growth conditions. The majority germinate relatively quickly, but a few lie dormant for a longer time. One hundred and forty-four days is the maximum period of dormancy recorded here * * *.

Burke states:

> The primary factors which cause the spore to lie dormant for long periods of time under optimum growth conditions are believed to be inherent in the spore itself. It is thought that relative permeability of the spore wall is one of the factors. Environmental conditions may secondarily modify the period of dormancy.

Burke, Sprague, and Barnes (*6, p. 560*) observed the same phenomenon with such non spore-bearing bacteria as *Bacillus coli* (=*Escherichia coli*). They found that spores of *B. subtilis* remained dormant 39 days and those of *B. megatherium* 90 days, although a large majority developed in 4 or 5 days. They believe:

> Dormancy must be considered a factor in infection. It reduces the chances of infection by reducing the number of organisms that would otherwise start to grow at one time. Since the cells begin to multiply at different times, the body has an opportunity to initiate defensive reactions before all the cells develop. If dormant for a sufficient period, the organisms will be excluded from the body before development takes place.

Swann (*28*) has observed that there is a variation in the germination time of anthrax spores, depending on the age and condition of the spores.

Morrison and Rettger (*24, p. 339*) recently stated—

> Because of the marked variability of germination, depending upon the stimuli supplied in the environment, the deduction is made that bacterial spores in the process of germination are vitally active bodies having requirements for metabolic function which are the same as or more exacting and specific than those of the vegetative cells.
>
> Experimental evidence is presented to show that the dormancy of aerobic bacterial spores is largely, if not entirely, determined by conditions in the environment of the spores, and that these factors must be taken into consideration, perhaps specifically for each species, before so-called "inherent" or "normal" dormancy of bacterial spores can be established.

This phase of the work with *Bacillus larvae* is being repeated with the organism obtained from a single source in an effort to determine the importance of this variable factor of dormancy.

SPORES OF BACILLUS LARVAE IN COMMERCIAL HONEY

A few instances have been reported in the bee journals, such as that by Merrill (*22*), in which American foulbrood has developed as a result of bees having access to cans of infected honey that have been carelessly thrown out. Without doubt in some cases honey has been allowed to get on the market from infected colonies through negligence of the beekeepers and without being diluted by mixing or blending with honey from disease-free apiaries. On the other hand, Fracker (*10, p. 379–380*) has shown, by a study of disease-inspection statistics for Wisconsin:

> 1. In Wisconsin the introduction of this disease into the State and into many individual localities is definitely known to have been in specific importations of bees and equipment.
>
> 2. Cases of infection in which the source appears to be infected honey in the channels of trade are comparatively rare.
>
> 3. Even near such a large center as Milwaukee the infection percentage is greatest in localities of active movement, such as greenhouse areas, and is relatively low within the city itself.
>
> 4. Towns and cities of from 3,000 to 40,000 which have been natural markets for infected honey from near-by counties, have remained for years free from disease either until the present or until infected bees and equipment were introduced.
>
> 5. No new centers of infection are known to have been started since the policy of limiting movement of bees and equipment was begun in 1919.
>
> 6. These observations appear to be confirmed by conditions in the South, in spite of the fact that the period of active flight of the bee tends to continue through the peak of honey distribution.

Furthermore, F. L. Thomas, State entomologist of Texas, in an unpublished manuscript states:

> The largest of the estimates with reference to the quantity of honey that is brought into Texas in a year is 19 carloads. Most of this honey is produced in California, Colorado, New Mexico, Utah, and Wyoming. * * *
>
> If 19 carloads of foulbrood-infected honey are distributed annually in this State, it seems reasonable to suppose that our inspectors would have a hard time to keep this disease within bounds. In fact, I would expect to find that the inspectors would be gradually losing ground in their attempts to eradicate this menace. A large share of the honey which is imported is sold in west and northwest Texas where practically no bees are kept. The amount which is distributed in the beekeeping territory of the State is evidently less dangerous than is commonly supposed. The following facts, I think, will prove this statement.
>
> During the period September 1, 1920, to August 31, 1926, the inspection work has been carried into 100 counties. Fifty-six counties were found to be free

from contagious or infectious diseases of bees, but in the other 44 counties American foulbrood has been present.

An average of 668 beekeepers have been visited each year and 38,661 colonies examined with the result that an average of 430 colonies, or 1.11 per cent, have been found to be diseased.

American foulbrood is found now in only 23 counties, 21 of the 44 counties having been cleaned up. In 12 of the counties where disease occurs, only 30 colonies were found to be infected out of 7,642 examined—less than 0.4 of 1 per cent. Six counties had one diseased colony each.

About 40 per cent of the beekeepers and 60 per cent of the colonies are reinspected from year to year; the remainder, being free of disease and considered out of danger, are dropped and "new territory" is taken over and examined for presence of foulbrood. By "new territory" is meant beekeepers and their colonies visited and inspected for the first time. An average of 228 diseased colonies are discovered each year in "new territory." This is 1.6 per cent of the total number of colonies examined in this territory.

The reinspection which has been made in the counties where disease has been present shows that there have been both gains and losses. But a net gain has resulted which has averaged 21 beekeepers and 368 colonies freed from American foulbrood and quarantine annually.

From these facts it is easily seen that definite and really rapid progress in eradicating the disease is being made. Rarely do our inspectors find new outbreaks of disease that can not be traced to careless beekeeping methods, bees robbing infected and weakened colonies, or to the use of old and infected equipment.

It is not my intention to imply that honey is not a carrier of American foulbrood. The above evidence simply indicates that the honey which has been imported into Texas has not been as dangerous a source of disease to bees as is sometimes thought.

Practically no work has been reported on the microbiology of honey other than that in connection with the spoilage of honey through fermentation by yeasts (*19*, *21*), and no work appears to have been done on the *Bacillus larvae* spore content of commercial honey. In 1925 the writer undertook to devise a method for demonstrating, at least qualitatively, the presence or absence of spores of *B. larvae* in honey and their significance in relation to the results of the spore-feeding experiments. Difficulties were encountered in obtaining cultures of *B. larvae* from honey. It was impossible to obtain vegetative growth of this organism, even when a considerable number of spores had previously been added to honey, because of the difficulty of eliminating contaminating organisms that developed rapidly in the honey, completely overgrowing any possible vegetative growth of *B. larvae* before it could get well started. Therefore, methods of concentrating the spores from the honey and of identifying them by means of microscopic examination were attempted. Because spores of *B. larvae* have a characteristic appearance in stained smears (*20*, *p. 9*), it was assumed that this method might give at least tentative evidence.

METHODS OF PROCEDURE

The first method attempted was the filtration of honey diluted with water through a membrane of ether-alcohol collodion or through filter paper impregnated with an acetic acid solution of collodion (*9*). Apparatus was devised in which both suction and pressure were tried in this filtering process. Stained smears were made of the sediment retained on the surface of the filter. In several cases spores of *Bacillus larvae* were observed in stained smears of the sediment filtered out of honey known to have a large spore content. However, with honey containing fewer spores it was found impossible to concentrate them on a small enough area of filter in sufficient

numbers to recover and identify them under the microscope. Even with a comparatively large filtering surface, the process was so slow that the diluted honey would frequently start to ferment before it had all passed through the filter. A filter of smaller area would become clogged, preventing the passage of a sufficient quantity of honey.

Several unsuccessful attempts were made to recover spores of *Bacillus larvae* from honey by centrifuging samples diluted with an equal quantity of water. After considerable experimentation with honey of known spore content, it was found that it was necessary to dilute the honey to a much greater extent—1 part to at least 9 of water—in order to throw the spores down with the sediment. Apparently the specific gravity of these spores is so low that on centrifuging they remain in suspension in only slightly diluted honey.

The procedure finally used for demonstrating the presence of spores of *Bacillus larvae* in honey is as follows: Five c c of warmed honey is thoroughly mixed with 45 c c of distilled water in a 50 c c cone-shaped centrifuge tube made of heat-resistant glass. Duplicate quantities of each sample of honey are made up for examination. The diluted honey is then centrifuged at 2,000 revolutions per minute for one-half hour. Because of the difficulty of obtaining a satisfactory stained smear from the sediment thrown down in the presence of the sugars of the honey solution, all but 2 c c of the solution in each centrifuge tube is drawn off by means of a 50 c c pipette. Another 45 c c of distilled water is added, the sediment is thoroughly shaken up in the water, and the tubes are centrifuged again for 20 minutes. After all but 2 c c or less of the wash water has been removed, 0.01 c c of the sediment is removed by means of a capillary pipette and smeared on a cover glass over a surface of 1 cm^2, a small loopful of carbol fuchsin being mixed with the material before it is allowed to dry. After drying by gentle heat, the cover glass is mounted on a slide by means of a drop of distilled water and the smear is examined with an oil-immersion objective. Spores of *B. larvae* are identified by their size and shape in conjunction with their distinctive habit of breaking loose from the stained mass of the smear and of showing a delicate Brownian movement in the thin film of water between the two pieces of glass. In a few samples only one or two spores were seen in numerous fields examined or the spores did not have the typical appearance of spores of *B. larvae*. In such cases another test, in which twice as much honey was used, was made from the sample.

OBSERVATIONS

One hundred and ninety-one samples of honey were examined by this method. (Table 7.) Of these, 187 were regular commercial samples purchased in the open market and 2 were from the experimental apiary at Laramie. The other two were miscellaneous samples, one of which was obtained from a brood comb from a diseased colony and the other from a cappings melter which had been used with combs from an infected apiary.

TABLE 7.—*Results of the examination of samples of honey for the presence of spores of Bacillus larvae*

Source	Samples tested	Samples showing positive presence of spores resembling *Bacillus larvae*	Samples showing no evidence of spores [a]
Commercial samples from 30 States	187	15	172
Experimental	2		2
Miscellaneous	2	2	
Total	191	17	174

[a] 29 of these samples were doubtful on the first examination, but repeated examinations gave negative results in each case.

Of the 187 samples of commercial honey obtained from 30 different States or Territories, 15, or 8 per cent, showed the presence of a sufficient number of spores resembling spores of *Bacillus larvae* to be designated as positive. In 29 of the commercial samples, or 15.5 per cent, one or two doubtful spores were seen in each case, but on repeated examinations none of these samples could be considered positive. Two of the four miscellaneous samples from infected sources were also found to contain spores of *B. larvae*.

Five of the samples showing the presence of spores of *Bacillus larvae* were fed to healthy 5-frame colonies during the summer of 1930. These samples consisted of from a pint to a quart of honey. No evidence of American foulbrood appeared in any of the five colonies during the entire brood-rearing season.

In order to determine the approximate number of spores in the samples of honey in which the presence of *Bacillus larvae* was demonstrated, a series of dilutions of spores was prepared as described for the work with cultures. A stained smear was made of 0.01 c c of each dilution spread over a 1-cm^2 surface of cover glass mounted with water and examined with the oil-immersion objective. By this means a definitely recognizable number of spores could be found down to the dilution of 2,000,000 spores per cubic centimeter, with a few single spores seen in occasional fields down to the dilution of 500,000 spores per cubic centimeter. (Table 8.) Then 1 c c of each dilution was added to 5 c c of distilled water in 15 c c centrifuge tubes and centrifuged at 2,000 revolutions per minute for 20 minutes. A stained smear made from 0.01 c c of each sediment showed a definitely recognizable number of spores down to the 5,000-spore dilution, with one or two doubtful spores in several fields from the 500-spore dilution. The sample containing the 50,000-spore dilution, which would be comparable to the sugar sirup containing the minimum number of spores per cubic centimeter fed to colonies in the spore-feeding experiments that produced infection, showed a great many more spores in each field examined by this method than did the sample of commercial honey that showed the greatest number of spores. Therefore, until a better quantitative method is devised, it seems reasonable to believe, from the indications of the preliminary work on this problem, that, even though the presence of a few spores of *B. larvae* may be

demonstrated in 5 c c quantities from a comparatively small percentage of samples of commercial honey, the numbers are far below the minimum necessary to produce infection when such honey is used in healthy colonies of bees. Before definite conclusions can be drawn, it will be desirable to examine many more samples of commercial honey and to feed to healthy colonies samples of honey in which the presence of spores has been demonstrated.

TABLE 8.—*Microscopic examination of dilutions for spores of Bacillus larvae* [a]

Number of spores per cubic centimeter in each dilution	Direct examination of 0.01 cubic centimeter	Examination of sediment after centrifuging 1 cubic centimeter	Number of spores per cubic centimeter in each dilution	Direct examination of 0.01 cubic centimeter	Examination of sediment after centrifuging 1 cubic centimeter	Number of spores per cubic centimeter in each dilution	Direct examination of 0.01 cubic centimeter	Examination of sediment after centrifuging 1 cubic centimeter
5, 000, 000, 000	+	+	10, 000, 000	+	+	100, 000	−	+
4, 000, 000, 000	+	+	9, 000, 000	+	+	90, 000	----------	+
3, 000, 000, 000	+	+	8, 000, 000	+	+	80, 000	----------	+
2, 000, 000, 000	+	+	7, 000, 000	+	+	70, 000	----------	+
1, 000, 000, 000	+	+	6, 000, 000	+	+	60, 000	----------	+
500, 000, 000	+	+	5, 000, 000	+	+	50, 000	−	+
400, 000, 000	+	+	4, 000, 000	+	+	40, 000	----------	+
300, 000, 000	+	+	3, 000, 000	+	+	30, 000	----------	+
200, 000, 000	+	+	2, 000, 000	+	+	20, 000	----------	+
100, 000, 000	+	+	1, 000, 000	±?	+	10, 000	----------	+
90, 000, 000	+	+	900, 000	----------	+	5, 000	−	+
80, 000, 000	+	+	800, 000	----------	+	4, 000	----------	----------
70, 000, 000	+	+	700, 000	----------	+	3, 000	----------	----------
60, 000, 000	+	+	600, 000	----------	+	2, 000	----------	----------
50, 000, 000	+	+	500, 000	±?	+	1, 000	----------	----------
40, 000, 000	+	+	400, 000	−	+	500	−	±
30, 000, 000	+	+	300, 000	−	+	50	−	−?
20, 000, 000	+	+	200, 000	−	+	5	−	−

[a] + indicates that spores were found; − indicates that spores were not found, by microscopic examination; ± indicates that the result was doubtful; ±? indicates that the positive was more doubtful than the negative; −? indicates that the absence of spores was not definite.

SUMMARY AND CONCLUSIONS

As a result of five years' study it has been found that, in order to produce American foulbrood infection in a healthy colony of bees, the sugar sirup used for inoculation must contain a certain initial number of spores of *Bacillus larvae*. Seventy-three colonies were inoculated during this time with numbers of spores ranging from approximately 5,000,000,000 to 100,000 per colony; 30 of these colonies receiving 50,000,000 spores or less. Of these 30 colonies, 2 out of 11 receiving 50,000,000 spores showed infection, but no colony receiving less than that number of spores developed disease. Therefore, the minimum infectious dose of *B. larvae* for a colony of bees seems to be approximately 50,000,000 spores in 1 liter of sugar sirup.

Preliminary experiments in which individual bee larvae were given known numbers of spores of *Bacillus larvae* in 0.01 c c quantities of sugar sirup show that infection can be produced by this method, but with considerable difficulty. From 50 to 100 larvae were inoculated with each dilution of spores, ranging in number from approximately 50,000,000 spores to, theoretically, 1 spore per larva. The minimum infectious dose was found to be 10,000,000 spores per larva fed in 0.01 c c of sugar sirup. These results indicate that the

minimum dose of spores of *B. larvae* that will produce American foulbrood infection must be large.

The germination of spores of *Bacillus larvae* and vegetative growth on a suitable artificial culture medium resulting from the inoculation of 556 culture tubes with seedings varying from approximately 50,000,000,000 to 500 spores per culture also shows that a certain minimum initial number of spores in the inoculum is necessary in order to produce growth. This minimum number of spores producing vegetative growth on a medium consisting of yeast-carrot extract, egg-yolk suspension, and agar was found to be approximately 50,000 in 1 c c of suspension inoculated.

The production of nitrite in this medium by the vegetative growth of *Bacillus larvae* serves as a fairly delicate and reliable indicator of such growth.

There was a tendency for the seedings containing the smaller numbers of spores of *Bacillus larvae* to require a longer period of incubation than the larger seedings in order to produce vegetative growth. However, there was a considerable variation in the germination time of many of the seedings of spores, in one case a seeding of 9,000,000 spores requiring 27 days' incubation to produce growth and another of 70,000 spores requiring only 6 days. This variation, thought to be due to the variable character known as dormancy in bacterial spores, prevented more than a slight correlation.

In the group of cultures comprising seedings between 5,000,000,000 and 9,000,000 spores, only 1.67 per cent required more than 10 days' incubation to produce vegetative growth, 100 per cent having shown growth after 30 days. In the group of cultures comprising seedings between 8,000,000 and 500,000 spores, 71.93 per cent required more than 10 days' incubation, while 53.81 per cent showed no growth at the end of 30 days' incubation. In the group of cultures comprising seedings between 400,000 and 50,000 spores, 97.18 per cent required more than 10 days' incubation, while 91.55 per cent of the group showed no growth at the end of 30 days. Below 50,000 spores no growth was obtained. In other words, below a seeding of 9,000,000 spores an increasing number of the smaller spore seedings required a longer period of incubation. About 80 per cent of all the positive cultures were obtained during the first 10 days of incubation, although this was approximately only 30 per cent of all the cultures made; at the end of 30 days' incubation only about 38 per cent of all the cultures had shown any growth.

It was found possible to demonstrate the presence of spores of *Bacillus larvae* in 15 out of 187, or in 8 per cent, of the samples of commercial honey examined by means of the centrifuge and the microscope. The preliminary results indicate that, even though spores of *B. larvae* may be demonstrated in a certain percentage of samples of commercial honey, in most instances they are probably present in such small numbers as to be less than the minimum number, 50,000,000 per liter, found to be capable of producing disease, and therefore are ineffective in the spread of American foulbrood.

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QUANTITATIVE DEMONSTRATION OF THE PRESENCE OF SPORES OF BACILLUS LARVAE IN HONEY CONTAMINATED BY CONTACT WITH AMERICAN FOULBROOD

BY

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QUANTITATIVE DEMONSTRATION OF THE PRESENCE OF SPORES OF BACILLUS LARVAE IN HONEY CONTAMINATED BY CONTACT WITH AMERICAN FOULBROOD[1]

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INTRODUCTION

In a previous paper[3] the writer showed that it is possible to demonstrate the presence of spores of *Bacillus larvae*, the cause of American foulbrood, in samples of commercial honey that have had contact with American foulbrood in the course of their production or preparation for the market. Since this work was reported, 25 additional samples, making a total of 212 samples of commercial honey, obtained on the open market from 28 States and 2 Territories have been examined by the same method, and spores of *B. larvae* have been found in 17, or 8 percent, of these samples.[4] In most cases the spores were present in relatively small numbers.

The method of examination used in the work thus far reported gave only a qualitative indication of the number of spores present, the observations being recorded as showing "the presence of a sufficient number of spores resembling spores of *B. larvae* to be designated as positive."[5] This amounted to from one or two definite spores to a very few spores seen in numerous microscopic fields of each stained sediment examined. The primary object was to demonstrate only their presence or absence. It was assumed that in most cases the number of spores found was considerably smaller than would be found in honey containing numbers comparable with the observed minimum infective dose of 50,000,000 per liter.

The only way of demonstrating the accuracy of this assumption has been to feed such "positive" samples of commercial honey to healthy colonies of bees. This was done with 15 of the 16 samples in which spores were demonstrated, and only 1 sample, or 6.7 percent, was found to contain sufficient infection to produce the disease in a healthy colony. These investigations indicate that the requirement of certification of honey, as has been proposed and even placed in operation in certain States, is not a justifiable measure in the control of American foulbrood under the present conditions of inspection and control of disease in this country.

To permit a more accurate, quantitative study of the infectivity of honey that has been in contact with American foulbrood, on the

[1] Received for publication Jan. 27, 1936; issued June 1936. This investigation was carried on at the Intermountain States laboratory of the Division of Bee Culture, which is maintained cooperatively by the University of Wyoming and the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

[2] Acknowledgments are due to F. R. Hall, associate professor of commerce, University of Wyoming, for advice and assistance in the statistical analysis of the data.

[3] STURTEVANT, A. P. RELATION OF COMMERCIAL HONEY TO THE SPREAD OF AMERICAN FOULBROOD. Jour. Agr. Research 45: 257–285, illus. 1932.

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[5] STURTEVANT, A. P. See footnote 3.

basis of its spore content—that is, a detailed study of the distribution of spores of *B. larvae* in the honey from infected hives or apiaries, or in commercial honey obtained on the open market, or of the effect of mixing infected honey with disease-free honey in the course of production or blending and preparation for the market—a more detailed investigation has been made of the spore content of honey containing approximately known numbers of spores. This has been accomplished by an improved and more accurate method of determining the number of spores in such honey, and the accuracy of the results and method has been demonstrated by means of a statistical analysis of the data obtained.

METHOD OF OBTAINING THE DATA

PREPARATION OF SAMPLES OF HONEY

A series of samples of honey containing approximately known numbers of spores per cubic centimeter were prepared in the manner described previously,[6] by adding to 100-cc quantities of spore-free honey the necessary quantities of various dilutions of a stock suspension of spores of *Bacillus larvae* containing approximately 5,000,000,000 spores per cubic centimeter. Five samples of honey were prepared in this way containing approximately 1,000,000, 800,000, 500,000, 300,000, and 50,000 spores per cubic centimeter, respectively. These samples, each considered as a unit and not as a dilution of the 1,000,000-spore sample, were heated in a water bath to 120°–130° F., and then thoroughly mixed with a mechanical stirrer for 5 minutes. Duplicate 5-cc quantities of each sample were then placed in 50-cc conical centrifuge tubes, and 45 cc of distilled water of approximately the same temperature was added. When the honey and water were completely mixed, the samples were centrifuged at 2,000 revolutions per minute for 45 minutes. All but about 1 cc of the supernatant honey-water solution of each sample was then removed by means of a pipette and suction. Again approximately 45 cc of distilled water was added, and after thorough mixing the suspensions were centrifuged for 30 minutes longer. The removal of the supernatant solution was repeated until all but approximately 0.1 cc[7] of the water had been removed from each centrifuge tube, and each sample of sediment was completely suspended in this remaining quantity of water by blowing gently through a capillary pipette dipped into the water. Duplicate 0.01-cc quantities of each suspension were then transferred with the capillary pipette (calibrated to deliver 0.01 cc) to microscope cover glasses. Circular cover glasses, size 12, no. 1 thickness, having an area of 1.13 cm^2, proved satisfactory for this purpose. A small (2 to 3 mm) loopful of carbolfuchsin stain was added to the drop of suspension on the cover glass and thoroughly mixed with it. This stained liquid was then spread uniformly over a 1-cm^2 area of the cover glass, a narrow ring at the outside edge being left uncovered. The smears were allowed to dry in the air and were then mounted on microscope slides either with water or, preferably, with Canada balsam, for examination under the microscope. These stained smears were not washed in water, as this might have caused some spores to be lost.

[6] STURTEVANT, A. P. See footnote 3.
[7] A mark was placed on the outside of the conical centrifuge tubes to indicate the 0.1-cc volume.

The foregoing process gives a concentration of spores in the sediment from the 5-cc samples of honey suspended in 0.1 cc of water, or one-fiftieth the original volume.

METHOD OF COUNTING SPORES

A method similar to that of Breed and Brew [8] for counting bacteria in milk was used for counting the spores of *Bacillus larvae* in these stained smears. This method is similar to that described in a previous paper [9] and is represented by the formula

$$\text{Number of spores per cubic centimeter} = \frac{KNX \times 100 \times D}{N}$$

where K is the factor for the number of circular fields per 1-cm² area, N is the number of circular fields counted, X is the actual mean number of spores per field, 100 is the factor that gives the number of spores per cubic centimeter from 0.01 cc of the suspension, and D is the dilution.

TABLE 1.—*Spore counts in stained smears of the sediments resulting from the centrifuging of duplicate 5-cc portions of five samples of honey containing known numbers of spores of Bacillus larvae*

Field no.	Spore counts in samples [1] containing the indicated number of spores per cubic centimeter									
	50,000		300,000		500,000		800,000		1,000,000	
	A	B	A	B	A	B	A	B	A	B
1	2	1	7	8	14	15	19	21	24	21
2	2	2	8	9	12	12	18	20	24	29
3	1	1	8	10	12	13	24	18	26	38
4	0	2	7	5	10	15	22	18	23	24
5	2	0	9	6	10	11	20	20	30	34
6	1	3	9	7	12	13	18	17	23	29
7	0	1	8	7	15	14	21	18	26	24
8	2	1	7	8	12	13	27	17	29	21
9	1	0	8	6	16	16	19	21	19	26
10	0	2	9	12	17	13	21	20	30	31
11	1	1	7	11	14	12	16	19	25	36
12	1	3	10	6	16	12	21	20	28	26
13	1	3	9	5	13	10	22	22	27	33
14	2	1	7	9	18	14	25	24	25	22
15	1	0	8	10	12	13	21	25	29	26
16	2	1	8	5	10	11	20	25	24	26
17	1	0	6	9	11	16	18	28	35	23
18	2	0	5	6	13	14	26	23	24	28
19	0	2	7	10	17	16	24	23	25	34
20	0	1	9	5	13	18	26	28	27	30
21	2	1	10	15	13	15	16	23	25	32
22	2	3	11	10	8	16	19	18	26	25
23	1	1	7	8	18	10	20	16	27	25
24	3	1	6	5	15	12	18	22	28	34
25	1	1	7	9	12	15	21	21	29	28
26	0	1	6	7	15	10	21	20	27	22
27	1	1	5	10	10	14	26	18	34	23
28	2	1	7	8	12	15	22	26	22	27
29	2	2	8	10	11	11	22	28	29	28
30	2	2	10	8	12	11	25	22	21	30
Total	38	39	233	244	393	400	638	641	791	835
Total for 60 fields	77		477		793		1,279		1,626	
Mean number of spores per field	1.2833		7.9500		13.2167		21.3167		27.1000	

[1] A and B represent duplicate portions of the samples.

[8] BREED, R. S., and BREW, J. D. COUNTING BACTERIA BY MEANS OF THE MICROSCOPE. N. Y. State Agr. Expt. Sta. Tech. Bull. 49, 31 pp., illus. 1916.

[9] STURTEVANT, A. P. See footnote 3.

An ocular micrometer disk, such as is used for counting bacteria in milk, was used in counting spores in the fields of the stained smears. The area of the circle etched on this disk was found to be 0.00006082 cm^2 when used in a binocular microscope with 15 × paired eyepieces and a 1.8-mm oil-immersion objective. Therefore, the factor K became 16,441.96.

The spores in 30 fields from each of the duplicate smears were counted, making a total of 60 fields (N) for each honey-spore sample. The fields were counted at random from various parts of the smear. From these counts the actual mean number of spores per field recovered in 60 fields for each honey-spore sample was determined (table 1).

Substituting the values for K and N and 0.02 (1/50) for D, the spore dilution in the foregoing formula gives

Number of spores per cubic centimeter

$$=\frac{16,442\times 60X\times 100\times 0.02}{60}=32,884X$$

COMPUTATION OF THEORETICAL MEAN NUMBER OF SPORES PER FIELD

The theoretical mean numbers of spores per field that should be recovered from each of five honey-spore samples used, under ideal conditions where there is no loss of spores during the process, were calculated by the foregoing formula, which for this purpose may be stated as follows:

$$X=\frac{\text{Number of spores per cubic centimeter}}{32,884}$$

X now designates the theoretical mean number of spores per field. In table 2 these values are given in comparison with the corresponding actual mean number of spores per field for each honey-spore sample.

TABLE 2.—*Relation between the actual and the theoretical mean numbers of spores of Bacillus larvae per field recovered from five samples of honey containing known numbers of spores per cubic centimeter*

Spores per cubic centimeter in sample (number)	Mean spores per field			Ratio of actual mean to theoretical mean
	Theoretical	Actual	Standard deviation	
	Number	*Number*	*Number*	*Percent*
1,000,000	30.4100	27.1000±0.3554	4.0812	89.12
800,000	24.3279	21.3167± .2751	3.1596	87.62
500,000	15.2050	13.2167± .2011	2.3100	86.92
300,000	9.1230	7.9500± .1708	1.9615	87.14
50,000	1.5205	1.2833± .0747	.8582	84.40

RESULTS OBTAINED BY USE OF THE METHOD

By the method used, the actual mean number of spores per field obtained by counting 60 fields from each honey-spore sample differed from the calculated theoretical mean number of spores per field by 10.88 percent for the honey containing 1,000,000 spores per cubic centimeter to 15.60 percent for the honey containing 50,000 spores per

cubic centimeter (table 2). This difference, which is relatively constant for each sample, may be due to the fact that some spores are lost during the centrifuging, but more probably to the fact that a certain proportion of the spores in each smear are covered up and not seen in the masses of stained debris always present even in honey of the highest quality.

DETERMINATION OF ACCURACY OF THE METHOD

STATISTICAL ANALYSIS OF THE DATA

Since the data obtained for the actual mean number of spores per field (table 1) for each honey-spore sample, if plotted against the data calculated for the theoretical mean number of spores per field (table 2), give practically a straight line having a trend similar to that of a line plotted for the theoretical data alone, the relation between the theoretical means and the actual means, for the five honey-spore samples used, was determined by the customary statistical methods.

The standard deviation and the probable error for the actual mean number of spores per field were determined from frequency tables prepared from the original data (table 1) for each honey-spore sample used [10] (table 2). The actual means were derived from large samples (60 fields each), and the calculated probable errors and standard deviations were shown statistically to be small.

The coefficient of correlation [11] between the values for the actual mean number and those for the theoretical mean number of spores per field for each sample as given in table 2 was found to be 0.9999 ± 0.0001.

The relation between the actual mean number of spores per field recovered from each honey-spore sample and the corresponding most probable values estimated from the theoretical mean number of spores per field for each sample was determined by use of the regression equation for the actual mean number of spores. This was found to be $\overline{Y}=0.8905X-0.1791$. Substituting the various values of the theoretical mean number of spores per field (table 2) for X in this equation gave the most probable estimated values for the actual mean number of spores per field ($\overline{Y}$) that should have been recovered from each sample (table 3). These most probable estimated values were found to be in excellent agreement with the actual values obtained.

TABLE 3.—*Theoretical and actual mean numbers of spores per field and the most probable estimated theoretical and actual mean numbers of spores per field*

Number of spores per cubic centimeter in sample	Mean number of spores per field			
	Theoretical	Estimated theoretical	Actual	Estimated actual
1,000,000	30.4100	30.6313	27.1000	26.9010
800,000	24.3280	24.1378	21.3167	21.4850
500,000	15.2050	15.0431	13.2167	13.3610
300,000	9.1230	9.1297	7.9500	7.9449
50,000	1.5205	1.6443	1.2833	1.1749

[10] CHADDOCK, R. E. PRINCIPLES AND METHODS OF STATISTICS. pp. 160–164, 240–241. Boston, New York [etc.]. 1925.

[11] CROXTON, F. E., and COWDEN, D. J. PRACTICAL BUSINESS STATISTICS. p. 416. New York. 1934.

The purpose of this investigation, however, was to develop an equation with which, if the actual mean number of spores per field is obtained with sufficient accuracy, the theoretical number of spores per field may be estimated, thereby giving the data necessary for estimating the number of spores per cubic centimeter in an unknown sample of honey. The regression equation or the theoretical mean number of spores per field can be used for this purpose, and was found to be $\overline{X}=1.1228Y+0.2034$. Substituting for Y in this equation, the various values of the actual mean number of spores per field, as obtained in table 1, gave the most probable estimated values for the theoretical mean number of spores per field that should be obtained from the actual counts for each honey-spore sample (table 3). By this method of estimation these values were found to agree closely with the original calculated values for the theoretical mean number of spores per field for each honey-spore sample (table 2).

DETERMINATION OF PERMISSIBLE LIMITS OF ERROR

The analysis of the data so far indicates the accuracy of the method outlined above for determining the most probable actual mean spore count per field from the mean of 60 fields counted. Variations in the counts may occur in individual samples, however, owing to the failure to recover all the spores, as stated previously.

The permissible limits of error in the statistical analysis of such cases are customarily determined by use of the standard error of estimate. This, for the most probable estimated actual means derived from the theoretical means, was found to be small, ± 0.1298 spore, and indicates the closeness with which new estimated values may be expected to approximate the true but unknown values. Since two of the five actual means fall within ± 0.1298 spore of the estimated actual means while the other three are only from 0.11 to 0.26 percent outside this zone, within which approximately two-thirds of the observations may be expected to fall in relation to the most probable values, a sufficient accuracy for the method is indicated.

The standard error of estimate for the most probable theoretical means derived from the actual means (which were found to agree closely with the estimated actual means) was found to be ± 0.1458 spore. As is to be expected in this case, again two of the original theoretical means fall within the zone of ± 0.1458 spore while the other three are only from 0.11 to 0.25 percent outside this zone. However, since ± 3 times the standard error of estimate, which should include 99.7 percent of all observations, is used customarily in delineating the largest error to which statistical analyses of this type are subject, it is found that all the theoretical means fall well within this zone, or within ± 0.4374 spore. This indicates the probable accuracy of estimating the number of spores per cubic centimeter in an unknown sample by calculating the most probable theoretical number of spores per field from the actual mean number counted.

PRACTICAL APPLICATION OF THE METHOD

In a previous paper [12] it was shown that during observations covering 5 years no cases of American foulbrood developed in 19 colonies of bees fed less than approximately 50,000,000 spores of *Bacillus*

[12] STURTEVANT, A. P. See table 1 of reference in footnote 3.

larvae in 1 liter of sugar sirup, or less than 50,000 spores per cubic centimeter. Of 11 colonies fed 50,000 spores per cubic centimeter, 2 developed disease and 9 remained healthy; of 6 colonies fed 75,000 per cubic centimeter, 3 developed positive disease and 1 probable disease, and 2 remained healthy; of 6 colonies fed 100,000 per cubic centimeter, 2 were positive, 1 probable, and 3 remained healthy; of 4 colonies fed 200,000 spores per cubic centimeter, 3 were positive and 1 probable. Thus it was assumed that 50,000 spores per cubic centimeter of sirup could be considered the critical number or minimum infectious dose of spores that will produce disease, when 1 liter is used as the unit volume to be fed.

Since the foregoing analysis of the data indicates, by the method of estimating used, that the actual mean number of spores per field falls well within the limits of permissible error for the estimated actual means (± 3 times the standard error of estimate), the most probable value for such a mean for use in determining the number of spores per cubic centimeter of an unknown sample is the actual mean number of spores per field determined by counting 30 fields each from stained smears from two centrifuged sediments of this sample. If the formula $\overline{X}=1.1228Y+0.2034$ is used to estimate $\overline{X}$, the most probable theoretical number of spores that should have been recovered, when Y represents the actual mean number of spores per field, and if this value is then multiplied by 32,884, the most probable number of spores per cubic centimeter in the unknown sample can be calculated. Applying the limits of error for $\overline{X}$, ± 3 times the standard error of estimate, or ± 0.4374 spore, and carrying it through into the second formula will give the possible range in which the number of spores per cubic centimeter might fall within the precision of the method.

Further work is in progress to determine whether the same accuracy will be obtained by counting a smaller number of fields to obtain the mean number of spores per field from a larger number of smears from sediments.

Since in the experimental work the samples of known spore content contained approximately round numbers of spores—multiples of 50,000—it probably would be sufficiently accurate to designate the number of spores as the nearest multiple of 50,000 to the actual figures derived from the formulas. When using the limits of error 0 ± 0.4374 spore per field, for the estimated mean number of spores per field, it will be found that for numbers below 100,000 there will be some overlapping between 10,000-spore increments, and the value will have to be expressed approximately (for example, the honey contains between 40,000 and 60,000 spores per cubic centimeter); neverthless the honey can still be designated either as dangerous or as not dangerous.

SUMMARY

Previous work on the qualitative demonstration of the presence or absence of spores of *Bacillus larvae* in honey that has been in contact with American foulbrood has been followed by the development of a quantitative method for determining the approximate number of spores per cubic centimeter in such honey. The method is represented by the formula

$$\text{Number of spores per cubic centimeter}=\frac{KNX\times 100\times D}{N}$$

where K is the factor for the number of circular fields per 1-cm^2 area, N is the number of circular fields counted, X is the actual mean number of spores per field, 100 is the factor that gives the number of spores per cubic centimeter from 0.01 cc of the suspension, and D is the dilution. The mean number of spores of *Bacillus larvae* per field counted in 60 fields of stained smears made from the sediments obtained by centrifuging 5-cc quantities of honey containing approximately known numbers of spores have been determined by this method.

The mean actual spore count per field was determined for a series of samples of honey prepared to contain approximately 1,000,000, 800,000, 500,000, 300,000, and 50,000 spores per cubic centimeter. The mean theoretical spore count per field that should have been recovered was determined by use of the formula

$$X=\frac{\text{Number of spores per cubic centimeter}}{32,884}$$

The actual mean numbers of spores per field were similar in trend to the calculated theoretical means but were from 10.88 to 15.60 percent smaller. A statistical analysis of the data to determine the accuracy of the method showed that the calculated probable errors and standard deviations were small. The coefficient of correlation between the actual and the theoretical mean number of spores per field for each sample was found to be 0.9999 ± 0.0001.

The relation between the actual mean number of spores per field ($\overline{Y}$) and the corresponding most probable values that should have been recovered, estimated from the theoretical mean number of spores per field (X), was determined by means of the regression equation $\overline{Y}=0.8905X-0.1791$. These most probable estimated values were found to be in excellent agreement with the actual values obtained, well within the customary limits of ± 3 times the standard error of estimate, which was found to be ± 0.1298 spore.

The most probable theoretical mean number of spores per field ($\overline{X}$) was estimated by means of the regression equation $\overline{X}=1.1228Y+0.2034$. These values were found to be in excellent agreement with the original calculated values for the theoretical mean, well within ± 3 times the standard error of estimate, ± 0.1458 spore.

The statistical analysis of the data therefore indicates that the method used is sufficiently accurate for determining the spore content of unknown samples of honey. For this purpose the following formulas are used:

$$\overline{X}=1.1228Y+0.2034\pm 0.4374$$

where Y=the actual mean number of spores per field counted from 60 fields, and

$$\text{Number of spores per cubic centimeter}=32,884\overline{X}.$$

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THE DEVELOPMENT OF AMERICAN FOULBROOD IN RELATION TO THE METABOLISM OF ITS CAUSATIVE ORGANISM

BY

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(Contribution from Bureau of Entomology)

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THE DEVELOPMENT OF AMERICAN FOULBROOD IN RELATION TO THE METABOLISM OF ITS CAUSATIVE ORGANISM

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INTRODUCTION

American foulbrood is one of the two serious diseases affecting the brood of the honeybee. The specific cause of this disease is a pathogenic, spore-forming microorganism, known as *Bacillus larvae*. The occurrence of this organism in uniformly pure culture, accompanied by the gross effects of its activity, as manifested by the characteristic appearance and age of the diseased and dead larvæ, differentiates American foulbrood from the other serious brood disease of bees, European foulbrood. The latter disease is caused by an entirely different non-spore-forming organism, *Bacillus pluton*, which causes a different manifestation of gross symptoms, complicated by the action of various secondary invaders.

Certain limited facts concerning the characteristics of the various types of bacteria concerned in causing or associated with these brood diseases have been studied, from which various practical applications have been derived. As has been stated by Phillips (*39*)[2], "Bacteriological studies of bee diseases have been useful to practical beekeepers in explaining the reasons for success or failure with various treatments attempted. These studies have been especially important, however, because through them methods of laboratory diagnosis of the different diseases have been worked out."

Advancement in knowledge concerning the etiological and biochemical relationships of the brood diseases, particularly concerning differences in characteristics as related to gross symptoms, has been limited, however, because of the peculiar growth requirements of the causative organisms. There are fundamental differences between American foulbrood and European foulbrood, particularly as to characteristics of development, which, although recognized, have not been adequately explained by the incomplete data so far obtained on the metabolism of the causative organisms.

The present investigation was undertaken to obtain further data concerning the growth requirements of *Bacillus larvae*, the cause of American foulbrood, by which to explain these differences in the symptoms and development of the two diseases. Through improved methods of cultivation, a study has been made of factors concerned in the metabolism of *Bacillus larvae* correlated with certain hitherto unrecognized biochemical factors associated with the metabolism of the normal honeybee larva. The results obtained add materially to the knowledge of the biology of the brood diseases.

[1] Acknowledgments are due to Dr. E. R. Whitmore, professor of bacteriology and preventive medicine of the George Washington University, for much valuable advice and many suggestions, and to Dr. E. F. Phillips, apiculturist, Bureau of Entomology, United States Department of Agriculture, under whose direct supervision this work was done. Presented in part satisfaction of the requirements for the degree of doctor of philosophy at the George Washington University, April 21, 1923. This work was completed April 10, 1923.

[2] Reference is made by number (italic) to "Literature cited," p. 165–168.

THE RELATION OF CONTRIBUTING CAUSES TO THE COMPARATIVE DEVELOPMENT OF THE TWO SERIOUS BROOD DISEASES OF BEES

In order to understand the basis upon which the consideration of this problem has been developed, it is necessary to make a comparative study of certain of the characteristics of the two brood diseases, American foulbrood and European foulbrood, aside from their etiology. It will be apparent from this study that certain contributing causes, although recognized and described, have not been further analyzed to any extent, particularly in relation to specific etiology. The experimental work of the present investigation is concerned primarily with American foulbrood, however, since the causative organism, *Bacillus larvae*, can be isolated and grown in pure culture, while as yet no artificial medium suitable for the growth of *Bacillus pluton*, the cause of European foulbrood, has been devised.

RACE

It is an accepted fact that in American foulbrood the race or strain of bees has little or no relation to the development of or the resistance to the disease. This, aside from apparent lack of immunity or resistance of any of the races, may be explained partially by the fact that the decomposed material resulting from the death of the larvæ is of such a nature that the bees can not to any extent remove it from the combs after the disease has once become established. The dried-down masses (scales) are practically glued to the cell walls. *Bacillus larvae* forms resistant spores which allow the disease to be carried and spread almost indefinitely by means of the honey and old scales.

In European foulbrood, on the contrary, Italian bees seem to have some characteristic which makes them more resistant or vigorous in combating infection under the proper conditions. The results of bacterial decomposition of the diseased remains, even at their worst, are such that, if the colony is able to build up or is made sufficiently strong in worker bees, they are able to remove these remains, thereby removing the infection sufficiently to prevent its further development. *Bacillus pluton* does not form spores and lives only a comparatively short time under unfavorable conditions for growth, as in honey or on long drying. Furthermore, as has been demonstrated by the writer in a previous paper (*45*), this apparent resistance of the Italian bees was observed to be due largely to the racial characteristic of removing all foreign materials more promptly from the hive than do common black bees or hybrids, rather than to any natural resistance or immunity to the disease.

STRENGTH OF COLONY

If a colony of bees has been exposed to infection from American foulbrood, the strength of the colony apparently has no direct relation to the development of the disease, except that strong colonies are usually the ones which rob the weaker infected colonies, thereby spreading the infection through the apiary. As suggested above, European foulbrood attacks primarily the weak colonies which have an insufficient force of bees to remove the infected material. Diseased combs from such a colony can be placed in a strong healthy colony of Italian bees with no resulting disease. This would be fatal in the case of American foulbrood.

SEX

There has been slight mention in the literature of the relation of the sex of the bee larvæ to the development of disease. Phillips (*38*) states with regard to European foulbrood: "A symptom of greatest importance is the fact that the

disease attacks drone and queen larvæ nearly as quickly as those of the workers. The tendency of this disease to attack queen larvæ is a serious drawback in treatment. Frequently bees of a diseased colony attempt to supersede their queen but the larvæ in the queen cells often die, leaving the colony hopelessly queenless. The colony is thus depleted rapidly."

In American foulbrood, according to Phillips (*39*), "Usually the disease attacks only worker brood, but rare cases are found in which queen and drone brood are diseased." White (*55*) states, however: "That worker, drone, and queen larvæ are all susceptible to the disease has been demonstrated during these [White's] studies. Affected drone brood is encountered less often in the diagnosis of this disease than in that of European foulbrood. The writer has encountered queen larvæ affected by American foulbrood in experimental colonies only, although very probably diseased queen larvæ do occur in nature also." A few samples of diseased brood containing American foulbrood sent to the Bee Culture Laboratory for diagnosis have been found to contain affected drone larvæ as well as one or two cases of diseased queen larvæ. Although beekeepers believe that in American foulbrood drone brood is so seldom affected that the absence of diseased drone brood is a diagnostic character, the fact that occasionally drone larvæ do die of the disease makes it possible that some other factor than nonsusceptibility of sex is concerned. No accurate data are available on this subject. The work of this paper is concerned only with worker brood, because the great preponderance of worker brood affected gives slight importance to the comparatively few drone larvæ in the average colony.

AGE

The general characteristic difference in age between larvæ dying of American foulbrood and those dying of European foulbrood, mentioned at the beginning of this paper, has been one of the chief factors in the differentiation between the two diseases. Originally there was considered to be only one disease, "foulbrood." Although beekeepers have long known that brood of various ages is attacked by brood disease, it seems not to have been until about 1880 that the difference in age at the time of attack was used to separate foulbrood into two distinct forms, one "easily curable" and the other "virulent." Dzierzon (*21*) was the first thus to differentiate definitely into two types of disease, according to the difference in symptoms and age at time of attack. He stated that in the curable disease, "More of the larvæ die still unsealed, while they are still coiled in the bottom of the cell * * *. The brood which does not die before sealing mostly attains to perfection * * *. This is exactly the reverse in the malignant kind of foulbrood. In this the larvæ do not generally die before they have raised themselves from the bottom of the cell, have been sealed and begun to change into nymphs."

Cheshire (*13*) who probably was the first to investigate the bacteria associated with what, in the light of present knowledge, is known as European foulbrood, was inclined to agree at first with the distinctions made by Dzierzon. He soon stated (*14*), however, that Dzierzon was in error and that there is only the one disease, foulbrood, which he supposed was caused by an organism to which he gave the name *Bacillus alvei*. Cheshire and Cheyne (*15*) described *Bacillus alvei* as a spore-forming bacillus which they constantly found associated with a diseased condition of the brood and recognized only as "foulbrood." The results of this work caused considerable confusion to beekeepers and investigators, both in this country and abroad, for more than a decade.

In this country some time after 1890 it became evident to certain beekeepers, particularly in New York State, that they were dealing with two distinct diseases. The newly recognized form, which was found to attack the coiled larvæ,

was at first erroneously called "black brood," to distinguish it from the "foulbrood" of sealed larvæ. "Black brood" assumed epidemic proportions in New York State by 1897. This gave rise in American beekeeping literature to descriptions of two distinct diseases, as far as the age of the larvæ attacked and the appearance from the resulting decomposition were concerned.

RESULTING DETERMINATION OF ETIOLOGY

As a result of the increasing devastation by this new disease, work was started in New York State in 1902 (*33*), which was later carried on by White (*49, 50*), on the bacteriology of these brood diseases, by which doubt was cast upon *Bacillus alvei* being the cause of any disease, although it was found to be associated only with European foulbrood. Furthermore, a new spore-forming bacillus distinct from *Bacillus alvei* was observed and cultivated on special culture media from the disease attacking the sealed larvæ. This organism was at first designated *Bacillus* X but was later named *Bacillus larvae* (figs. 1 and 2). Subsequently this was found to be the cause of American foulbrood by experimental inoculation of healthy colonies with pure cultures (*51*). The symptoms

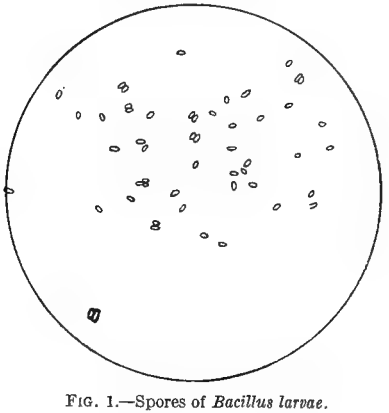

FIG. 1.—Spores of *Bacillus larvae*. (McCray (*31*))

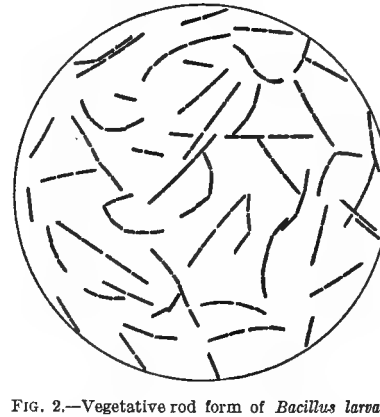

FIG. 2.—Vegetative rod form of *Bacillus larvae*. (White (*55*))

were accurately described and differentiated by Phillips (*37*), definite new names being used for the first time in order to eliminate confusion, as follows: American foulbrood, formerly known as "foulbrood" ("Usually the larvæ are attacked at about the time of capping, and most of the cells containing infected larvæ are capped"); and European foulbrood, originally called "black brood" ("This disease attacks the larvæ earlier than does American foulbrood, and a comparatively small percentage of diseased brood is ever capped").

Maassen (*27*) in Germany described at about the same time what is now accepted as the same organism as *Bacillus larvae*, a spore-forming organism constantly found to be present in the diseased brood dying after sealing, "Nymphenseuche." He gave the name *Bacillus brandenburgiensis* to this organism. Burri (*12*) in Switzerland also recognized the fact that the spores present in large numbers in scales in the "nymph" disease were a new species that was difficult of cultivation.

White (*52*) later showed conclusively that *Bacillus alvei* is not the cause of European foulbrood but is only one of several secondary invaders. He demonstrated that the probable cause of European foulbrood is a nonspore-forming organism which he called *Bacillus pluton*. This organism develops before the

death of the larva in the intestinal tract and usually kills before sealing takes place, as differentiated from American foulbrood as described above. Unfortunately, as yet it has been impossible to grow this organism in pure culture on artificial culture media.

Further work has been done by various investigators on certain laboratory phases of the bacteriology and diagnosis of the two diseases, but no additional information has been obtained concerning the etiological and biochemical relationships of the causative organisms which would aid in the solution of the present problem.

BASIS FOR INVESTIGATIONS

Throughout all the discussion of symptoms of the brood diseases in the literature, particularly in relation to the different ages at which the diseases attack during the life history of the larvæ, there has been no adequate explanation of the reason for this apparent fundamental difference.

Maassen (*28*) in the case of American foulbrood made the observation that, "according to the microscopic findings from section preparations, *Bacillus brandenburgiensis* [*Bacillus larvae*] does not come to luxuriant development in the intestine of the larva, though this is the case with *Bacillus alvei* and with *Streptococcus apis* [in 'sourbrood']. It finds much more promising nourishment in the fat bodies of the larva. Apparently the bacillus finds opportunity to press its way into the fat bodies shortly before the pupation of the bee, at the beginning of the natural changes in the intestinal tube. From this it seems clear why the larvæ containing *Bacillus brandenburgiensis* die after sealing." In part this is probably correct, since it may easily be observed that soon after capping the tissues of the healthy larva become more or less granular and watery in consistency, at which time it is almost impossible to distinguish the intestinal tract. It is also difficult to remove the larva in this condition from the cell without rupturing the skin envelope. This process is described more in detail later. It does not explain, however, why the spores of *Bacillus larvae* do not germinate and increase in numbers sufficiently to kill the larva much earlier during the feeding period, as in the case of European foulbrood. A vague and only partially correct suggestion was given in an earlier paper by the writer (*46*), in which the following theory was stated: "*Bacillus larvae* gains entrance to the larva generally in the spore stage, in the larval food. This occurs at about the same stage as in European foulbrood, while the larva is still coiled in the cell. Only rarely, however, do coiled larvæ die. This is apparently because it takes some time for the resting stage spores to germinate into the active vegetative rods. This causes death, as a rule, to occur later in the life history of the larva."

RELATION OF THE BROOD DISEASES TO THE LIFE HISTORY OF THE HONEYBEE LARVA

The development of the honeybee may be divided in general as follows: After the egg is laid there is a period of three day's incubation before it hatches into the larva. The larval stage, during which active feeding and growth occur, comprises four and a half to five and a half or six days. At the end of the feeding period the larva is sealed in the cell, where it spins its cocoon. Metamorphosis then occurs, and the fully formed adult bee emerges in about 12 days, making a complete developmental period of approximately 21 days. According to White (*53*), there is a prepupal period in healthy brood of four days after sealing occurs before the actual change in the external form to that of the adult bee takes place. During the first two days after capping, the larva is active in the cell, consuming any remaining food and spinning a cocoon. Some time during this period according to Straus (*43*), or just previous to capping according to Zander (*57*)

the larval intestine, which up to this time has been a blind sac, is connected with the end gut, allowing defecation to take place. There is then two days of quiescence, during which the larva extends in the cell and lies motionless, while internal changes preparatory to metamorphosis occur (figs. 3 and 4). These changes (*7*) consist of the almost complete histolysis of the fat body of the larva in order to furnish nutriment for the formation of imaginal tissues. This is made possible by the physiological and morphological changes occurring in this stage of the development of the larva. Extended investigations have been made of these physiological and morphological changes, but they need not be summarized further here, since the present work has been solely of a biochemical character. It is noticeable, however, that the intestines of mature larvæ even for a short time after capping are full of material colored by the pollen content, while the intestines of the prepupæ, after they have extended in the cell, are colorless.

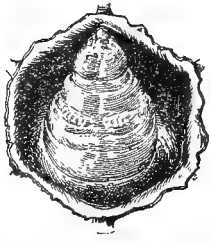

FIG. 3.—Healthy prepupa approximately 8 days old, having reached the quiescent stage. This is the age at which the majority of larvæ die from American foulbrood. End view. (White (*55*))

It is during the latter two-day prepupal period that according to Maassen (*28*) the invasion of the fat body by *Bacillus larvae* occurs and that according to White (*55*) the majority of the brood dies in American foulbrood.

In European foulbrood, on the contrary, the majority of the larvæ in typical cases of this disease die before sealing and after reaching an age of 3½ to 4 days from the time of hatching of the egg (*56*) (fig. 5). In certain abnormal cases in European foulbrood death may occur after capping (*46*), but this almost always occurs during the first two days of the prepupal stage, when the larva in most cases is still moving about in the cell, usually causing a gross appearance quite different from that of dead of American foulbrood.

PRELIMINARY EXPERIMENTS

While studying the bacterial flora associated with the early stages of European foulbrood in the larval intestine certain results were obtained which suggested a possible explanation of the delayed development in American foulbrood. Until death takes place in European foulbrood the growth of the organism causing the disease and certain secondary associated forms occur only within the intestine (*52*); that is, within the peritrophic membrane, but not in actual contact with living tissues of the larva. It is only after death that the secondary invaders, particularly *Bacillus alvei*, invade the body tissues (*45*).

FIG. 4.—Healthy prepupa. Side view. (White (*55*))

Another important distinction which must be considered is that the feeding of the larva is not the same throughout larval life. Von Planta (*40*) has shown that for the first part of the feeding period one type of food is used by the larva and that at a later stage a food different in chemical and physical composition is provided. Young larvæ receive a food for a time after hatching that is much richer in fat and albuminous material but lower in sugar content than that fed to older larvæ. The food of the older larvæ, which is known to consist mainly of honey or nectar and pollen, is much higher in sugar content, while there is a considerable decrease in fat and albuminous material. The sugar in the food of the older larvæ, particularly

that of larvæ at the age when European foulbrood makes its attack, comprises nearly 45 per cent of the dried substance, or nearly 14 per cent of the fresh substance. From these facts it may be assumed that, because of the great amount of food given the larva at this age, there must be present in the larval intestine, at all times during the active feeding period, considerable amounts of this food rich in sugar unassimilated, up to and even after active feeding ceases. A number of larval and prepupal intestines were dissected from healthy larvæ and tested roughly with Benedict's qualitative solution (*34*) for the presence of reducing sugars. The results indicated the presence of relatively large amounts of reducing sugar in the intestines of larvæ just prior to sealing. Little or no reducing sugar could be demonstrated in the intestines of sealed larvæ or prepupæ.

It may therefore be assumed that certain of the organisms associated with the early stages of European foulbrood are able to grow in the presence of a high sugar concentration. Experiments were devised in which a medium containing 10 per cent dextrose was used. It was found that while a few types of organisms, such as one resembling *Streptococcus apis* (*28*), could be grown in varying numbers, an organism similar to that described by Maassen (*29*), resembling the larger forms of *Bacillus pluton*, called *Bacillus lanceolatus*, could be isolated and grown from over 50 per cent of the samples cultured. As described by the writer (*47*), "This organism was found to grow best on a 10 per cent dextrose yeast extract agar with a reaction slightly acid. It is differentiated from *Bacillus pluton* and *Streptococcus apis* in being gram-negative, and does not grow readily if at all in media without sugar." From these studies it was suggested that possibly this comparatively high sugar content of the unassimilated food in the larval intestine may have an influence on the germination of the spores and growth of *Bacillus larvae* and that a change may occur when the sugar content is sufficiently reduced by assimilation in the larval intestine after it has been capped and when the intestines have been emptied by the opening of the ventriculus into the end gut. Therefore, with these preliminary observations as a basis, experinental work on this subject was begun during the spring of 1922.

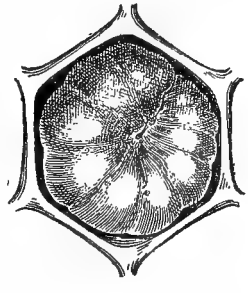

FIG. 5.—Healthy coiled larva at age of maximum intestinal sugar content and approximately the age when the majority die from European foulbrood. (White (*56*))

GROWTH OF BACILLUS LARVAE IN CULTURES IN RELATION TO VARIATION IN SUGAR CONCENTRATION

The first step in the substantiation of this theoretical assumption is to determine whether there is a correlation between germination of the spores of *Bacillus larvae* and vigor of vegetative growth and variations in concentration of reducing sugars in culture media. Ordinary culture media are unsuitable for the growth and isolation of *Bacillus larvae;* in fact, one of the confirmatory tests for this organism in laboratory diagnosis of American foulbrood (*31*) is the absence of growth on plain beef infusion agar plates, since the spores will not germinate thereon. There are rarely any secondary invaders associated with *Bacillus larvae* in the decayed material, and these plates practically never show growth.

GROWTH REQUIREMENTS OF BACILLUS LARVAE

Various special culture media have been devised which answer more or less satisfactorily the requirements for the ordinary growth of the organism. The

spores of *Bacillus larvae* will germinate and grow feebly on an agar medium in the preparation of which healthy bee larvæ are used as is meat in ordinary culture media, sterilizing as usual by heat in an autoclave (*49*). However (*51*), if a broth made by macerating healthy bee larvæ in several times their volume of water is sterilized without heating by filtering through sterile bacteria-proof filters and then is pipetted aseptically into tubes of previously sterilized liquefied agar cooled to 50° C., the resulting medium gives much better growth. This medium is nevertheless unsatisfactory, owing to difficulties of preparation, and particularly because of lack of material for its preparation except during the brood-rearing season. White (*54*) therefore devised a medium which consists of a suspension of the yolk of an egg aseptically in 70 cc. of sterile water, 1 cc. of which suspension is added by sterile pipette to each 5 cc. of ordinary sterilized tubed agar medium which has been melted and cooled to 50° C. Growth occurs on this medium quite abundantly, although with the technic described great care must be taken to prevent contamination.

Maassen (*28*) has also devised a medium made from a mixture of equal parts of a broth from calf or pig brain and a solution of egg albumin in water, to which 1.8 per cent agar and 1 per cent each of Witte's and Chapoteaut's peptone are added, after which it is filtered, tubed, and sterilized. This medium gives an almost neutral or weakly acid reaction to blue litmus paper. Maassen also found that the vegetative forms develop abundantly if grown on a meat and water medium if it is acid in reaction and if 0.25 per cent of pollen and 1.5 per cent of Aschmann's or Chapoteaut's peptone are added, but that the former medium is more favorable. Both media are found to deteriorate on too much heating. It is also stated that in acid peptone bouillon, in bouillon of bee larvæ, and in the brain bouillon, the bacillus may be cultivated, although growth is slow, the bouillon becoming weakly turbid and a thick slimy deposit gradually being formed.

For the purpose of the present experiments, after consideration of the advantages or disadvantages of the various media so far described, a modification of the egg-yolk suspension medium of White was adopted as the most satisfactory general medium. During the course of the experiments some modifications were made both in the medium and in the technic of preparation.

PREPARATION OF YEAST-EXTRACT AGAR BASE

Because of most satisfactory results in other work with various brood disease cultures, a yeast-extract agar described by Ayers and Rupp (*2*) was used instead of beef infusion agar as a base, because of the ease of preparation and the uniformity of the medium. Spores of *Bacillus larvae* on the surface of a slant of this agar germinate to some extent on this medium alone, and vegetative cultures from egg-yolk suspension agar transferred to the yeast medium grow fairly vigorously. The addition of egg-yolk suspension to the yeast-extract agar increased the vigor of growth and longevity of cultures.

One liter of the yeast extract agar is prepared as follows:

Dried yeast	grams	10
Peptone	do	10
Buffer (sodium glycero-phosphate)	do	5
Water	cc	500

This is heated in flowing steam for one-half hour, then adjusted to a hydrogen-ion concentration of $P_H = 7.6$ to 7.8 by the colorimetric method of Clark and Lubs (*16, 17*). The broth is then boiled for one minute over an open flame and filtered through filter paper on a perforated porcelain funnel, using siliceous earth to clarify. To this broth is added an equal amount (500 cc.) of double strength

(3 per cent) solution of agar, washed and filtered by the method described by Ayers, Mudge, and Rupp (*3*). The final hydrogen-ion concentration reaction is adjusted so that upon addition of 1 cc. of the egg-yolk suspension to 10 cc. of the yeast-extract agar the reaction is about $P_H = 6.8$. The normal hydrogen-ion concentration value of the contents of the larval intestine at various ages during the active feeding period with honey and pollen and just after sealing averages $P_H = 6.8$, varying to slightly more acid with the amount and type of pollen in the food material. Intestines were dissected out from the larvæ and macerated in 10 cc. of neutral distilled water and compared colorimetrically with known buffer solutions, using brom thymol blue as an indicator. Fabian and Parks (*22*) found this value to be $P_H = 6.6$ by macerating the entire larva in water. From earlier unpublished work by the writer, as well as by the above-mentioned investigators, the optimum hydrogen-ion concentration for the growth of *Bacillus larvæ* was found to be approximately $P_H = 6.8$. The yeast extract medium is tubed, sterilized in the autoclave at 15 pounds pressure for 15 minutes, and stored until needed.

PREPARATION OF EGG-YOLK SUSPENSION

The egg yolk can be diluted much more than was directed in the original formula with even better results, the more dilute suspension giving a more transparent medium with fully as profuse growth. A wide-mouthed flask containing 200 cc. water, sterilized with a cotton plug protected by a paper cap, is used for each egg yolk. At times, from 0.5 per cent to 1 per cent of a neutral buffer salt is added to the water previous to sterilization. This holds in check the slow increase in acidity observed on long standing. A small amount of normal sodium hydroxid (2 to 3 cc.) is also added to the flasks before sterilization to bring the resulting reaction of the egg suspension nearer to the desired reaction for the final medium.

APPARATUS TO REPLACE PIPETTING

An apparatus was devised (fig. 6) which to a great extent eliminates the danger of contamination of agar tubes when adding egg-yolk suspension, and also makes possible the preparation of a large quantity of medium in a short time. As a rule egg-yolk suspension can be stored or withdrawn at any time after the apparatus has been set up, until all used up, unless the egg yolk itself is not sterile. A two-holed rubber stopper, of correct size to fit the flask containing the egg-yolk suspension, is fitted with two tubes, one of small bore to reach nearly to the surface of the liquid when placed in the egg-yolk flask, and a second larger tube fitted flush to the inner surface of the stopper, protruding outward about 1½ inches. A piece of rubber tubing 5 inches long is fitted to this tube, closed with a pinchcock. To this rubber tube is attached a delivery tube which passes through another rubber stopper placed in one end of a glass cylinder 1¼ inches in diameter and 4 inches long, to about half its length. This forms a protective bell for the delivery tube similar to that used in filling vaccine or antitoxin ampules. The

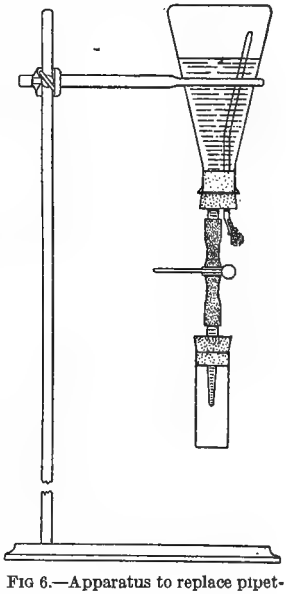

FIG 6.—Apparatus to replace pipetting of egg-yolk suspension

entire apparatus is sterilized in the autoclave, using a temporary empty flask into which the stopper for the culture flask is placed, and all is wrapped in paper with a paper protective cap over the open end of the delivery bell. Before use, the apparatus is removed from the paper and the stopper is carefully removed from the empty flask so as to prevent contamination and is fastened firmly in the flask containing the egg-yolk suspension. After placing the pinch cock in position, the apparatus is carefully inverted and hung on a ring stand. The small-bore glass tube in the flask now reaches a little above the surface of the liquid and serves for an air inlet. By means of this apparatus, sterile egg-yolk suspension can be added to tubes of sterile base medium, with little danger of external contamination, by inserting the tube under the protective bell.

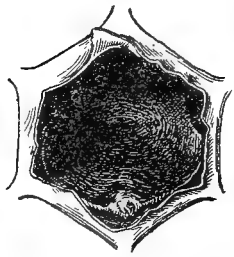

FIG. 7.—American foulbrood scale. End view. (White (55))

METHOD OF ISOLATION OF PURE CULTURES OF BACILLUS LARVAE

When medium is desired for the isolation or cultivation of *Bacillus larvae*, tubes of the yeast-extract agar are melted in a water bath and cooled to 55° C., after which from 1 to 2 cc. of egg-yolk suspension is added for each 10 cc. of base, by means of the apparatus described above. The contents of the tubes are well mixed and then slanted.

From a comb containing decaying material dead of the disease, a dried scale (figs. 7 and 8) is removed with a sterilized needle scalpel (also used for removing cappings) and dropped into the water of condensation in the culture tube to soften. It is then smeared over the surface of the agar with an inoculating needle. If ropy gluelike material is available it is more satisfactory (fig. 9). A large loopful of this is removed from the cell, from which the capping has been aseptically removed by means of an inoculating needle, and is streaked over the surface of the agar. A heavy initial inoculum gives best results, as it is often difficult to obtain growth with a small amount. It is quite easy to obtain pure cultures by this procedure, since almost never are secondary contaminations found associated with *Bacillus larvae*. Plating may be carried out from these initial cultures if absolute surety is desired, but initial growth is obtained much more easily by the tube culture method. Germination of spores and some growth take place during the first 24 hours' incubation at 37° C., but maximum growth is not obtained much before 48 hours.

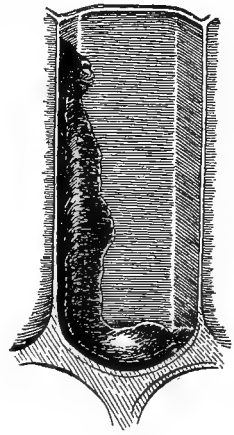

FIG. 8.—American foulbrood scale. Side view. (White (55))

EXPERIMENTAL PROCEDURE, USING AGAR SLANTS

To determine whether there is a correlation between germination of spores and vegetative growth of *Bacillus larvae* and the concentration of sugar in the culture medium, a series of tubes is prepared with varying percentages of dextrose, from 0.5 per cent to 10 per cent (Table I). These are prepared by adding the required amounts of dextrose to 50 cc. portions of the yeast-extract agar base, which is then tubed and sterilized at 10 pounds pressure for 15 minutes. On cooling to 55° C., 1 cc. of sterile egg-yolk suspension is

added to each tube and it is then slanted. Series of agar slants varying in sugar concentration are inoculated with either vegetative cultures or diseased material containing only spores. To determine spore germination an approximately uniform amount, about one 2-mm. loopful of ropy material, when available, is used for inoculation of slants, otherwise a scale softened as described above. If no visible growth takes place after 48 hours' incubation, stained smears are made, to determine whether any germination has occurred. In the case of the determination of growth from vegetative culture, a single uniform streak is made on the agar slant, using one 2-mm. loopful of growth from a 48-hour culture of *Bacillus larvae* prevously isolated and cultivated. After 48 hours' incubation, as well as after about one week, comparative observations are made of the relative amount and character of the growth. Where little or no growth has occurred, stained smears are made from the streak to see what has happened to the organisms. These experiments were carried out with a number of different strains of vegetative cultures and from a number of different samples of American foulbrood.

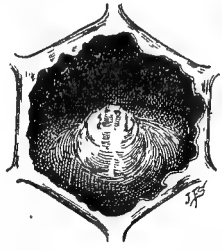

FIG. 9.—Partially decomposed American foulbrood larva at the stage of ropy consistency. (White (55))

TABLE I.—*The effect of varying the sugar concentration in egg-yolk suspension medium (1) on germination and vegetative growth from spores; and (2) on vegetative growth from vigorous vegetative cultures of Bacillus larvae.*[a]

Test material	Per cent dextrose in medium							
	Control	0.5	0.7	1.0	1.3	1.5	1.75	2.0
Spores	++++	++	+++	++++	++++	+++	+++	++
Vegetative cultures	++++	+++	+++	++++	++++	++++	+++	+++

Test material	Per cent dextrose in medium									
	2.25	2.5	2.75	3.0	3.5	4.0	4.5	5.0	7.5	10.0
Spores	+	+	+	+	− G	− G	− G	− G	− G	− G
Vegetative cultures	++	++	++	++	+	+	±	−	−	−

[a] The following symbols are used:
+ Slight growth.
++ Fair growth.
+++ Good growth.
++++ Heavy growth.
± Doubtful.
− No evidence of growth.
G Slight germination of spores.

EXPERIMENTAL PROCEDURE, USING PLATE CULTURES

The egg-yolk suspension agar is not entirely satisfactory for counting colonies in plate cultures, since the egg yolk gives the medium a cloudy, semiopaque appearance. However, by using the supernatant fluid from the egg-yolk suspension or a somewhat smaller amount of the suspension for each tube of yeast extract agar (10 to 15 drops), a fairly satisfactory plate culture is obtained if the proper amount of inoculum is used. The following procedure is used: To a series of melted tubes of yeast-extract agar containing varying amounts of dextrose as described above (Table II) the egg-yolk suspension is added and the desired inoculation of the tube made while the medium is still liquid. The tubes are agitated to mix the contents thoroughly and then poured into sterile Petri dishes.

These on cooling are inverted and incubated for 48 hours at 37° C., after which counts are made. If the plates are flooded with a dilute solution of fuchsin or eosin before counting, the colonies are more easily differentiated for counting in the semiopaque medium. Vegetative cultures only were used for plating. A suspension of one loopful of culture in 3 cc. of sterile broth is made and one loopful of that is used to inoculate each plate. Dilution in sterile water was also tried, using 1 cc. of the dilution for each plate, but without success, since there seems to be a minimum amount of initial inoculum required, below which it is difficult to obtain growth.

TABLE II.—*Average number of colonies per 4-mm. loopful of vegetative culture suspension in broth on plates of varying sugar concentration*

Per cent dextrose	Average number of colonies	Per cent dextrose	Average number of colonies
Control (o)	1,500	2.0	
0.5	1,590	2.5	150
1.0	1,560	3.0	0
1.5	914	3.5	0

OBSERVATIONS

SPORE GERMINATION AND GROWTH IN RELATION TO SUGAR CONCENTRATION

At different times during the investigation seven different series of culture tubes were made, using as material for inoculation either scales or, in most cases, ropy remains heavily laden with spores of *Bacillus larvae*, but no vegetative rods. This material was taken from six different samples of diseased brood from different localities. From these series of cultures, varying in sugar concentration from 0.5 per cent to 10 per cent dextrose, it was found that active growth occurs up to and including 2.5 per cent dextrose, although some growth occurs occasionally up to 3 per cent (Table I). The exact limits varied slightly with different strains as well as with variation in the amount of inoculum. Even up to 10 per cent dextrose concentration, a varying small number of spores germinate, as is demonstrated by stained smears, but they give no further evidence of vegetative growth upon the culture medium.

GROWTH FROM ACTIVE VEGETATIVE CULTURES

In a similar manner five different series of tubes with varying sugar concentrations were made, using 24-hour cultures of three different characteristic vegetative cultures of *Bacillus larvae*, previously isolated and accustomed to growth on artificial culture media for different lengths of time. Good growth occurs on the average up to 2.5 per cent to 3 per cent dextrose concentration, with evidence of varying slight growth up to 4 per cent and in one case up to 4.5 per cent (Table I). In the latter case much of the variation is due to variation in the amount of initial inoculum. If a heavy inoculation is made on the surface of the agar tubes, the upper sugar concentration limits for inhibition of growth are increased, although in these cases the growth was meager at best. Stained smears, however, made after a few days, from the higher sugar concentrations particularly, soon showed the peculiar disintegration of the rods noted by White (*55*) as taking place in old cultures and where spore formation is inhibited, such as in the presence of sugar. This, according to observations of Sturges and Rettger (*44*) on other organisms, suggests that this disintegration of the rods is the result of autolysis.

QUANTITATIVE GROWTH IN PLATE CULTURES

Great difficulty is found in obtaining satisfactory plate cultures. Only two series of plate cultures were obtained which could be counted successfully. The average number of colonies showed a definite decrease with increased sugar concentration, with no growth at 3 per cent or higher. (Table II, fig. 10). As stated above, the plate method, with the small amount of initial inoculum necessary for accurate counts, is not a satisfactory method for obtaining growth of *Bacillus larvae* under these conditions, although the method may be used for obtaining pure cultures.

From these observations (Table I) it is, therefore, safe to conclude that a concentration of reducing sugar of approximately 3 to 4 per cent or more inhibits the growth of *Bacillus larvae*, although slight germination of spores may take place at higher sugar concentrations.

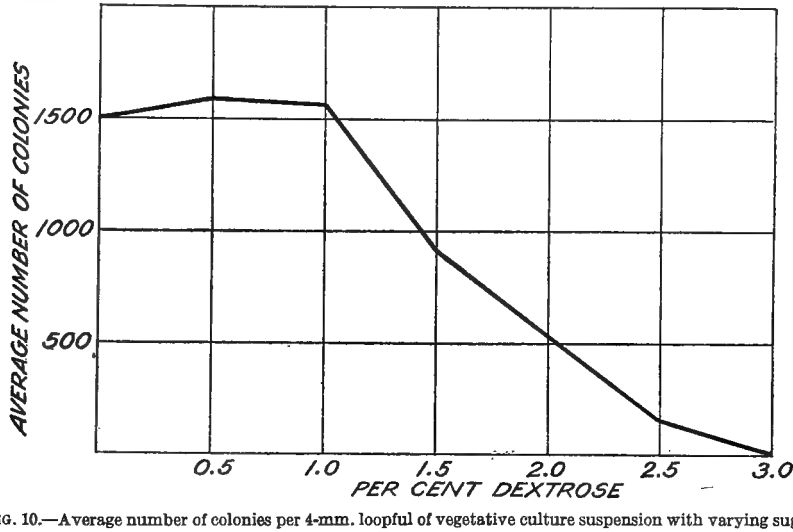

FIG. 10.—Average number of colonies per 4-mm. loopful of vegetative culture suspension with varying sugar concentration (Table II)

QUANTITATIVE DETERMINATION OF UNASSIMILATED SUGAR IN THE LARVAL INTESTINE AT VARIOUS AGE PERIODS

In the preliminary experiments it was shown that unassimilated sugar is present in the intestinal contents of the actively feeding larva, which apparently is assimilated completely by the time the prepupa has extended in the cell and has become quiescent. Since it is demonstrated that a direct relation exists between the growth of *Bacillus larvae* in suitable culture media and its reducing sugar concentration, it is now necessary to determine quantitatively the amount of unassimilated sugar in the intestine of the feeding larva and in the intestine of the prepupa, in order to determine whether reducing sugar concentration has any bearing on the time of attack by American foulbrood.

COMPOSITION OF BROOD FOOD

The older bee larvæ (*40*) receive a food consisting of a mixture of honey or nectar and pollen, rich in sugar, chiefly reducing sugar. This sugar constitutes about 45 per cent of the dried substance, or over 13 per cent of the fresh substance. The food of younger larvæ contains only about 5 per cent of sugar in the fresh

material (Table III, fig. 11). Nelson and Sturtevant (*35*) and Lineburg (*26*) have shown that the change in the composition of this food comes definitely soon after the second day, instead of the fourth day, as stated by Von Planta, after which increasingly large amounts of honey and pollen are fed up until the time of sealing. The larva is fed during this period about as fast as it can ingest the food. From this it is reasonable to suppose that there must be a constant surplus of unassimilated food in the larval intestine until after feeding has ceased.

TABLE III.—*Percentage composition of worker brood food, calculated from Von Planta (40), and on the basis of his assumption of 70 per cent water content*

Substance	Under four days		Over four days	
	Dried substance	Fresh substance	Dried substance	Fresh substance
	Per cent	*Per cent*	*Per cent*	*Per cent*
Nitrogenous	53.38	16.01	27.87	8.36
Fat	8.38	2.51	3.69	1.11
Glucose	18.09	5.43	44.93	13.48

COMPOSITION OF HONEY

The average chemical analysis of American honeys has been shown by Browne (*9*) to be as follows: Moisture 17.59 per cent, invert sugar 74.41 per cent, sucrose 1.98 per cent, ash 0.23 per cent, dextrin 2.09 per cent, undetermined 3.70 per cent. Approximately the same percentages have been found by all other workers in this field. The maximum sucrose content of honey is given in American standards for food analysis as 8 per cent, although a few samples have been found with a slightly higher sucrose content. In the utilization of honey as food by either the adult bee or the larva, it may be assumed that sucrose is rapidly hydrolized. In any analysis of the stomach content of the bee larva for sugar content, therefore, after the change in larval food has occurred and when honey enters directly into its composition, it may safely be assumed that a determination of the amount of reducing sugar will indicate the amount of unassimilated sugar in the intestine, since there will be but a small additional sugar content from sucrose, if any of the latter sugar still remains. In determining the sugar content of the whole larva, as was done in most of the present work, it may be assumed that there is a comparatively small amount of reducing sugar in the blood stream, because of the exceedingly rapid transformation of these sugars into fat and glycogen which are known to occur in the bee larva. It is therefore concluded that the sugar found in the whole larva is virtually that which occurs in the intestine alone, and this greatly simplifies the work of analysis.

COMPOSITION OF THE LARVA AT DIFFERENT AGE PERIODS

The work of Straus (*43*) on the chemical composition of the worker and drone brood during their different developmental stages gives the results of the metabolism of this food, as indicated by the presence of fat and glycogen stored in the so-called fat body of the larva (Table IV, fig. 12). He was unable to demonstrate more than a trace of what he terms reducing substances, except in one case in which only a slight amount was found. He believes that this is because the sugar of the larval food is assimilated so rapidly, as is indicated in the larval composition by the exceedingly rapid increase in the amount of glycogen and fat until after feeding has ceased.

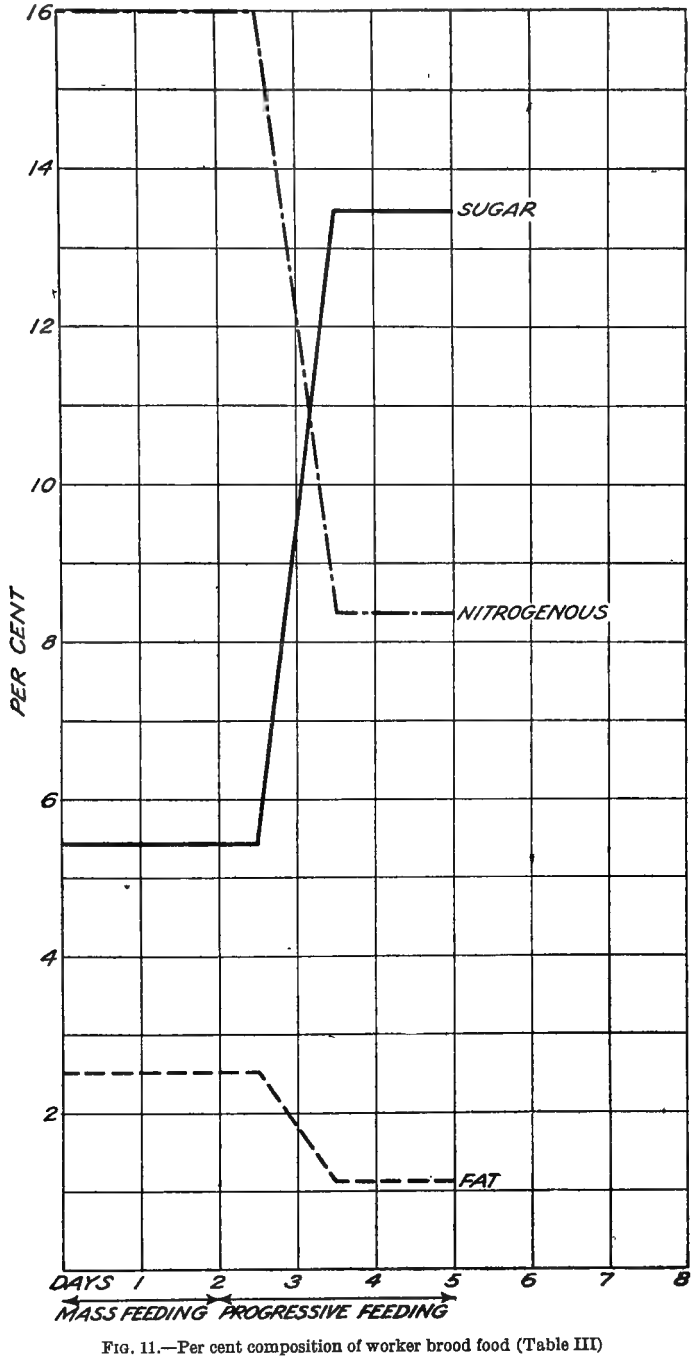

FIG. 11.—Per cent composition of worker brood food (Table III)

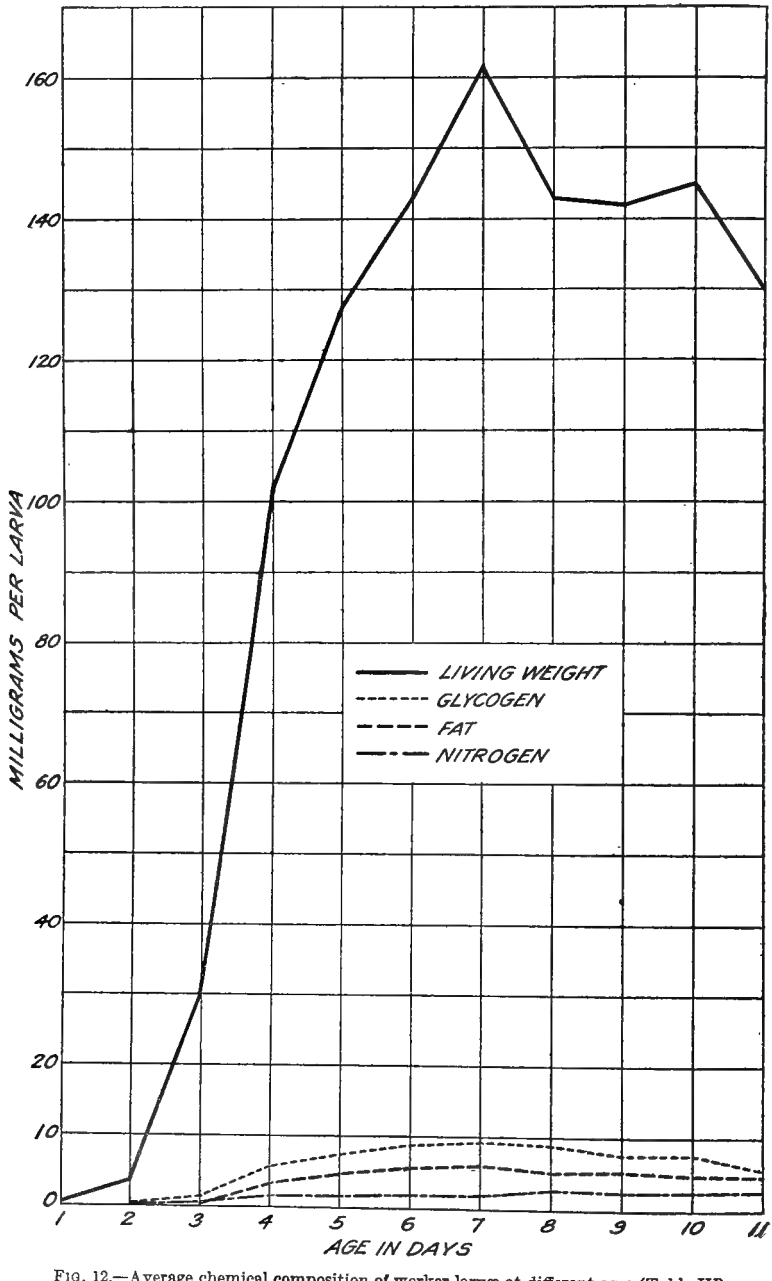

FIG. 12.—Average chemical composition of worker larvæ at different ages (Table IV)

TABLE IV.—*Average chemical composition of worker larvæ at different ages, compiled from Straus (43)*

Age	Weight of larva	Glycogen		Fat		Nitrogen		Reducing sugar
		Grams per larva	Per cent of fresh substance	Grams per larva	Per cent of fresh substance	Grams per larva	Per cent of fresh substance	
Days	*Grams*							
1	0.00030							
2	.00340	0.00008	2.50	0.00004	1.53	0.00009	2.86	
3	.03000	.0012	2.75	.00005	1.64	.0005	2.04	
4	.10010	.0055	5.58	.0031	3.60	.0016	1.44	
5	.12775	.0072	5.57	.0047	3.64	.0016	1.47	0
6	.14290	.0088	6.95	[a].0057	[a]3.98	.0019	1.45	
7	.16140	.0092	6.43	.0060	3.71	.0018	1.22	Trace.
8	.14300	.0089	6.35	.0051	3.53	.0027	1.51	Trace.
9	.14200	.0075	5.21	.0052	3.66	.0022	1.60	
10	.14500	.0075	5.24	.0049	3.60	.0022	1.58	0.0002
11	.13000	.0056	4.21	.0047	3.26	.0023	1.68	

[a] Calculated by interpolation and averaging.

CHOICE OF REAGENT

It was necessary to devise a special technic for the determination of the unassimilated reducing sugar in the larva by the application of procedures used in other analyses where small amounts of reducing sugars must be determined, such as in urine analysis. After studying the various methods of sugar analysis, a volumetric titration method seemed the most promising.

For the purpose of determining quantitatively the unassimilated sugar in the bee larva at different ages, the modified copper sulphate solution of Benedict (*5*) was chosen, mainly because, as in urine analysis, it has proved more satisfactory than any other titration method for determining small amounts of reducing sugars quantitatively, and because this solution keeps indefinitely without deteriorating. The potassium sulphocyanate in the solution produces, upon reduction of the sugar, a white precipitate of cuprous sulphocyanate, which permits the end point of the reaction to be more accurately determined than with Fehling's solution. A trace of ferrocyanid is added to prevent precipitation of red cuprous oxid which may be caused by certain impurities, which would interfere with the determination of the end point. The test solution is standardized to a known solution of dextrose so that 5 cc. equals 0.0102 grams of dextrose.

CHOICE OF LARVAE

Since there is little likelihood of there being any appreciable amount of sugar elsewhere than in the intestine, analyses were made of entire larvæ, because of the great difficulty attending the dissection of the intestines. Larvæ for analysis were chosen from combs having large areas of brood of uniform size and age. In most cases 25 larvae as nearly of the same size as possible were carefully removed from the cells by means of a pair of fine forceps, care being taken to remove as little uningested food as possible. Any visible amount of adhering food was removed with filter paper and the 25 larvæ were weighed. Several series were weighed for each age above the two-day age period through to about the fourth day after capping.

DETERMINATION OF AGE OF LARVAE

When choosing larvæ for the analysis, the approximate age was determined by comparison with drawings to scale by Nelson and Sturtevant (*35*) of larvæ of known age at various age periods, 24 hours apart. Nelson and Sturtevant, as

well as Straus (Table IV), also give weights for larvæ of known age, but in order to eliminate the danger of variations due to the effect of different seasonal and environmental conditions, the average age of the larvæ analyzed from various groups of 25 was determined by comparison with a series of weighings of larvæ of known age that were made during this same period (*35*). The various series of weights, with the corresponding determinations of reducing sugar, were arranged in age groups, 24 hours apart, as shown in Table V. In some cases, such as the small two-day larvæ, or the quiescent prepupæ, where the amount of unassimilated sugar is small, 50 larvæ were taken for analysis, but usually 25 proved satisfactory.

PREPARATION OF MATERIAL FOR ANALYSIS

Several difficulties were encountered in the preparation of material for sugar determination. At first, attempts to extract the sugar were made by macerating the larvæ with distilled water and filtering through filter paper. This produced a cloudy opalescent liquid, indicating the presence of colloidal material, and this solution did not give the characteristic reaction with the Benedict reagent. Various clarification methods were tried. Precipitation with both neutral and basic lead acetate (*10, p. 276*) solutions proved unsatisfactory, something still remaining to interfere with the reaction. Mercuric nitrate solution, which is sometimes used to clarify liquids of animal origin such as blood, urine, and milk, was tried (*10, p. 447*). This method occasionally gave good results, mainly with the younger larvæ, but often with older larvæ and prepupæ the colloidlike material still remained in the filtrate, interfering with the reaction. Furthermore, because of the numerous filtrations necessary to remove successive precipitates, it was feared that more or less sugar is lost by adsorption to those precipitates, even with careful washing. An attempt was made to clarify by filtration with suction through a celloidin membrane, and this gave a clear solution which reacted well with the test solution, but the method required too great time. The method finally adopted was by extraction with 50 per cent alcohol, similar to the method used in the extraction of sugars from grains and similar products (*11*). This method proved successful, since the alcohol causes precipitation of all solid matter, giving a clear filtrate which reacted properly with the Benedict's reagent. Since glycogen in water solution is colloidal in nature, and thereby difficult to remove by filtration from such a solution, it is doubtless the glycogen present in the larva which prevented clarification and interfered with the reaction. It is possible for this reason that Straus (*43*) failed to demonstrate reducing sugars. To determine this point, a small amount of glycogen was added to a known solution of dextrose and tested with the copper sulphate solution, and the known reducing sugars could not now be demonstrated quantitatively. Since glycogen is insoluble in alcohol (*10, p. 443*) the 50 per cent alcohol precipitates the glycogen and thereby removes materials interfering with the reaction in the filtrate. Even though there may still be a small loss of reducing sugar by adsorption or by some other means, the results obtained are of value for purposes of comparison. If any reducing sugar is lost by the method adopted, the amount is exceedingly small and may therefore be disregarded, since repeated washings failed to demonstrate its presence.

TECHNIC ADOPTED

After weighing, the larvæ are removed to a small porcelain mortar and macerated in 30 cc. of 50 per cent alcohol. This material is then washed carefully into a small flask and allowed to stand from two to three hours before filtering. The precipitate is washed with 50 per cent alcohol. The filtrate is then made up to 50 cc. with distilled water, and run into a burette. Five cc. of the stand-

ardized Benedict's solution are placed in a white porcelain casserole and diluted with an equal amount of distilled water. To this are added about 5 grams of anhydrous sodium carbonate and a small amount of ground pumice. This solution is brought to a boil and the larval extract is run in slowly, drop by drop at the end, until the blue color disappears and a white precipitate forms. From the number of cc. of larval extract used, the milligrams of sugar per larva and the per cent of sugar per larva are calculated (Table V).

TABLE V.—*Unassimilated sugar in intestinal content of larvæ at different ages*

Larvæ of known age, Sturtevant (*55*)			Larvæ analyzed for presence of unassimilated sugar (weights in grams)									
Age in days	Average weight	Limits by weight for age groups	Date	Weight of sample	Number of larvæ	Average weight of 1 larva	Extract used[a]	Equivalent number of larva	$CuSO_4$ solution	Equivalent dextrose	Dextrose per larva	Sugar per larva
	Gram	*Gram*	*1922*	*Gram*		*Gram*	*Cc.*		*Cc.*	*Gram*	*Gram*	*P. ct.*
2	0.004745	Up to 0.014685.	7–18	0.6233	50	0.01247	50	50	5	No reaction.	0	0
3	.024626	0.014685 to 0.059308.	7–25	.4967	25	.01987	50	25	5	No reaction.	0	0
			7–25	1.1072	25	[b] .04429	44	22	5	0.01020	0.000463	1.13
			8–2	[b] 1.0979	25	[b] .04392	23.5	23.5	5	.01020	.000434	.98
			5–9	[c] 2.2906	50	.04581	90	45	10	.02040	.000453	.94
			8–11	1.1706	25	.04682	42	21	5	.01030	.000490	1.04
			7–27	1.4009	25	.05604	20.25	10.125	5	.01020	.001007	1.79
	Average					.043222					.000475	.98
4	0.093990	0.059308 to 0.120369.	8–2	1.6749	25	.06700	21	10.5	5	.01020	.00097	1.44
			8–11	[d] 1.6817	25	.06727	11	9.16	5	.01030	.00112	1.66
			8–11	1.9372	25	.07749	12.75	6.375	5	.01030	.00161	2.07
			8–11	1.9916	25	.07966	18.1	9.05	5	.01030	.00113	1.41
			7–31	2.3044	25	[b] .09218	6.8	3.4	5	.01020	.00300	3.25
			8–2	2.3566	25	[e] .09426	8.6	4.3	5	.01020	.00237	2.51
			8–30	2.3733	25	.09493	6.58	3.29	5	.01030	.00313	3.29
			7–25	2.4901	25	.09960	5.8	2.9	5	.01020	.00351	3.52
			8–2	2.6748	25	.10699	8.0	4.0	5	.01020	.00255	2.37
			8–17	2.6843	25	.10737	4.75	2.38	5	.01030	.00431	4.01
			8–18	2.7781	25	.11112	4.35	2.175	5	.01030	.00473	4.25
			8–2	2.7919	25	.11168	7.0	3.5	5	.01020	.00291	2.61
			8–18	2.8205	25	.11282	4.35	2.175	5	.01030	.00473	4.19
			6–1	2.8332	25	.11333	12.7	6.35	8.9	.018156	.00286	2.52
			8–11	2.8972	25	.11589	6.8	3.4	5	.01030	.00303	2.61
			8–4	2.9274	25	.11710	7.1	3.55	5	.01020	.00287	2.45
			8–2	2.9505	25	.11802	7.1	3.55	5	.01020	.00287	2.43
			8–31	2.9749	25	.11900	5.2	2.6	5	.01030	.00396	3.32
			8–2	2.9908	25	.11963	6.0	3.0	5	.01020	.00340	2.84
			8–17	3.0038	25	.12015	4.5	2.25	5	.01030	.00457	3.80
			8–4	3.0105	25	.12042	6.5	3.25	5	.01020	.00314	2.61
	Average					.10314					.00299	2.82
5	0.146748	0.120369 to 0.150876.	6–1	[c] 3.0961	25	.12384	27.0	6.75	10	.02040	.00317	2.57
			8–18	3.1148	25	.12459	4.2	2.1	5	.01030	.00490	3.93
			8–18	3.1953	25	.12781	4.2	2.1	5	.01030	.00490	3.83
			8–11	3.2141	25	.12856	6.4	3.2	5	.01030	.00322	2.51
			7–26	3.3153	25	.13261	5.6	2.8	5	.01020	.00364	2.75
			7–31	[e] 3.3278	25	.13311	5.0	2.5	5	.01020	.00408	3.06
			8–11	3.3479	25	.13392	6.5	3.25	5	.01030	.00317	2.36
			8–10	3.3602	25	.13441	6.15	3.075	5	.01030	.00334	2.45
			7–31	3.3689	25	.13476	4.1	2.05	5	.01020	.00497	3.69
			8–11	3.3706	25	.13482	6.1	3.05	5	.01030	.00337	2.49
			7–27	3.3721	25	.13488	4.5	2.25	5	.01020	.00453	3.35
			7–25	3.4029	25	.13612	3.4	1.7	5	.01020	.00600	4.41
			7–25	3.4620	25	.13848	5.25	2.625	5	.01020	.00388	2.80
			8–17	3.4644	25	.13858	3.5	1.75	5	.01030	.00588	4.23
			8–31	3.4775	25	.13910	4.6	2.3	5	.01030	.00448	3.22
			7–25	[f] 3.6156	25	.14462	7.92	3.96	6.07	.01238	.00312	2.15
			8–17	3.6394	25	.14558	3.6	1.8	5	.01030	.00572	3.92
			8–17	[f] 3.6971	25	.14788	4.2	2.1	5	.01030	.00490	3.31
			8–18	[f] 3.7164	25	.14866	5.2	2.6	5	.01030	.00396	2.66
	Average					.13591					.00428	3.14

[a] Unless otherwise stated, total cc. of extract equals 50
[b] Total extract, 25 cc. only.
[c] Total extract, 100 cc.
[d] Total extract, 30 cc. only.
[e] Just sealed, early.
[f] Just sealed, still coiled.

TABLE V.—*Unassimilated sugar in intestinal content of larvæ at different ages*—Continued

Larvæ of knownage, Sturtevant (35)			Larvæ analyzed for presence of unassimilated sugar (weights in grams)									
Age in days	Average weight	Limits by weight for age groups	Date	Weight of sample	Number of larvæ	Average weight of 1 larva	Extract used	Equivalent number of larva	$CuSO_4$ solution	Equivalent dextrose	Dextrose per larva	Sugar per larva
	Gram	*Gram.*	*1922*	*Grams*		*Gram*	*cc.*		*cc.*	*Gram*	*Gram*	*P.ct.*
6	0.155005	0.150876 to maximum and down to 0.148326.	7–18	[c] 3.8012	25	0.15205	18.05	4.51	9.6	0.19584	0.00434	2.85
			8–31	3.8038	25	.15215	5.4	2.7	5	.01030	.00381	2.50
			8–11	3.8925	25	.15570	4.35	2.175	5	.01030	.00473	3.03
			8–31	[g] 3.9783	25	.15913	6.0	3.0	5	.01030	.00343	2.15
			8–31	4.1249	25	.16500	7.0	3.5	5	.01030	.00294	1.78
			7–27	3.7706	25	.15082	6.65	3.33	5	.01020	.00306	2.03
	Average					.15581					.00372	2.39
7	0.141648	0.148326 to 0.139406.	8–18	3.6980	25	.14792	7.3	3.65	5	.01030	.00282	1.91
			8–10	3.5600	25	.14280	8.9	4.45	5	.01030	.00231	1.56
			8–10	[h] 3.5835	25	.14334	11.5	5.75	5	.01030	.00179	1.25
			8–4	3.5572	25	.14229	7.4	3.7	5	.01030	.00275	1.93
			8–18	[i] 3.4871	25	.13948	45.0	22.5	5	.01030	.00050	.36
	Average					.14397					.00203	1.40
8	0.137165	0.139406 to 0.135158.	7–26	3.4358	25	.13743	50.0	25	5	No reaction.	0	0
			7–18	[j] 3.4453	25	.13781	50.0	25	5	No reaction.	0	0
	Average					.13762					0	0
9	0.133152		8–4	[k] 3.3232	25	.13293	50.0	25	5	No reaction.	0	0

[c] Total extract, 100 cc.
[g] All sealed, coiled or with backs out. Feeding ended and spinning of cocoons started.
[h] Cocoon partially spun, still some color in the intestine.
[i] Cocoon not quite finished, still moving somewhat, no color in intestine.
[j] Quiescent prepupæ, intestines colorless, empty, histolysis started.
[k] First indication of change in external form.

OBSERVATIONS

Over 60 samples of 25 larvæ each of various ages, containing over 1,600 individual larvæ, were analyzed for the presence of reducing sugars. The largest number of analyses were made on larvæ from $3\frac{1}{2}$ to $5\frac{1}{2}$ days of age during the active honey and pollen feeding period. At least five analyses were made of each of the other age periods which might show the presence of sugar. To obtain averages with a small probable error, the analyses are grouped by age periods of 24 hours each, as described earlier (Table V, fig. 13). All larvæ in the two-day group, as well as one sample of larvæ nearly as heavy as the three-day average larva, showed no reducing sugar. Larvæ in the three-day group, averaging 0.043222 gm. in weight, gave 0.000475 gm. of reducing sugar per larva, or 0.98 per cent concentration. Larvæ in the four-day group, averaging 0.10314 gm. in weight, gave 0.00299 gm. of reducing sugar per larva, or 2.82 per cent concentration. Larvæ in the five-day group, comprising those just prior to sealing, with a few just sealed, averaging 0.13591 gm. in weight, gave 0.00428 gm. of reducing sugar per larva, or 3.14 per cent concentration. In the five-day group there were two samples which gave a concentration of over 4 per cent, the maximum being 4.41 per cent. The six-day group, comprised entirely of larvæ that had been sealed, had finished feeding and had started spinning, averaging 0.15581 gm. in weight, gave 0.00372 gm. of reducing sugar per larva, or 2.39 per cent concentration. This group contains larvæ of maximum size (fig. 14). From

this point on the gross weight decreases as preparation for metamorphosis begins. The seven-day group, comprising larvæ which are still moving about in spinning, and most of which show only a slight remaining color in the intestines, indicating

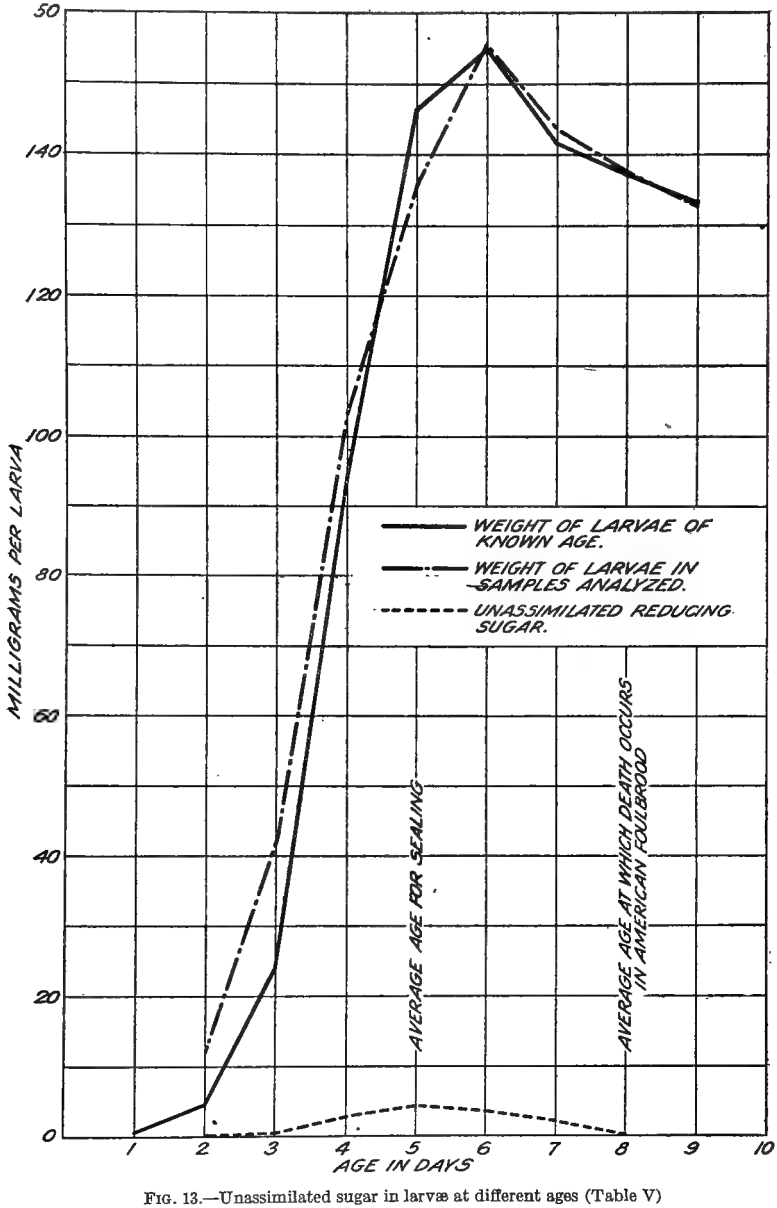

FIG. 13.—Unassimilated sugar in larvæ at different ages (Table V)

that the connection between ventriculus and end gut is made, averaging 0.14397 gm. in weight, gave 0.00203 gm. of reducing sugar per larva, or 1.40 per cent concentration. One sample in this group gave as low as 0.36 per cent. Larvæ

of the eight-day group, averaging 0.13762 gm. in weight, showed a total absence of reducing sugar. These larvæ represent the two-day quiescent prepupal stage (fig. 3 and 4). They have stretched out motionless in the cell, the intestines are entirely empty and colorless, and the histolysis of the tissues preliminary to metamorphosis has begun.

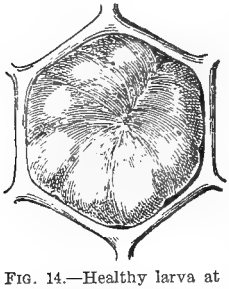

FIG. 14.—Healthy larva at age of maximum size, just after sealing and before the start of the cocoon-spinning period (White (*56*))

From these observations it is seen that there is an amount of reducing sugar in the entire actively feeding larva which would seriously interfere with the germination and growth of *Bacillus larvae*, provided the entire bee larva were to serve as the medium for its growth. Since this reducing sugar does not exist equally distributed throughout the bee larva, and since at this stage the organisms are found almost solely in the intestinal tract, it is certain that the reducing sugar concentration of the intestine is sufficient to prevent the germination of *Bacillus larvae*, so that death from American foulbrood is delayed until after the larva has been sealed in the cell and has become quiescent. This will be discussed more in detail later.

SUPPLEMENTARY STUDIES ON THE BIOCHEMICAL REACTIONS OF BACILLUS LARVAE

Up to the present time few facts have been determined concerning the biochemical reactions of *Bacillus larvae*, mainly because of a lack of suitable culture media. White states (*55*), "Carbohydrate liquid media as ordinarily prepared are not suitable for the growth of *Bacillus larvae*. In some of these after a considerable period a slight growth may appear at the bottom of the tubes. A little brood-filtrate or egg-suspension added to the media improves it. No visible gas is formed, but in some instances slight acidity is produced. No growth takes place in plain or in brood-filtrate gelatin at temperatures at which it remains congealed." Maassen states (*28*), "The bacillus also grows on nutrient gelatin. Upon a nutrient gelatin medium which had been made from the previously mentioned nutrient liquids, and an almost completely neutralized gelatin (a so-called emulsion of gelatin), there resulted growth although very slowly, from which a quite gradual liquefaction of the gelatin resulted. Liquefaction did not occur in the presence of grape sugar (dextrose). Through the addition of 1 per cent grape sugar the growth-producing ability of the gelatin as well as of other nutrient media was noticeably improved. On the most favorable media no special chemical properties were shown, with the exception of the ability to peptonize. The destruction of the albuminous bodies occurred very slowly and with little characteristic appearance. Only in worn-out cultures could any odor resembling foul glue be detected after a time." There are, however, certain characteristic manifestations in American foulbrood resulting from the growth and metabolism of *Bacillus larvae*, aside from the gross symptoms and appearances, which only a more complete knowledge of the biochemical activity of the organism can explain.

From the previous cultural experiments (Table I) it may be seen that apparently *Bacillus larvae* can utilize in its metabolism a certain amount of reducing sugar (dextrose), although this sugar is not necessary to the development of the organism. In the larva which is attacked by American foulbrood there may be two sources of sugar, that present unassimilated in the intestine and that hydrolyzed from the stored glycogen. Hydrolysis of glycogen may occur in connection with histolysis of the tissues preparatory to metamorphosis through enzym

action, or *Bacillus larvae* itself may have the ability to produce enzymes which hydrolyze the glycogen, or it may be a combination of both. Through the utilization of this reducing sugar one would expect that there at least would be a considerable production of acid, but, as stated earlier, the hydrogen-ion concentration of dead ropy material is never found to vary much from P_H=6.6 to 6.8. Since the data available concerning the biochemical reactions of *Bacillus larvae* offer no explanation of this hydrogen-ion concentration, a series of experiments was devised, the results of which add materially to the knowledge concerning the biochemical reactions and relationships of *Bacillus larvae*. In certain cases where, because of the limitations on growth, cultural growth has failed, it was found possible to obtain the desired information by examination of the diseased larval remains.

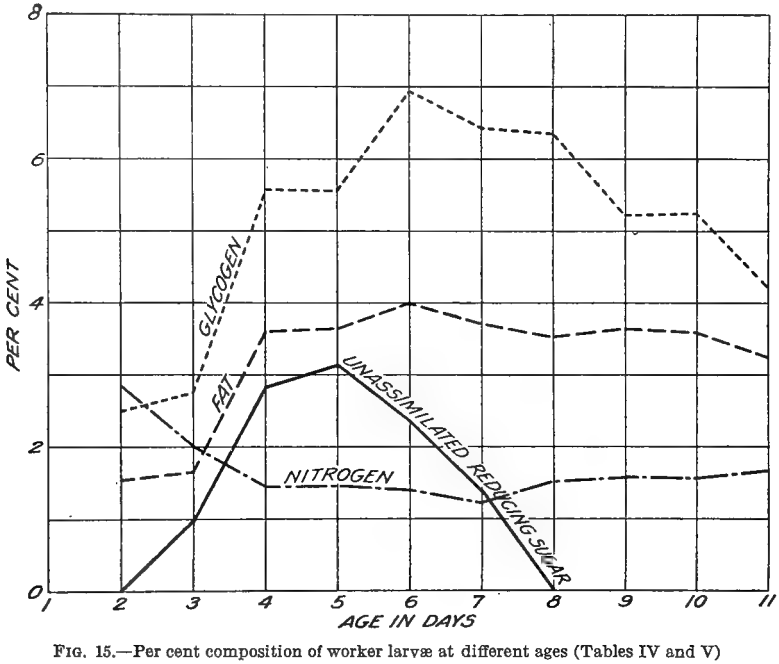

FIG. 15.—Per cent composition of worker larvæ at different ages (Tables IV and V)

UTILIZATION OF GLYCOGEN

According to Straus (Table IV, fig. 15) the greatest percentage of stored glycogen occurs just after sealing, when feeding has ceased. If an emulsion of the tissues of a larva of this age, or slightly older, at the age when prepupæ usually die of American foulbrood, is tested for the presence of glycogen with iodin solution,[3] the resulting deep reddish brown color shows that there are large amounts of glycogen present. If a prepupa which has just died from disease, slimy in consistency, light brown in color, and which in the microscopic picture still shows the presence of vegetative rods, is tested with iodin solution, it will

[3] Glycogen treated with iodin solution gives a color varying from brown to wine red, which disappears upon heating to 60° C., but returns again upon cooling. Soluble plant starch with iodin solution gives the following reactions: Amylodextrin, first dextrin of conversion, dark blue; erythrodextrin, second dextrin of conversion, red; intermediate steps give various shades of purple or lavender.

be found that most of the glycogen has disappeared, although the iodin solution gives a light yellowish brown color. The presence of a trace of reducing sugar also occasionally can be demonstrated with Benedict's solution in diseased material of this type where vegetative organisms are still actively present. In material which has decomposed completely, has reached the dark brown ropy stage (fig. 9), and contains only spores of *Bacillus larvae*, glycogen is found to be completely absent, nor can any reducing sugar be demonstrated, the sugars having been completely destroyed.

This type of material stained with Sudan III or osmic acid (*32, p. 78*) shows fat globules in practically the same condition and amount as in healthy larvæ, so that fat is apparently not acted upon by *Bacillus larvae* even after drying down to the scale stage.

Glycogen of the fat body of the healthy larva is hydrolyzed to dextrose to be used in metamorphosis, by the action of enzyms during the histolytic processes subsequent to sealing and prior to metamorphosis. This enzym action is demonstrated by the following experiments:

EXPERIMENTAL PROCEDURE

Several series of 50 healthy prepupæ each that had reached the period of quiescence were macerated in 25 cubic centimeters of 50 per cent alcohol and incubated at 37° C. for from 3 to 24 hours. The extract was then filtered and diluted with an equal amount of water. A series of test tubes were prepared, using for each tube 5 cubic centimeters of this extract and 5 cubic centimeters of 0.4 per cent glycogen in water, and also another series using 5 cubic centimeters each of a 0.1 per cent soluble starch. Both glycogen and starch were used, since it has been shown by Bradley and Kellersberger (*8*), as well as by experiments by the writer using commercial Taka-diastase, that diastase acts similarly on both glycogen and starch. These tubes were incubated for various periods and then tested with iodin solution for the presence of glycogen and starch (Table VI). Hydrolysis of both glycogen and starch seems to be complete after incubation for about five hours, and positively complete after incubation overnight, demonstrating the presence of diastase in the prepupæ.

In another experiment 50 prepupæ were macerated in 50 cc. of water and incubated at 37° C. for 24 hours. Then sufficient 95 per cent alcohol was added to precipitate any glycogen present, and the solution was filtered and tested with both the qualitative and the quantitative Benedict's solutions. In both cases definite traces of reducing sugar could be demonstrated, none having been present in the original solution before incubation, again demonstrating enzym activity of the larval tissues. This may have been due to action by bacterial contamination, but if such had been the case the sugar would probably have been fermented and could not have been demonstrated.

In a similar manner extracts with 50 per cent alcohol were made of ropy diseased material, enzym activity being demonstrated in the same manner as above. This, however, does not indicate whether the organism causing the disease has any diastatic power or whether the reaction was due to enzyms remaining in the decomposed tissues. Further extracts were made with 25 per cent and 50 per cent alcohol of several 48-hour vegetative cultures of *Bacillus larvae* grown on egg-yolk suspension medium. These extracts showed definite enzym activity with glycogen after a few hours' incubation, and more positive activity after incubation overnight (Table VI), while with starch marked hydrolysis was shown after only a few hours' incubation.

TABLE VI.—*Test for diastatic action with alcoholic extract*[a]

Test material	Control tubes		Color with iodin after incubation of—									
			0 hour		½ hour		2½ hours		5½ hours		18 hours	
	Glycogen	Starch	Glycogen	Starch	Glycogen	Starch	Glycogen	Starch	Glycogen	Starch	Glycogen	Starch
Extract of healthy prepupæ	++++ (brown)	++++ (blue)	+++	++	++	+	+	±	——	——	——	——
Extract of decomposed ropy remains	++++	++++	——	——	++	+	+	±	——	——	——	——
Extract of vegetative cultures	++++	++++	——	——	+++	++	++	+	——	——	——	——

[a] The following symbols are used:
++++ Deep color, brown or blue.
+++ Slightly lighter brown than check or wine color.
++ Light coffee brown or lavender.
\+ Trace faint brown or trace faint lavender.
—— No color or only iodin color, showing complete diastatic action.

To further determine the production of diastase by *Bacillus larvae*, a series of Petri dishes were poured, using yeast-extract egg-yolk suspension agar, to which had been added respectively 0.25 per cent and 1 per cent of glycogen and 0.25 per cent and 1 per cent of starch, this being an adaptation from methods described by Vedder (*48*) and by Allen (*1*). After solidification of the media in the Petri dishes, smears were made upon the surface of the agar from 48-hour cultures of various previously isolated strains of *Bacillus larvae*. After several days the plates were examined, first by holding up to the light and then later by flooding with iodin solution, and comparing with control plates containing no starch or glycogen. In nearly all the plates good growth had occurred, causing clear areas to be produced in the cloudy culture medium extending slightly beyond the edge of the area of growth. When flooded with iodin the halo around the culture growth, although not wide, was more prominently differentiated from the surrounding medium, showing in both glycogen and starch plates. These results, in conjunction with those of the extraction experiments, demonstrate that weak diastatic action is produced by *Bacillus larvae*.

ACID PRODUCTION

It has been shown that there is still an appreciable amount of sugar (reducing sugars in the food remaining in the intestines and dextrose available from glycogen) present in the larva after sealing and in the prepupa at the age when American foulbrood attacks, available for fermentation (Tables IV and V). In the various cultural investigations both by others and by the present writer, there is no evidence of carbon dioxid production. It would be expected, however, that at least some acid would be produced from the bacterial fermentation of these sugars, which is known to be present. To determine this more definitely than heretofore, a culture medium was devised for the qualitative determination of acid production, which gave good vigorous growth of *Bacillus larvae*.

The method used is an adaptation of the method of using agar slants for detecting acid formation, instead of liquid medium, described by Conn and Hucker (*18*), in which the change in reaction can readily be seen. The regulation yeast-extract egg-yolk suspension agar was prepared for this purpose by adding to the yeast extract base before sterilization an indicator in the proper amount both to the plain medium and also to a portion to which was added 1 per cent of dextrose.

Brom thymol blue was first used, as it covers the range of the supposed optimum reaction for *Bacillus larvæ* as described earlier. Baker (*4*) also has shown that brom thymol blue, used in about a 0.0024 per cent concentration in culture media, gives the most desirable color for comparison, without inhibiting acid fermentation. This concentration was obtained by using 12 cc. of a 0.2 per cent alcoholic solution of the indicator per liter. After marked acid production in the dextrose tubes was demonstrated with brom thymol blue, brom cresol purple was used as suggested by Conn and Hucker (*18*) in a 0.001 per cent concentration as a check on the end point. This concentration was obtained by using 8 cc. of a 0.2 per cent alcoholic solution of the indicator per liter. The yeast-extract base, both with and without dextrose, was adjusted so that after the addition of the egg-yolk suspension the final medium would have a primary reaction of approximately P_H=7.2, a definite blue grass green in the case of brom thymol blue and a marked purplish tinge with brom cresol purple, except in one series, where the primary reaction of the plain medium was P_H=7.6. These tubes after being slanted were inoculated as usual, both with vegetative cultures and with diseased material containing spores. The change in reaction was noted after different lengths of incubation, and the final reaction was determined by comparison with standard buffer tubes used in combination with tubes of plain egg-yolk suspension media slanted in the same manner. The approximate increase in hydrogen-ion concentration was determined by this comparison (Table VII).

TABLE VII.—*Acid production by Bacillus larvae*

Culture No.	Brom thymol blue indicator				Brom cresol purple indicator			
	Plain medium		1 per cent dextrose		Plain medium		1 per cent dextrose	
	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H
9693–1	7.6	6.8	7.2	6.0	(b)	(c)	(d)	5.8
9834–1	7.2	6.6	7.2	6.0				
9834–2	7.6	[a] 7.4	7.2	6.2	(b)	(c)	(d)	6.0
9853	6.8–7.0	±6.6	6.6	6.0				
9857	7.2	6.6–6.8	7.2	6.0–6.2				
9867	7.6	[a] 7.4	7.2	6.4	(b)	(c)	(d)	6.2
9869	7.6	[a] 7.4	7.2	6.0	(b)	(c)	(d)	5.8
9874	7.6	7.0–7.2	7.2	6.0	(b)	(c)	(d)	5.8

[a] Doubtful growth.
[b] Beyond end point, no growth.
[c] No change in color, good growth.
[d] Beyond end point, good growth.
[e] No change in color, no growth.

OBSERVATIONS

Several interesting facts were observed from these experiments. Addition of buffer salts to the media delayed the approach to the final hydrogen-ion concentration reaction somewhat, but eventually practically the same end point was reached. Also, in one series of media in which the plain medium was adjusted to about P_H=7.6, little if any growth occurred in these tubes except with two strains of *Bacillus larvae*, indicating that the alkaline limit for growth is about at this point. In cases where the initial reaction of the plain medium was P_H=7.2, the final reaction averaged P_H=6.6 to P_H=6.8 (Table VII). In the case of the medium to which 1 per cent dextrose had been added, the final reaction averaged about P_H=6.0 for brom thymol blue and from P_H=5.8 to P_H=6.0 for brom cresol purple (Table VII). While, therefore, only a slight change in reaction occurred in media without sugar, a marked production of

acid was indicated in the tubes to which 1 per cent dextrose had been added. The maximum production of acid, however, required approximately 48 hours or more, the fermentation of the sugar apparently being relatively slow. As has been stated, however, the reaction of diseased material in various stages of decomposition and drying down is never found to reach a hydrogen-ion concentration of more than $P_H = 6.6$, and usually averages $P_H = 6.8$.

PROTEIN DECOMPOSITION

It is known that certain organisms have the ability to break down protein material under proper conditions, with the production of amino acids and alkaline decomposition substances, which latter tend to neutralize any acid produced from fermentation of sugar. If it can be shown that *Bacillus larvae* has this ability, it will explain the fact that the remains of larvæ dead from American foulbrood do not show a greater acid reaction resulting from the fermentation of the sugar of the intestinal contents. A series of experiments was devised to demonstrate whether such is the case with *Bacillus larvae*.

The prepupa at the age attacked by American foulbrood contains nitrogenous substances as shown by the Kjeldahl nitrogen determination equivalent to 1.45 per cent nitrogen (*43*). The source of this nitrogen is mainly albuminous material, one of the constituents of the larval fat body. Its exact composition has not been determined, but without doubt it is complex in nature. There are certain color reaction tests by means of which the constitution of this nitrogenous material may be indicated.

A delicate test for the presence of coagulable protein is that of Heller (*32, p. 1067*). A suspension of healthy prepupæ in water, treated by pouring about 4 cc. of concentrated nitric acid down the side of the inclined test tube, causes a white ring to form at the junction of the two liquids. Decomposed ropy material tested in this way gives no indication of such a ring, indicating that the complex protein has disappeared.

One of the most characteristic reactions for complex protein is the biuret test (*32, p. 915*). If some healthy prepupæ are suspended in a few cubic centimeters of 10 per cent sodium hydroxid and are treated with a few drops of a 0.5 per cent copper sulphate solution, a distinct pinkish-violet color is formed, again indicating the presence of complex protein material. Decomposed ropy material tested in this way gives no indication of this color, again indicating the complete disappearance of the complex protein.

There is also the xantho-proteic reaction (*32 p. 916*), which is given both by solid and by dissolved protein, and indicates the presence of the amino-acids, tryptophan, tyrosin, or phenylalanin in the protein molecule, or in solution. Tryptophan gives the reaction most intensely. Both healthy prepupæ and ropy material, boiled with concentrated nitric acid, produce a lemon-yellow color which on cooling and neutralizing with sodium hydroxid changes to an orange, denoting a positive reaction.

An even more delicate reaction for protein is that with Millon's solution (*32, p. 916*). A few cubic centimeters of a suspension of healthy prepupæ, treated with a few drops of Millon's reagent and boiled, cause a brick-red precipitate to form, leaving the liquid practically clear. A solution of decomposed ropy material, treated in the same way with Millon's reagent and boiled, causes a somewhat similar reddish precipitate, but the solution is also distinctly colored similarly, indicating that the protein has been changed in some way, part at least being soluble in water. Tyrosin is the only amino acid in protein that gives this reaction.

Since tryptophan is probably one of the principal constituents of the protein molecule in the healthy prepupa as well as in solution in diseased material, certain tests were made to determine its presence, because this amino-acid is easily utilizable by bacteria and gives decomposition products indicating the nature of bacterial action. The following tests are specific tryptophan reactions:

Adamkiewicz reaction (*32, p. 917*).—A suspension of healthy prepupæ or of diseased material in glacial acetic acid, treated by pouring concentrated sulphuric acid down the side of the inclined tube, causes a violet ring to form at the junction of the two liquids, indicating the presence of tryptophan, either as part of the complex molecule or in solution.

Rhodes reaction (*41*).—To a suspension of healthy prepupæ or of diseased material in water, a few drops of a weak solution of dimethyaminobenzaldehyde is mixed and concentrated sulphuric acid poured down the side of the inclined tube. This produces a violet ring at the junction of the two liquids which, if shaken, produces a reddish violet coloration in the mixture.

PROTEIN DECOMPOSITION PRODUCTS

It is therefore evident that the composition of the nitrogenous material in the healthy prepupæ is more or less complex but that certain amino-acids are available for bacterial metabolism, or are produced as a result of bacterial action.

In the decomposition of nitrogenous material, however, certain bacteria have the power of breaking down these amino-acids, such as tryptophan, to more simple compounds, some of them alkaline in nature, and often more or less foul smelling, or even to break them up into ammonia, the final product of nitrogenous decomposition. Indol is one of the products of such action of bacteria on material containing tryptophan. Its determination is largely used in the characterization of various organisms (*36*). Two indol tests were used, Ehrlich's aldehyde test (*19*) and the vanillin test (*19*), using for both suspensions of diseased material as well as cultures. Test of suspensions of diseased material gave positive results for the presence of indol, both with the Ehrlich method and even more definitely with vanillin. For testing in pure culture a broth consisting of 2 per cent peptone, 10 per cent yeast extract, and a few cubic centimeters of egg-yolk suspension was inoculated, incubating at 37° C. for about one week. Growth took place in this broth sufficiently to give a slight positive pink color with the Ehrlich aldehyde test, increasing on standing, and a much more positive result with the vanillin test.

AMMONIA PRODUCTION

Test of a suspension of diseased material as well as some of the above culture broth with Nessler's reagent (*32, p. 1084*) for presence of ammonia gave indications, from the resulting slight production of characteristic yellowish color, that the decomposition had passed even to the ammonia stage. A more delicate qualitative test was devised, using the modification of the microchemical method of Folin and McCallum (*32, p. 1093*) for the determination of urinary ammonia as described by Steel (*42*). To 25 cc. of a suspension of diseased material, or to broth culture similar to the above, 1 gram of sodium hydroxid and 15 grams of sodium chlorid are added and ammonia-free air bubbled through into 20 cc. of an approximately N/20 sulphuric acid, to which 10 drops of the indicator thymol blue are added. This showed the sulphuric acid solution to have a primary hydrogen-ion concentration of about $P_H=2$. After bubbling air through for an hour or more, in the case of the decomposed ropy material, sufficient ammonia had been carried over to neutralize part of the acid and change the hydrogen-ion concentration reaction from $P_H=2$ to $P_H=2.8$ or 3. Also one culture out of

three showed a change from $P_H=2$ to $P_H=2.8$. Therefore apparently *Bacillus larvae* has the ability of producing at least small amounts of ammonia. It seems probable that the rather pungent volatile gluelike odor often associated with American foulbrood receives some of its characteristics from this ammonia as well as from certain of the protein digestion products.

GELATINE LIQUEFACTION

The ability of putrefactive bacteria to liquefy gelatin is difficult to demonstrate with *Bacillus larvae* because of the cultural limitations. Maasen states (*28*) that slow liquefication takes place, while White (*55*) was unable to demonstrate any growth in gelatin. The writer inoculated a number of tubes of plain gelatin with several strains of *Bacillus larvæ*, all of which showed slight growth, and one or two showed a slight softening of the gelatin about the culture growth. Tubes of gelatin to which some egg-yolk suspension was added showed this softening more markedly, but in no case was there sufficient liquefication to enable one to say that it was positive. Decomposed ropy material inoculated into plain gelatin, on the other hand, gives a marked liquefaction in a short time. This, however, probably is due not to enzyms produced by *Bacillus larvae* so much as to enzyms from the body tissues functioning in the histolysis previous to metamorphosis.

This series of experiments, however, demonstrates that sufficient alkaline decomposition products are formed by the action of *Bacillus larvae* in the prepupa to neutralize most of the acid formed by the fermentation of the sugar in the intestinal contents and the dextrose resulting from the hydrolysis of the stored glycogen.

DISCUSSION

PER CENT CONCENTRATION OF SUGAR

In the data presented it may be seen that there is not an exact correlation between the percentage of dextrose which inhibits the germination or prevents the growth of *Bacillus larvae*, and the percentage of unassimilated sugar in the larva as expressed. The reason for this is that the percentage of unassimilated sugar is calculated in relation to the entire weight of the larva, like the figures of Straus (*43*) on the percentage composition of the larva (fig. 15). The percentage of dextrose in the culture media gives the actual effective concentration of the sugar in the medium by weight. Since the unassimilated sugar is contained almost entirely in the intestine from which it is absorbed, the true concentration of sugar in the intestine should be determined in relation to the weight of the intestinal content. Furthermore, as suggested by Maassen (*28*), growth of *Bacillus larvae* occurs only inside the intestine until after the histolysis has begun, making possible the invasion of the body tissues by the organisms. It is therefore in the intestinal contents during the last part of the feeding period that the presence of sugar is primarily effective in inhibiting the growth of the organisms. The actual concentration of sugar in the intestine is, however, difficult to determine accurately, since the actual weight of food consumed by the larva for each 24 hours of the feeding period is unknown. Furthermore, the weight of the intestinal content is difficult to determine, because of the difficulty of dissecting the intestine free from the surrounding body tissues or of removing the contents intact.

Several attempts were made, however, to remove intestines with as little adhering tissue as possible from larvæ of different sizes during the last two days prior to sealing, in order to obtain an approximately accurate figure for the relation between the weight of the intestine and the weight of the larva. This in the several larvæ dissected was found to be almost always about 1 to 5. Using this factor,

the percentage concentration in the intestine, at least during the progressive feeding period, should be approximately five times as great as the value calculated (in Table V) on the basis of the entire larval weight. The calculated percentages for the third and fourth days are now 4.90 and 14.10, respectively, and on the fifth day, just before sealing, the sugar concentration in the intestine should approximate 15.70 per cent (fig. 16). There is, of course, the factor of dilution

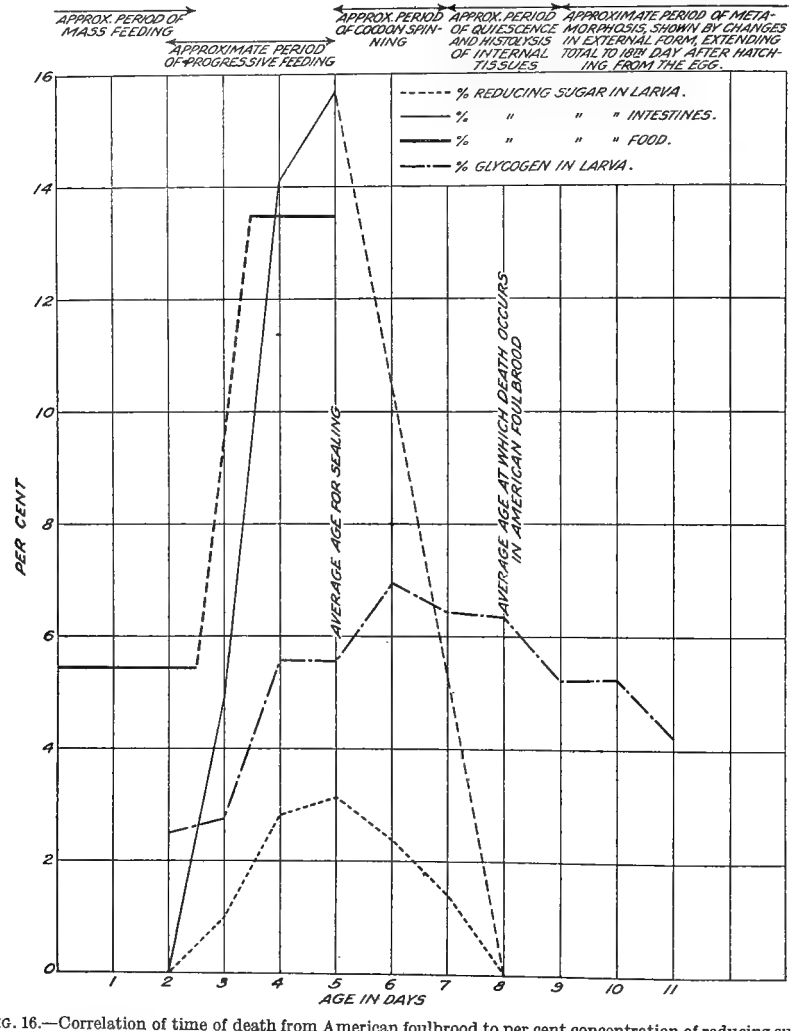

FIG. 16.—Correlation of time of death from American foulbrood to per cent concentration of reducing sugar in entire larva and to calculated per cent concentration in the intestine. Per cent sugar in the food and per cent glycogen in the larva are shown for comparison

particularly toward the end of the feeding period, caused by the accumulation of undigested pollen shells, which may lower this figure somewhat.

Still another approximate check may be calculated from the molecular weights of dextrose, glycogen, and fat, and from the percentage composition of the constituents of the larva (Tables IV and V), in order to obtain the percentage of sugar which was present in the intestine at any one time previous to assimilation neces-

sary for the formation of the stored glycogen and fat, on the basis of the relation of their carbon atoms.

	Formula	Molecular weight	Equivalent sugar per cent	
Dextrose (unassimilated)	$C_6H_{12}O_6$	180		[4]3. 14
Glycogen	$C_5H_{10}O_5$	162	$\frac{180-162}{180}\times 5.57+5.57=$	6. 13
Fat (oleic acid)	$C_{18}H_{34}O_2$	282	$\frac{3\times 180-282}{3\times 180}\times$[5]$3.64+3.64=$	5. 38
Total				14. 65

Since more sugar is used for energy in the production of a molecule of glycogen or of a molecule of fat (*32, p. 77*) than is indicated by the actual relation of the carbon atoms, this figure should be somewhat higher, thereby more nearly corresponding with the figure calculated from the weight ratio between intestine and larva.

The average concentration of sugar in the food of a larva of the age during which inhibition of bacterial growth takes place is 13.48 per cent (*40*) (Table III, fig. 11). The percentage composition of the larva at the different age periods (Table IV, fig. 15) in relation to food composition indicates, however, that the food probably is not assimilated as rapidly as it is ingested by the larva. If this is the case, an increase in the unassimilated sugar in the intestine would occur, as is indicated by the results obtained and the calculated percentage figures. The percentage of glycogen and fat increases slightly by the third day, as a result of the change in the composition of the food and the resulting increase in nursing (*26*). This is accompanied by the appearance of unassimilated sugar in the larva (Table V, fig. 15). There is then a marked increase by the fourth day, when apparently the limit for assimilation is reached, as shown by the constant percentage of glycogen in the larva as a whole between the fourth and fifth days in spite of the increase in weight, until after feeding ceases, when there is another increase in storage during the next 24 hours (sixth day). The latter is the result of the consumption of the food remaining in the cell after capping. The amount of unassimilated sugar increases continually, however, until feeding ceases, soon after the larva is sealed in the cell. This fact probably accounts for the slight difference between the percentage of sugar in the food and the calculated percentage in the intestine, but the correspondence is so striking as to substantiate the assumption.

Even though these calculations are only approximately accurate, it is known that some such condition must exist, since observations which have been made on the nursing habits of the honeybee (*26*), considered in relation to the figures for unassimilated sugar and food composition, give adequate foundation to the conclusion that there is considerably more than enough sugar in the intestine at the time infection occurs, or soon after, to inhibit the growth of the organism causing American foulbrood.

THE ACTIVE FEEDING STAGE IN THE LIFE HISTORY OF THE LARVA

The feeding stage of the honeybee larva has been divided into two parts, described by Lineburg (*26*) as the mass feeding period and the progressive feeding period. These two periods are characterized by a difference in the manner of feeding and in the amount of time spent by the nurse bees in the process, as well as by the change in chemical composition and character of the food (*40*) (Table III), and by the chemical composition of the larvæ themselves (*43*) (Table IV). It has been determined, however, that this change in composition of food occurs

[4] From Table V.
[5] From Table IV, Straus.

much earlier than stated by Von Planta (*35*, *26*). The young larvæ receive a food rich in nitrogenous material and relatively low in sugar and in which pollen grains are absent during about the first two and a half days of larval life. A large part of this, which is several times in excess of the weight of the larva during the first 24 hours or more (*35*) (Table VIII), seems to be placed in the cell with the newly hatched larva at one time soon after hatching, which justifies the assumption of mass feeding. During this period, assimilation must be very rapid, because the greatest relative growth occurs during the first two to three days (Table VIII) and also because no unassimilated sugar can be demonstrated in the larva during this period, even though the food contains about 5 per cent reducing sugar (Table V). The high nitrogenous content of the food apparently serves for rapid cell building, while the sugar is largely consumed in producing energy for this rapid growth; little storage of glycogen or fat occurs during this period. The nature and composition, as well as the biochemical reactions of this early food, as described by Koehler (*25*), suggest that it is a glandular secretion rather than a regurgitation of predigested honey and pollen from the ventriculus. The chances of larvæ of this age during mass feeding receiving infective material are, therefore, slight.

TABLE VIII.—*Ratio of weight in milligrams of unconsumed food in cell to weight of larva at different approximate ages (from Sturtevant (35))*

Approximate age (days)	Number of observations	Average weight of food per cell	Average weight of larvæ	Average ratio, food to larva
		Milligrams	*Milligrams*	
Egg	Several.	None.	[a] 0.10	0
1.1	33	3.96	1.02	3.88
1.4	131	3.23	1.36	2.37
2.1	24	9.10	7.20	1.26
2.6	35	11.79	17.48	.67
3.0	25	5.05	25.22	.20
3.5	65	7.75	63.46	.12
4.4	17	8.76	115.15	.08

[a] From Nelson (*35*).

Soon after the second day, a change in the composition of the food of the larva occurs, accompanied by a change in the method of feeding it by the nurse bees (*26*). The larva is now fed at approximately the rate at which the food is ingested by it, the demand for food rapidly increasing, accompanied by the great increase in actual body weight, until the time of sealing. The food now contains many entire pollen grains and has a much higher sugar content, nearly 14 per cent, and a relatively lower nitrogenous content (Table III). The principal ingredients are now honey or nectar and pollen. It is well known that honey which is gathered while disease is present in the hive usually carries infection. There is, therefore, a much greater opportunity for infection to occur when the larvæ are being given a food containing unmodified honey as one of its chief ingredients. Furthermore, the constant care of the larvæ during the period of progressive feeding and the large number of nurse bees which visit the cell still further increase the chances of infection being introduced during the period of progressive feeding. It is the young bees in the colony which act as nurses, and these bees are also the ones which clean the hive, so that they are more apt to have infected material on their mouth parts and elsewhere than are old field workers. There can, therefore, be little doubt that it is almost exclusively during the period of progressive feeding that infection normally occurs. Under normal feeding conditions the disease organisms can not develop in the larval intestine until after

feeding has ceased and the sugar-containing food has largely been assimilated, because, with the rate at which the larva is given food containing about 14 per cent sugar, the sugar concentration in the intestine must rapidly increase beyond the 3 to 4 per cent concentration which inhibits growth, so as to prevent the development of the disease in the larva. From Tables IX and X it may be seen that from the second day on, increasing numbers of visits are made to the larva with an increasing amount of time spent in nursing (Table X), so that on the last day of feeding a nursing visit, averaging six seconds in duration, is made approximately every 30 seconds, or an average total during the last 24 hours of 2,855 visits, or 36 per cent of the average 7,858 visits made during the entire feeding period (Table IX). The rapid consumption of this great amount of food, which is supplied in almost a steady stream, is indicated by the fact that the amount of food in the cell with the larva decreases to less than 10 per cent of the weight of the larva just prior to sealing. Even though the exact amount of food consumed may not be known, the actual concentration of sugar in the intestine during this period must rapidly rise, because of the high concentration of sugar in the food and the volume at which it apparently is fed to the larva, to several times the concentration necessary to inhibit the growth of *Bacillus larvae*. There is, as mentioned above, a diluting factor due to the accumulation of undigestible material until the connection is made with the end intestine, but, as shown by the microscopical examination of the intestinal contents, this, because it is largely insoluble, is probably of relatively slight importance in its effect upon the actual sugar concentration in the intestine during the period when inhibition occurs.

TABLE IX.—*Relation of nursing to increase in weight of larva* [a]

Age (days)	Average weight of larvæ	Average daily increase	Average daily ratio of increase to preceding weight	During active feeding period	
				Number of visits in 24 hours	Average increase in weight per visit per 24 hours
	Gram	*Gram*			*Gram*
Egg	0.000100				
1	.000650	0.000550	5.5	921.6	0.000000597
2	.004745	.004095	6.3	833.8	.00000492
3	.024626	.019881	4.19	1163.5	.00001709
4	.093990	.069364	2.82	2083.7	.00003329
5	.146748	.052758	.56	2855.5	.00001848
6	.155005	.008257	.06		

[a] From Sturtevant (*35*) and Lineburg (*26*).

TABLE X.—*Time spent in feeding* [a]

Age (days)	Average time spent in nursing per visit per 10 minute period	Average number of visits per 10 minute period	Average number of seconds per visit	Average frequency of visits	Average number of seconds between visits
	Seconds		*Seconds*	*Seconds*	*Seconds*
1	20.73	6.40	3.2	93.7	90.5
2	5.93	5.79	1.0	103.6	102.6
3	11.42	8.08	1.4	74.3	72.9
4	41.00	14.47	2.8	41.5	38.7
5	118.08	19.83	5.9	30.3	24.4

[a] Calculated from Lineburg (*26*).

Since it is shown that the concentration of reducing sugars in the larval intestine is usually sufficient to inhibit the growth of *Bacillus larvae* and thus to prevent the manifestation of American foulbrood until after sealing, it is now necessary to explain the rare cases in which advanced stages of the disease are seen in younger coiled larvæ. Such cases are exceedingly rare, except in colonies where almost every cell in the brood combs is filled with a dried scale, and where the bees have deserted the brood-nest because of this diseased material. There can be no doubt that in these cases the earlier manifestation of the disease is due to the fact that in such colonies the progressive feeding of the larvæ is seriously reduced by the fact that the colony has already been depleted in numbers of adult bees. Since there must be a decrease in progressive feeding in such cases, the concentration of reducing sugars in the intestine of the larva is obviously reduced, causing a condition to exist in these intestinal tracts which no longer inhibits the germination and growth of the causative organism. Such rare cases of young larvæ dead of American foulbrood do not, therefore, disprove the theory regarding the time of the development of the disease which has been here set forth, but rather serve as further substantiation of it.

THE COCOON-SPINNING STAGE

Sealing usually takes place on about the fifth day, at which time apparently the intestine contains a maximum amount of unassimilated sugar. After sealing occurs and feeding ceases, a different set of factors influence the concentration of sugar in the intestinal contents, so that there is a rapid steady decrease from this time on. The storage of glycogen and fat, however, continues for a short while from the assimilation of reducing sugar. Soon the movements of cocoon spinning and the histolysis of tissues make necessary the utilization of energy stored in form of glycogen and fat, so that the percentage of these substances begins to decrease as the larva loses weight. The emptying of the intestine of fecal material during this period also tends to decrease the sugar in the intestine, so that by the time the cocoon is finished, some time between the seventh and eighth days, the intestine is empty. The larva has straightened out and become quiescent by the eighth day, and all remaining sugar has now been assimilated. It is during this period that, as the concentration of sugar decreases, a point is reached where the growth of the organism can proceed. This apparently occurs when the sugar concentration in the intestine has decreased to about 3 to 4 per cent or less, probably not until some time between the sixth and seventh days. In the cultural experiments it was found that with 2 per cent or less dextrose, vigorous vegetative growth occurs. This vigorous growth requires, however, from 24 to 48 hours to develop, depending somewhat on the amount of initial inoculum. It is probable, as suggested by observations of Berman and Rettger (*6*), that this sugar in the food furnishes the energy for vegetative growth, while the soluble nitrogenous constituents of the intestinal contents furnish material for cell metabolism, until the time when the organisms have increased in number sufficiently to cause death and are able to invade the tissues of the larva, causing their subsequent decomposition.

THE QUIESCENT STAGE

As also is known from long observations of symptoms, the death of the larva and invasion of the body tissues do not in the majority of cases take place until after the larva has at least reached the age of 8 days and has become quiescent. This fact explains the characteristic uniformity of position and appearance of the majority of the larvæ dead from American foulbrood (figs. 7, 8, and 9). There are occasional cases in which death is still further delayed for some reason

until external transformation in form has begun, so that after death the pupa tongue is seen extended and often attached to the upper side wall of the cell in a characteristic manner (fig. 17.) It is possible that in such cases the initial inoculum was smaller than the average, thereby retarding maximum growth of the organism, as was noted in the cultural experiments, and delaying the production of sufficient toxin to kill until this stage of development had been reached.

While the biochemical relations of the bee larva to the disease are seemingly quite adequate to explain the delay in the time when American foulbrood is manifest, there is one other consideration which should be mentioned. The ability of the larva to resist the invasion of the bacteria is a subject on which virtually nothing is known, yet there must be some such ability, as is suggested by the fact that a slight initial inoculum in a colony may not cause the disease to be manifest. At the time when the biochemical conditions are most favorable for the germination and growth of the invading organisms, the larva itself has reached that stage of its development when its internal structure is materially modified by histolysis in advance of pupation, and it must follow almost necessarily that its power of resistance is reduced. The extent to which this factor is involved is, for the time being, purely a matter of speculation.

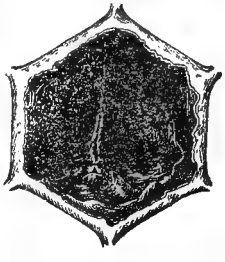

FIG. 17.—Decomposed, dried down remains of pupa dead from American foulbrood, showing characteristic tongue attachment to upper wall of cell (White (55)

The variation in the content of reducing sugar of the healthy honeybee larva and the inhibition of the germination and growth of *Bacillus larvae* by a concentration of over 3 or 4 per cent yield an interesting fact concerning the other serious disease of the brood of bees. European foulbrood makes its attack on the bee larva at an earlier stage in its development, while the content of reducing sugars is still high. It must, therefore, be concluded that *Bacillus pluton* has the ability to grow and rapidly to produce toxic substances sufficient to kill the larva in a medium of much higher reducing-sugar content than has *Bacillus larvae*, probably as high as 15 per cent.

THE EFFECTS OF BACTERIAL METABOLISM IN THE LARVA

There is little unanimity of opinion concerning the effect of dextrose (glucose) upon nitrogen metabolism by various bacteria. Kendall and Walker (*24*) concluded that the presence of glucose in the medium delays the production of proteolytic enzym, indicating the "protein-sparing" action of carbohydrates. Fischer (*23*) believed that proteolytic enzym is inactivated by glucose, indicated by inhibition of indol formation. Berman and Rettger (*6*) state that the presence of a carbohydrate in a culture medium may inhibit protein metabolism, depending on the nature of the medium and on the type of the organism as related to hydrogen-ion concentration. DeBord (*20*) believes that some bacteria destroy glucose without marked increase in the hydrogen-ion concentration and that the rate of production of amino nitrogen or ammonia nitrogen, which may be affected by the presence of carbohydrates, indicates different types of metabolism of bacteria.

The results of the present investigation, although more or less incomplete on this subject, seem to indicate that *Bacillus larvae* has the ability to decompose nitrogenous material in the presence of carbohydrate, since there must be dextrose available until the stored glycogen of the fat body is entirely hydrolyzed.

Many organisms are unable to attack complex protein unless there is some other source of food present, because, according to Berman and Rettger (*6*), to be

utilizable by bacteria sufficient growth is necessary by which to produce the enzym capable of splitting the complex protein molecule into its simpler amino-acid forms. In the healthy larva at the age when *Bacillus larvae* starts growth in the intestine, the remaining sugar in the intestinal contents and other material of nitrogenous nature in the food is sufficient, as stated earlier, to produce the energy for the initial growth of the organism. By the time the invasion of the tissues by the organisms occurs sufficient proteolytic enzym has been produced to attack the body proteins. Furthermore, the process of histolysis itself, as stated in relation to gelatin liquefaction by ropy material, has probably broken up sufficient of the body proteins to serve as food for bacterial metabolism.

Since *Bacillus larvae* belongs to the spore-forming group of organisms, its processes of metabolism may be similar to those of *Bacillus subtilis*, as described by Berman and Rettger (*6*), as follows: "The ability of *Bacillus subtilis* to break down protein in the presence of fermentable sugar, and in the absence of an added buffer, may be explained as follows. This organism attacks glucose slowly, and for this reason it is able to produce its proteolytic enzym before the hydrogen-ion concentration reaches a point unfavorable to further growth. When the enzym is thus formed the products of the nitrogen metabolism neutralize the acid, at least in a measure, and the metabolism therefore continues uninterruptedly."

The fact that the production of indol and even of ammonia can be demonstrated, although they may be produced slowly and in small amounts, indicates that even though considerable acid may be produced by the fermentation of the carbohydrate in the food and the hydrolysis of the glycogen, *Bacillus larvae* has putrefactive functions which bring about the formation of sufficient alkaline protein-digestion products from the larval tissues to neutralize this acid production, thereby maintaining the hydrogen-ion concentration at approximately P_H=6.8.

SUMMARY AND CONCLUSIONS

1. It has been shown by the work of others that the glycogen and fat content of the bee larva increases in a definite manner for a time and then decreases. In the present work it is shown that the per cent and amount of reducing sugar likewise increase after the third day of larval feeding, but decrease rapidly immediately after feeding has ceased, until by the eighth day no reducing sugar remains in the larva.

2. The presence of reducing sugar can be determined only after the progressive feeding of the larva begins.

3. The best method for extracting reducing sugars from the bee larva was found to be by the use of 50 per cent alcohol and the removal by filtration of the insoluble materials. By this means the interference of glycogen with the action of the reducing solutions is prevented.

4. The constitution of the protein molecule of the normal tissue content of the healthy bee larva is complex, containing, however, among other amino-acids, tryptophan.

5. The best medium for the growth of *Bacillus larvae* so far devised is a yeast-extract agar medium to which sterile egg-yolk suspension has been added. The optimum reaction for cultural growth is P_H=6.8.

6. Reducing sugars in the culture medium of more than 3 or 4 per cent usually inhibit germination of spores and growth of *Bacillus larvae*. A few spores may germinate at higher concentrations, but the resulting vegetative forms fail to increase in number and show granular disintegration due to autolysis. Less than 2 per cent of reducing sugars seems to stimulate growth of the vegetative forms.

7. The food of the older honeybee larva contains a high percentage of reducing sugar, which is derived from the honey or nectar used in its production. The concentration of reducing sugar in the larval intestine is more than sufficient to inhibit the growth of *Bacillus larvae* until after feeding has ceased. After feeding ceases, the remaining reducing sugar is rapidly assimilated, so that by the seventh day the concentration of sugar has been reduced sufficiently for the active growth of *Bacillus larvae* to occur.

8. The incubation period of *Bacillus larvae* is 24 to 48 hours, so that growth sufficient to kill the larva does not occur until it has completed the spinning of its cocoon and has extended quiescent in the cell, on or after the eighth day, by which time all reducing sugar has disappeared from the larva.

9. The delayed death of the larva in American foulbrood is, therefore, correlated with the inhibiting effect of unassimilated reducing sugar in the intestine upon the germination and growth of *Bacillus larvae*.

10. *Bacillus larvae* has the ability to produce considerable acid, but the hydrogen-ion concentration of the decomposing material is not thereby increased, because of the neutralizing effect of protein decomposition products. The hydrogen-ion concentration of the diseased larva throughout its decay varies only slightly from $P_H = 6.8$.

11. *Bacillus larvae* not only utilizes reducing sugar for its initial growth, but also completely hydrolyzes the glycogen of the larval body tissues in the process of decomposition.

12. *Bacillus larvae* has the ability to decompose nitrogenous materials, with the formation of amino-acids, indol, and ammonia, but the hydrogen-ion concentration is not decreased by this action, because of the concomitant production of acids from carbohydrates.

13. *Bacillus larvae* apparently has no action on fat.

14. The biochemical data herein presented for the first time explain the remarkable characteristics of American foulbrood, which were left entirely unexplained from observations on etiology alone.

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THE STERILIZATION OF AMERICAN FOULBROOD COMBS

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CONTENTS

INTRODUCTION

A new era in the treatment and control of American foulbrood has been opened by the use of disinfectants such as an alcohol-formalin solution for the sterilization of combs infected with this disease of the brood of bees. Widespread interest has been aroused throughout the beekeeping industry by the apparent success of this method of treatment, which eliminates to a great extent the large losses previously caused by the necessary destruction of all combs infected with American foulbrood.

Numerous attempts have in the past been made to use formaldehyde gas as a disinfectant, but it failed to sterilize infected combs completely except in a few carefully controlled cases, so that eventually the use of this material for disinfecting combs was entirely abandoned. White (*25*, *26*)[1] found that the gas penetrated the combs slowly, even when used in a tightly sealed jar, and that as usually applied in the apiary it was insufficient to disinfect the combs completely. More recently Maassen and Borchert (*18*) and Borchert (*1*), experimenting with formaldehyde gas generated from

[1] Reference is made by number (italics) to "Literature cited," p. 27.

Professor Jones (*15*) we learned that in every case water used with the formalin gave just as good results as when alcohol was used. The assistant apiarist, Mr. Jarvis, also substantiated these conclusions.

In a recent article G. L. Jarvis (*13*), of the Ontario Agricultural College, Guelph, Ontario, describes results of experiments in the apiary in collaboration with Doctor Jones concerning the effectiveness of water-formalin and alcohol-formalin solutions. Colonies made with 2-pound packages of bees were used in which to test combs treated in various ways. He says:

> From the results of these experiments and laboratory tests we have arrived at these conclusions: First, that both the formalin water and alcohol formalin solution will kill all germs causing American foulbrood in open cells. Second, that there is still a doubt as to the effectiveness of both solutions in the case of capped cells.
>
> With this in mind, we are ready to advocate the use of the formalin water solution for sterilizing super combs from diseased colonies after the honey has been extracted. The strength of the solution must not be less than 15 per cent formalin to 85 per cent water and the combs immersed for at least 12 hours. The uncapping must be well done; that is, there must be practically no sealed honey in the combs. The combs should be held, if possible, until the following spring before being given back to the bees.
>
> Although in our experiments so far we have had no reappearance of disease in treated brood combs, except in check colonies, some other beekeepers who have tried the alcohol-formalin treatment have not been so successful. However, the super comb is the doubtful comb, and we believe it is well worth while to know of a cheap solution, such as the formalin-water, which will eliminate this doubt. Again, if all combs from a diseased colony were melted or burned there would be approximately three super combs destroyed to every brood comb, and the super combs would likely average a better quality than those in the brood chamber.

G. H. Vansell, in California (*23*), recently has reported promising experiments in sterilizing foulbrood combs, in which he used various mixtures of formalin in soapy waters. The results were found encouraging as to sterility and cost. No details are given concerning methods or results.

Since the announcement of this new method of treating combs many beekeepers, as well as the investigators cited above, have been stimulated to experiment for themselves. In 1924, 33 samples of treated comb were submitted by beekeepers to the Bee Culture Laboratory for cultural examinations as to the sterilizing efficiency of the various solutions used. In a majority of cases the disinfectant used was not described. In about one-third of the cases some variation of the alcohol-formalin solution as devised by the person sending the sample was used. Three of these cases, however, were specifically said to have been treated with the commercial alcohol-formalin solution. Of the 33 samples, 10 gave cultures showing growth of *Bacillus larvae* from both open and sealed cells. Fourteen of the 33 samples, including 2 samples that had been treated in the commercial solution, gave cultures showing no growth from open cells but good vegetative growth of *B. larvae* from many of the sealed cells. Nine of the 33 samples, only 1 of which was known to have been treated with the commercial solution, gave cultures showing no growth from either open or sealed cells, thereby indicating complete sterilization.

It would, therefore, seem probable that, although the method of treating infected brood combs with an alcohol-formalin solution is a step in advance in the control of American foulbrood, there apparently

is room for improvement which will eliminate the danger of occasional cases of failure. Because of the widespread interest in this subject, preliminary work was started early in 1924 at the Bee Culture Laboratory with the purpose of making an exhaustive bacteriological study of the efficiency of various disinfectants, including the commercial alcohol-formalin solution as well as water-formalin solutions. It was hoped that the results of the investigation by laboratory methods would form a basis for practical work in the apiary. As the work has developed, numerous difficulties have been encountered which indicate that the problem of the perfect sterilization of American foulbrood combs is neither simple nor as yet fully solved.

The results given herein are of a preliminary nature, the data being in some cases incomplete; but they are given for what they seem to indicate. Since these investigations were started Doctor Hutzelman has taken out a patent (*12*) on the solution devised by him, issued October 14, 1924. In the light of the issuance of this patent it seems advisable to state that the United States Department of Agriculture can assume no responsibility for the use of any of the solutions or processes described and discussed in this paper if they in any way infringe the patent.

METHODS

DISEASED MATERIAL FOR TESTS

In devising methods for testing the efficiency of various disinfectant solutions, procedures were adopted corresponding as closely as possible, on a reduced scale, to the actual practice of beekeepers. Combs affected with American foulbrood, containing as many scales as possible both in sealed cells and in open cells, were obtained from various sources. From the brood areas of these combs test pieces were cut approximately of the standard size of 1½ by 2½ inches. Glass specimen jars of about 160 cubic centimeters capacity, with fitted glass covers, were used to hold the test solutions and pieces of comb treated. A standard volume of liquid of 100 cubic centimeters was used throughout the tests, the proportion of liquid to comb being approximately that in the the regular 10-frame tanks used by beekeepers for disinfecting combs. Loss of liquid after each consecutive comb had been removed was made up to 100 cubic centimeters with more solution before a new piece was immersed. Several pieces of comb were passed through each lot of solution consecutively, in keeping with the actual apiary practice of treating many combs in the same solution. The piece of comb to be tested was placed in the empty jar and fastened down with a wire spring to prevent its floating. The solution was poured into the jar slowly to permit the liquid more readily to enter the open cells. In accordance with the most approved apiary practice, there was no shaking of combs to aid in removal of air bubbles. The test combs were then allowed to soak for 24 or 48 hours, as the case might be. No immersions of less than 24 hours were tried in this series of experiments.

Upon removal of the pieces of comb from the disinfectant, as much as possible of the excess liquid remaining in the cells and on the surface of the comb was removed by vigorous shaking. Each

piece was then inclosed in a piece of filter paper to protect it from dust and was allowed to dry at the ordinary room temperature of the laboratory until no odor of formalin could be detected.

CULTURES

Yeast-extract egg-yolk agar medium, as described in a recent paper by the writer (*21, p. 136*) was used in the form of slants for the purpose of making the cultural tests of the scales treated by the various solutions. In order that there should be a sufficient amount of water of condensation at the base of the slants, the necessary number of tubes of fresh medium for each group of combs to be tested were made up by the addition of sterile egg yolk to tubes of the base medium. With each new lot of medium control cultures were made from untreated scales of American foulbrood.

The removal of scales or other diseased remains from the cells of the treated combs was accomplished by means of a lancet-shaped dissecting needle which had just been sterilized in a flame and allowed to cool. The scale (or other remains) was then carefully placed in the water of condensation of the tube of culture medium, one scale or material from one cell to a tube of medium. Cappings from sealed brood were removed with the hot needle, and after resterilizing the needle the scale contained in one cell was removed and placed in a culture tube. After the diseased material had been allowed to soak in the condensation water of the tube for about an hour, or longer if necessary, until the dried scale had softened, it was macerated and spread over the surface of the slant by means of a stiff platinum loop.

Cultures were at first made of material from one open cell and one sealed cell from each comb treated, on the assumption that for a given solution the sterilization would be uniform for a definite period of time, but it soon became evident that there was a variation in the rapidity with which the various solutions penetrated the brood cappings. At least five cultures were therefore made from open cells and five from sealed cells in each piece of comb, amounting to approximately 3 to 5 per cent of all cells in a piece of comb of the size used in these experiments. The percentage cultured of the scales actually present is really much higher than this, since a piece of comb of that size seldom has a scale in every cell.

After incubation for 48 hours at 37° C., cover-glass smears were made from the material on the surface of the slants. A large loopful of a mixture of material from different parts of the slant in a drop of distilled water was used in making a good-sized smear on the cover glass. After drying the cover glass in the air and passing it quickly through a flame three times the smears were stained for about 20 seconds with Ziehl-Neelson carbol-fuchsin diluted with an equal quantity of distilled water. The cover glasses were then carefully washed in water, dried, mounted with Canada balsam, and examined under the microscope. At least 20 to 30 fields were examined to determine whether there had been any germination of spores of *Bacillus larvae* or vegetative growth not visible on the slant. When no germination of spores was observed, as a rule only one examination of the culture was made at the end of 48 hours' incubation, since if there are any spores in a condition to germinate

they will do so within that period. When a few spores were found to have germinated, but without evidence of any vegetative growth, a subculture was generally made, using a generous quantity of material from the surface of the original slant. When good vegetative growth was observed it was so recorded as positive growth. In some instances when there was doubt regarding a culture several more cells of the same kind were cultured from the same piece of comb.

SOLUTIONS TESTED

In the earlier part of this investigation an attempt was made to find a substitute for alcohol as a carrier for the disinfectant, because of the difficulty of purchasing pure grain alcohol and the comparatively high cost of the commercial alcohol-formalin solution. Since certain substances are used in various insecticide sprays to increase the wetting and spreading powers of the spray solutions (*6*, *19*), it was suggested that if a liquid could be found which would spread easily over the surface of the wax comb and diseased remains, formalin added to such a liquid would be carried by it and brought into contact with those remains; and that it would penetrate sufficiently to kill the spores, even though the cells, particularly sealed cells, might not be completely filled with the liquid. This property in insect sprays is often obtained by the addition of soaps of various kinds to the solutions. The addition of soap also tends to lower the surface tension of a water solution. Dilute solutions of various types of soaps, both soft and hard, were used to form the carrier for the 20 per cent of formalin. By experimentation with dry pieces of comb it was found that only a comparatively small quantity of soap can be used in a solution, as too much causes the liquid to become viscous and jelly-like, inhibiting its entrance into the cells. In the tests with soap solutions varying quantities of half-normal sodium oleate soap, cottonseed-oil soap, and a 10 per cent solution of one of the common hard toilet soaps were used in making up the solutions for the disinfection of diseased combs. From 0.5 to 2 cubic centimeters of each of the various soaps was used per 100 cubic centimeters of a 20 per cent solution of formalin in water; if much more than 2 cubic centimeters was used the solution became too viscous.

In a recent article King (*16*) describes experiments in sterilizing American foulbrood combs by using a disinfectant solution consisting of the 20 per cent water-formalin solution, with sufficient soap to form suds (about 1 pound to 5 gallons of solution), the addition of the soap causing the solution to enter the cells more readily. After 48 hours' immersion, followed by extraction and a short drying period, the combs were given to a healthy colony. Only one colony was used in the experiment, but King reports that no disease appeared in it when observed carefully at intervals for seven weeks after it had been given the treated combs.

Among substances similar in physical properties to ethyl alcohol, a commercial methyl-ethyl ketone solution, acetone, and iso-propyl alcohol were tried as carriers for formalin, but acetone and iso-propyl alcohol were too expensive for practical use.

The use of hydrochloric acid in the sterilization of imported hides, as a preventive of anthrax (*20*), a disease caused by a spore-

forming organism similar in nature to *Bacillus larvae*, suggested its use in the treatment of American foulbrood. Various dilute solutions of iodine were suggested, since iodine is successfully used in the sterilization of drinking water. A solution of formalin in water, with the addition of acetic acid to increase its penetrating power, is often used for fixating histological preparations; for the treatment of American foulbrood a similar solution was accordingly suggested.

A number of tests were made to compare the germicidal efficiency of the commercial alcohol-formalin solution, now frequently used by beekeepers, with that of the various other solutions tried, and in particular with the 20 per cent solution of formalin in water. Krönig and Paul (*17*) have shown that increasing quantities of either ethyl or methyl alcohol added to a solution of formalin in water progressively decreases the germicidal efficiency of the solution. Tests were therefore made with various dilutions of denatured alcohol as the carrier for the 20 per cent solution of formalin. Formula No. 1 (*22, p. 100*) for specially denatured alcohol was used as a basis for these dilutions. Commercial alcohol-formalin solution was diluted in a similar manner by the addition of varying quantities of a 20 per cent solution of formalin in water. These tests were made to determine whether the presence of a greater proportion of water mixed with the alcohol changes the germicidal efficiency of the solution.

In this preliminary work no measurements were made of surface tension and other physical properties, but the bacteriological results were used as a criterion of the relative efficiency of the various disinfectant solutions tested.

OBSERVATIONS

SOAP SOLUTIONS

In the use of soap-formalin solutions it was found immaterial whether soft or hard soap is used. The greatest difficulty with the soap solutions, aside from the fact that they do not seem to penetrate sealed cells uniformly, which will be discussed later, is a rapid change in reaction from alkalinity to marked acidity during the period of immersion of one piece of comb. The acid reaction causes the soap gradually to precipitate, so that the solution is soon little better than ordinary water-formalin solution. Even the addition of sufficient normal sodium-hydroxide solution to bring the reaction back to alkaline after the comb is removed fails to remedy this fault, as the precipitated soap does not return into solution on the addition of the sodium hydroxide. The prolonged action of soap and alkali on the wax of the comb during an immersion of 48 hours causes more softening of the cell walls than is desirable, and makes the combs quite fragile. Since some of the sealed cells in practically every series of combs tested failed to be sterilized, as indicated from the cultures made (Table 1), this solution was dropped from consideration for use with combs having sealed brood.

In the case of the sodium-oleate-formalin solutions, 59 open and 59 sealed cells were cultured in 10 series, varying in length (Table

1). Cultures from 54 open cells showed no growth of *Bacillus larvae*, whereas 5 culture tubes were contaminated. Cultures from 45 sealed cells showed no growth of *B. larvae*, 2 culture tubes were contaminated, 11 showed good growth of *B. larvae*, and 1 showed only a few spores germinated, or 12 positive cultures in all. In brief, of the sealed cells 20.3 per cent were not sterilized.

TABLE 1.—*Cultural results of various tests with samples of comb treated 48 hours in water-soap-formalin solution*

Kind of soap	Composition of solution				Number of consecutive pieces of comb immersed in same lot of solution	Open cells					Sealed cells				
	Soap	Water	Formalin solution	Normal sodium hydroxide		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	Cultures contaminated	First comb in series containing open cells showing growth of *B. larvae* in cultures	Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	Cultures contaminated	First comb in series containing sealed cells showing growth of *B. larvae* in cultures
	C. c.	*C. c.*	*C. c.*	*C. c.*											
Half-normal sodium oleate.	0.5	80	20	0	4	4	0	3	1	----------	4	0	4	0	
	0.5	80	20	0	4	4	0	3	1	----------	4	0	2	2	
	1	80	20	0	5	5	0	5	0	----------	5	1	4	0	Third.
	1	80	20	0	5	5	0	4	1	----------	5	2	3	0	Do.
	1	80	20	1	6	6	0	6	0	----------	6	0	6	0	
	1	75	20	5	6	6	0	6	0	----------	6	0	6	0	
	1	80	20	1	9	9	0	8	1	----------	9	[1]1	8	0	Second
	1	80	20	1	5	5	0	5	0	----------	5	2	3	0	First.
	2	80	20	2	9	9	0	8	1	----------	9	2	7	0	Sixth.
	[2]2	80	20	1	6	6	0	6	0	----------	6	4	2	0	First.
10 per cent solution of hard toilet soap.	1	80	20	1	9	9	0	9	0	----------	9	1	8	0	
	2	80	20	2	9	9	0	9	0	----------	9	[3]2	7	0	Third.
	1	80	20	1	2	2	0	2	0	----------	2	[4]1	1	0	Second.
	1	80	20	1	4	4	0	3	1	----------	4	[3]2	1	1	First.
	1	80	20	1	7	[4]7	0	7	0	----------	7	4	3	0	Second.
	2	75	25	0	4	4	0	4	0	----------	4	3	1	0	First.
	2	75	25	0	4	4	0	4	0	----------	4	3	1	0	Second.
	2	80	20	2	6	[5]6	0	6	0	----------	[5]6	0	6	0	
	2	80	20	2	6	[5]6	0	6	0	----------	[5]6	0	6	0	
	0.5	90	10	0	4	4	0	4	0	----------	4	1	3	0	First.
	1	90	10	0	4	4	0	4	0	----------	4	[1]1	3	0	Fourth.
	0.5	85	15	0	4	4	[3]2	2	0	First.	4	2	2	0	First.

[1] Few spores germinated.
[2] Cottonseed-oil soap.
[3] One showed few spores germinated.
[4] Used entire brood combs in tank.
[5] Sealed cells with cappings perforated compared with open cells.

In the case of the solutions of hard toilet soap in 20 per cent formalin, 39 open and 39 sealed cells were cultured. Cultures from 38 open cells showed no growth, and the culture from 1 was contaminated. From the sealed cells, 22 cultures showed no growth, 1 was contaminated, 13 showed good growths of *Bacillus larvae*, and in 3 only a few spores had germinated; in all, there were 16 positive cultures, showing that 41 per cent of the sealed cells were not sterilized. The variation in the quantity of soap added to the solution seemed to have little effect on the variable penetration and sterilization of the scales in sealed cells, but the hard-soap solutions appeared somewhat less efficient than the solutions of soft soap.

Three short series (see bottom of Table 1) were tried, using smaller quantities of formalin, but these solutions proved inefficient as germicides in open as well as in sealed cells.

LIQUIDS OF LOW SURFACE TENSION OTHER THAN ETHYL ALCOHOL

The results of tests with these liquids are presented in Table 2. Acetone gave indications of being a rather more efficient carrier than those previously used, both in the proportions of 80 parts acetone to 20 parts formalin, and even when diluted with water in the proportions of 50 parts acetone to 30 parts water and 20 parts formalin. Two series, one of 4 open and one of 4 sealed consecutive combs, and one series of 9 sealed combs, showed no cells giving growth of *Bacillus larvae*, while each of three series of sealed combs showed only 1 cell giving growth. Two short series were tried with the use of smaller proportions of formalin, 15 parts and 10 parts, respectively, per 100 parts. These proportions proved to be inefficient as germicides in both open and sealed cells. In the light of later work, it seems possible that if more cells from each comb had been examined a few more positive cultures from sealed cells might have been found; but in comparison with other solutions tested under the same procedure of cultural examination, acetone gave about the best results as a carrier. The comparatively high cost of acetone eliminates it as a substance that can be used economically in apiary practice.

The ketone solution previously mentioned was mixed with formalin in the proportions of 80 parts ketone to 20 parts formalin. This solution, as may be seen from the table, sterilized the open cells, but was unsatisfactory in its action on scales in sealed cells.

TABLE 2.—*Cultural results of various tests with samples of comb treated for 48 hours in liquids of low surface tension, other than ethyl alcohol*

Liquid used	Composition of solution			Number of consecutive pieces of comb immersed in same lot of solution	Open cells					Sealed cells				
	Liquid used	Water	Formalin solution		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	Cultures contaminated	First comb in series containing open cells showing growth of *B. larvae* in cultures	Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	Cultures contaminated	First comb in series containing sealed cells showing growth of *B. larvae* in cultures
	C.c.	*C.c.*	*C.c.*											
Acetone	80	0	20	4	4	0	4	0	----------	4	0	4	0	
	80	0	20	10	10	0	10	0	----------	10	1	9	0	Tenth.
	80	0	20	10	10	0	10	0	----------	10	1	9	0	Do.
	60	30	20	9	9	0	9	0	----------	9	0	9	0	
	50	30	20	9	9	0	9	0	----------	9	[1] 1	8	0	Fifth.
	40	40	20	4	4	0	3	1	----------	4	0	4	0	
	85	0	15	3	3	[1] 1	2	0	Second	3	1	2	0	Second.
	90	0	10	3	3	[1] 1	2	0	Third	3	1	2	0	Third.
Methyl-ethyl ketone	80	0	20	5	5	0	5	0	----------	5	[2] 4	1	0	First.
	80	0	20	5	5	0	4	1	----------	5	[3] 3	1	1	Second.
	80	0	20	5	5	0	3	2	----------	5	1	3	1	Do.
Iso-propyl alcohol	50	30	20	7	7	0	7	0	----------	7	[1] 1	6	0	Fifth.
	90	30	20	7	7	0	7	0	----------	7	0	7	0	

[1] Few spores germinated. [2] Three showed few spores germinated. [3] Two showed few spores germinated.

Iso-propyl alcohol, another liquid manufactured on a commercial scale resembling ethyl alcohol in its physical properties, but too

expensive for practical use, was also tested. Only four series, each of seven cells, were tried with this carrier for purposes of comparison, two of open and two of sealed cells, the proportions used being 50 parts of iso-propyl alcohol, 30 parts of water, and 20 parts of formalin. Of all the 28 cells cultured only one sealed cell was found to contain viable *Bacillus larvae*. This solution is therefore in about the same class as that of acetone.

MISCELLANEOUS SOLUTIONS

The results of experiments with these solutions are presented in Table 3. The use of iodine solutions proved entirely impracticable for sterilizing infected combs. In dilutions of 1 to 50,000 in water and even 1 to 500, with an immersion of 48 hours, no germicidal action on the spores from diseased material in either open or sealed cells could be demonstrated. A solution of 1 to 20 iodine killed all the spores, both in open and sealed cells, but the comb was attacked to such an extent as to make it too soft for further use. The probable reason for the lack of germicidal action on the part of iodine is that this substance combines readily with the fatty-acid constituents

TABLE 3.—*Cultural results of various tests with samples of comb treated for 24 hours in various solutions*

Composition of solution	Duration of immersion	Number of consecutive pieces of comb immersed in same lot of solution	Open cells				First comb in series containing open cells showing growth of *B. larvae* in cultures	Sealed cells				First comb in series containing sealed cells showing growth of *B. larvae* in cultures
			Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	Cultures contaminated		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	Cultures contaminated	
	Hours											
Iodine, 1–50,000 water	48	2	2	2	0	0	First	2	2	0	0	First.
Iodine, 1–500 water	48	1	1	1	0	0	do	1	1	0	0	Do.
Iodine, 1–20 water	48	2	2	0	2	0		2	0	2	0	
Denatured alcohol (50 per cent), 93 c. c., hydrochloric acid, 5 c. c., formalin, 2 c. c.	24	4	4	0	4	0		4	4	0	0	Do.
Denatured alcohol, 90 c. c., hydrochloric acid, 5 c. c., formalin, 5 c. c.	24	4	4	1	3	0	Third	4	4	0	0	Do.
Denatured alcohol (50 per cent), 93 c. c., hydrochloric acid, 5 c. c., formalin, 2 c. c.	48	3	3	0	3	0		3	3	0	0	Do.
Denatured alcohol, 90 c. c., hydrochloric acid, 5 c. c., formalin, 5 c. c.	48	3	3	1	2	0	First	3	3	0	0	Do.
Acetic acid, 10 c. c., water, 70 c. c., formalin, 20 c. c.	48	8	8	0	8	0		8	3	5	0	Fourth.
Do	48	8	8	0	8	0		8	3	5	0	Do
Acetic acid, 5 c. c., water, 75 c. c., formalin, 20 c. c.	24	7	7	0	7	0		7	3	4	0	Do.
Do	48	3	3	0	3	0		3	2	1	0	Second.
Denatured alcohol, 80 c. c., formalin, 20 c. c., glycerin, 2 c. c.	24	4	4	0	4	0		4	4	0	0	First.
Do	48	5	5	0	5	0		5	1	4	0	Third.
1 per cent solution of gelatine, 80 c. c., formalin, 20 c. c.	48	3	3	0	3	0		3	[1] 2	1	0	Second.
Do	48	3	3	0	3	0		3	[2] 1	2	0	Do.
10 per cent ketone solution added to 95 per cent alcohol, 80 c. c., formalin, 20 c. c.	48	7	7	0	6	1		7	2	5	0	Do.

[1] One showed few spores germinated.

[2] Few spores germinated.

of the wax, as well as with the fatty residue in the diseased remains of the brood, thereby nullifying the action of the iodine as a germicide.

The use of denatured alcohol in various dilutions containing 5 per cent concentrated hydrochloric acid, and a small quantity of formalin to prevent deleterious action of the acid (*10*) on the supporting wires of the frame, gave unsatisfactory results with immersions of 24 and of 48 hours. Each of the sealed cells tested gave a good growth of *Bacillus larvae*; in two cases growth was obtained from open cells, although some spores apparently were killed. This type of solution was not tested further.

Varying quantities of acetic acid added to a 20 per cent solution of formalin in water contributed nothing to the germicidal or penetrating action of the solution so far as sealed cells were concerned, as several such cells in each series tested gave each a good growth of *Bacillus larvae*. These results are very similar to those obtained with plain water-formalin solution, which will be considered later.

Several other miscellaneous solutions were tried whose composition is indicated in the table, all containing 20 per cent of formalin, but varying in the composition of the carrier. All proved unsatisfactory when used with sealed cells, and will not be discussed further.

SOLUTIONS WITH DILUTED ALCOHOL

A few dilutions of denatured alcohol, as well as dilutions of alcohol-formalin solution, were made, varying the alcohol content from about 30 per cent to over 60 per cent, as indicated in Table 4, the 20 per cent formalin, however, being kept constant in the mixtures with denatured alcohol. Tests were made of combs treated for 24 hours and for 48 hours. In every case no growth was obtained from open cells, and, as would be expected, there were fewer positive cultures from sealed cells in combs treated 48 hours than from those treated 24 hours, although several sealed cells showed no growth, even in the 24-hour series. In each of the 48-hour series there was at least one sealed cell from which was obtained a growth of *Bacillus larvae*, but, with the few observations made, no significant differences could be found between the few dilutions tested of alcohol or alcohol-formalin solution. Further work is necessary to demonstrate whether results comparable with those of Krönig and Paul can be obtained.

For purposes of comparison, as a preliminary test, three series of six samples each from diseased combs were treated with a solution containing no alcohol, composed of formalin 20 parts and water 80 parts (Table 9). No significant difference could be seen between these results and those obtained with the various alcoholic solutions. No open cells treated with this solution gave cultures showing growth, but 8 sealed cells, 1 or more in each series, gave cultures showing growth of *Bacillus larvae*, the growths in 3 of the 8 cultures being noted as " few spores germinated."

TABLE 4.—*Cultural results of various tests with samples of comb treated for 24 hours and for 48 hours in various dilutions of alcohol-formalin and commercial alcohol-formalin solutions*

Composition of solutions	Duration of immersion	Number of consecutive pieces of comb immersed in same lot of solution	Open cells			First comb in series containing open cells showing growth of *B. larvae* in cultures	Sealed cells			First comb in series containing sealed cells showing growth of *B. larvae* in cultures
			Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	
Commercial alcohol-formalin solution, 50 c. c., 20 per cent formalin in water, 50 c. c.	*Hrs.* 24	4	4	0	4	----------	4	1	3	First.
Do	48	7	7	0	7	----------	7	2	5	Sixth.
Commercial alcohol-formalin solution, 40 c. c., 20 per cent formalin in water, 60 c. c.	24	7	7	0	7	----------	7	[1] 5	2	Third.
Do	48	9	9	0	9	----------	9	1	8	Seventh.
Denatured alcohol (62.5 per cent), 80 c. c., formalin, 20 c. c.	24	7	7	0	7	----------	7	[1] 3	4	Third.
Do	48	5	5	0	5	----------	5	[2] 1	4	Fourth.
Denatured alcohol (50 per cent), 80 c. c., formalin, 20 c. c.	24	7	7	0	7	----------	7	[1] 2	5	Third.
Do	48	5	5	0	5	----------	5	1	4	Fourth.
Denatured alcohol (40 per cent), 80 c. c., formalin, 20 c. c.	24	7	7	0	7	----------	7	3	4	Fourth.
Do	48	7	7	0	7	----------	7	2	5	Sixth.
Denatured alcohol (50 per cent), 75 c. c., formalin, 20 c. c., glycerin, 5 c. c.	24	4	4	0	4	----------	4	2	2	First.
Do	48	5	5	0	5	----------	5	[1] 2	3	Fourth.

[1] One showed few spores germinated. [2] Few spores germinated.

COMMERCIAL ALCOHOL-FORMALIN SOLUTION

Ten series of combs were treated with the commercial alcohol-formalin solution at various times. From these combs 61 open and 61 sealed sells were cultured. It was intended to use these series as controls for comparison with other solutions, since published reports of the success obtained with this commercial solution in apiary practice indicated that negative cultural results would be obtained. As may be seen in Table 7, however, in the case of every one of the 10 preliminary 48-hour series 1 or more sealed cells, 22 in all, or 36 per cent, were found to give a growth of *Bacillus larvae*. In 3 cases of the 22 the growths were noted as "few spores germinated." No growth was obtained from the 61 open cells cultured. At first it was thought that the solution might have deteriorated; fresh solution was obtained and tested, with practically the same results. A chemical analysis of the actual formaldehyde content of the fresh solution, and a similar analysis of solution through which some 500 combs had been passed, showed an actual increase with use in the percentage of formaldehyde. This increase was without doubt due to the more rapid evaporation of the alcohol than of the formaldehyde content. These results seem to indicate that there must be a variation in the permeability of cappings, the decrease in permeability slowing up or even preventing the penetration of disinfectants to the scales and spores.

PERMEABILITY OF CAPPINGS

A simple experiment was undertaken in an effort to learn whether the permeability of the cappings is variable. Such a variation might account for the fact that sealed cells are not always sterilized, and for the variation in the number of cultures of *Bacillus larvae* from sealed cells in different combs.

If cappings are carefully removed from a piece of comb and examined under the microscope, it is seen that their structure is apparently very variable. The cappings are composed of criss-crossing cocoon fibers, pollen grains, and granules of wax, and consequently vary in structure. Cappings from brood cells of different ages are found to vary greatly in thickness. Freshly sealed cappings are much thicker and more opaque than cappings from the cells of more nearly matured pupæ, the latter often being gnawed by adult worker bees.

A simple piece of apparatus was devised to test the variability in porosity of cappings. A piece of glass tubing slightly smaller in outside diameter than the inside diameter of worker cells was drawn to a fine capillary tube and broken off, making a capillary opening on the end of a 6-inch tube. Numerous cappings were then removed from various samples of comb, both diseased and healthy, with a sharp scalpel, and cut so as to leave on the capping a rim of cell wall about one-sixteenth to one-eighth-inch wide, care being taken not to rupture the capping. These cappings were then sealed on the larger end of the glass tube with liquefied beeswax. The end of the tube covered by the capping was then submerged 3 centimeters below the surface of the chosen disinfectant, so as to have a uniform upward pressure on all the cappings successively tested. The rise of the liquid in the tube was measured at the end of a five-minute period unless the liquid was able to pass rapidly through the capping and to rise to the level of the outer liquid in less than that time. The capillary opening in the upper end of the tube somewhat retarded the exit of air and, unless the capping was cracked, or slightly perforated, the contained liquid would not reach the level of the outside liquid in five minutes. When the rise was more rapid the apparent reason was recorded. A considerable number of cappings were tested with fresh and used alcohol-formalin solution, and with fresh and used water-formalin solution (Table 5). As will be seen, there was not much difference in the results between the fresh and used solutions. It was clearly indicated, however, that there was a great variation in the rapidity with which the solutions passed through the various cappings.

Twenty cappings were tested in the alcohol-formalin solution. In 5 cases no solution passed through the cappings within the five-minute period; in 6 cases the liquid in the tube rose 1 centimeter or less; in 2 cases it rose between 1 centimeter and 2 centimeters; in 4 cases there was a rise of between 2 centimeters and 3 centimeters; and in 3 cases the liquid rose to the 3 centimeter mark in less than five minutes. Twenty cappings were likewise tested in the water-formalin solution. In 9 cases no solution passed through the cappings in periods varying from 5 to 10 minutes, and, in 1 case, even 60 minutes; in 4 cases there was a rise of liquid in the tube of 1 centimeter or less, one of these after 10 minutes; there were 3 cases of a rise

of between 1 centimeter and 2 centimeters, 1 of these being attained after 5 minutes, 1 after 15, and 1 not until 60 minutes had elapsed; there were 3 cases of a rise of between 2 centimeters and 3 centimeters, 2 of them after 5 minutes, and 1 after 15 minutes had elapsed; in 1 case the liquid rose to the 3-centimeter mark in 3 minutes. When no liquid passed through within the test period, ropy, diseased material was found smeared on the inside of the capping, or the capping appeared unusually thick. As was to be expected, the alcohol solutions passed through more readily in most cases than did water solutions. One capping, not recorded in the table, was submerged in water-formalin solution for three days with no perceptible passage of liquid into the tube.

TABLE 5.—*Tests of the permeability of brood cappings to disinfectants*[1]

Description of cappings	Immersion	Rise in tube
	Minutes	*Centimeters*
Fresh commercial alcohol-formalin solution:		
Dark brown from diseased comb, medium thick	5	2.2
Dark brown from diseased comb, fairly thick	5	.9
Dark brown from diseased comb, quite thin	3	3.0
Dark brown from diseased comb, thick	5	.2
Dark brown from diseased comb, slightly cracked	2	3.0
Dark brown from diseased comb, thick	5	0
Dark brown from diseased comb, medium	5	1.1
Dark brown from diseased comb, thick	5	0
Dark brown from diseased comb, thin	5	2.8
Dark brown from diseased comb, thick	5	0
Used commercial alcohol-formalin solution:		
Light brown from diseased comb, dried, medium thick	5	2.1
Dark brown, old, from diseased comb, dried, thick	5	.9
Brown, old, from diseased comb, medium thin	5	1.2
Scale smeared over inside, thick	5	.1
Dark brown from diseased comb, thick	5	0
Dark brown from diseased comb, thin	3	3.0
Dark brown from diseased comb, some scale in capping	5	[2]0
Dark brown from diseased comb, dry, medium thick	5	2.8
Light brown from diseased comb, good condition	5	1.0
Dark brown from diseased comb, thick	5	.2
Fresh water-formalin solution:		
Dark brown from diseased comb, dried, thick	5	0.9
Dark brown from diseased comb, thick	10	0
Dark brown from diseased comb, thin	15	1.7
Dark brown from diseased comb, normal	5	0
Dark brown from diseased comb, dried, thin	10	0
Do	15	2.4
Dark brown from diseased comb, slightly cracked	5	2.8
Dark brown from diseased comb, thick	10	0
Do	10	0
Do	10	0
Used water-formalin solution:		
Dark brown from diseased comb, dried, thick	10	0.4
Dark brown from diseased comb, dried, cracked	5	1.5
Dark brown from diseased comb, thin	10	0
Dark brown from diseased comb, very thin	5	2.1
Dark brown from diseased comb, thin	60	1.1
Dark brown from diseased comb, small hole	3	3.0
Dark brown from diseased comb, dried, thin	60	0
Dark brown from diseased comb, thin	10	0
Do	5	.7
Dark brown from diseased comb, cracked	5	.8

[1] Tests made at a uniform depth of 3 centimeters below surface of solution.

[2] Trace.

To observe what actually takes place in a sealed cell when submerged in a disinfectant solution, some artificial cells were made with pieces of glass tubing of the same diameter as those used in the experiment just described. Pieces of tube about three-quarters of an inch long were sealed at one end and sterilized in a hot-air sterilizer, cotton plugs closing the open ends. Scales containing virulent spores of *Bacillus larvae* removed from diseased combs were then

with aseptic precautions placed in a number of these glass cells, and cappings were sealed on the open ends as in the previous experiment. The sealed glass cells were then submerged for 48 hours in alcohol-formalin and water-formalin solutions, after which they were allowed to dry for a few days. Observations made at the end of 48 hours showed that no perceptible quantity of liquid had entered the cells. Enough moisture had been absorbed through the cappings, however, possibly in the form of vapor or of an indistinguishable film, to cause the dried scales to become slimy or almost ropy, like diseased remains before they have dried down. After drying, cultures were made in the usual manner (Table 6). Three scales out of 20 so treated, 1 in alcohol-formalin solution and 2 in water-formalin solution, apparently were completely sterilized, and from several, most of which had been treated in the alcohol-formalin solution, cultures were made which showed only a comparatively few germinated spores and having a slight growth. This seemed to indicate that not much actual disinfectant gains access to some at least of the sealed cells.

TABLE 6.—*Cultural results of various tests with spores of Bacillus larvae inclosed in artificial glass cells, capped, and treated for 48 hours in formalin solutions*

Solution tested	Cells cultured	Cells showing *B. larvae*	Cells showing no growth	Cells contaminated	Remarks
Fresh alcohol-formalin	5	[1] 5	0	0	Scarcely perceptible growth.
Used alcohol-formalin	5	[2] 3	1	1	Mostly good growth.
Fresh water-formalin	5	[3] 5			Do.
Used water-formalin	5	[3] 3	2	0	Do.
Control, not treated	2	2	0	0	

[1] All showing few spores germinated; one very few.
[2] Two showing many spores germinated.
[3] One showing very few spores germinated.

VACUUM TREATMENT

A method of forcing disinfectant solution into the cells was devised to demonstrate whether alcohol fills all spaces in a submerged comb. Pieces of comb of the same size as those used in previous experiments and containing numerous sealed cells were cut from infected brood combs and submerged in 180 cubic centimeters of disinfectant solution in graduated cylinders. One of these was allowed to soak for 48 hours. The cylinder containing the submerged comb was then subjected to a vacuum of 28 inches, which caused the air still remaining in many of the open cells to rush out in considerable amount and the air in sealed cells to bubble through and in some cases to burst the cappings. When the pressure was allowed to become normal the free liquid in the cylinder had decreased by from 25 to 30 cubic centimeters in displacing the air in the cells. Similar results were obtained by applying the vacuum as soon as the combs were immersed. When combs so treated were subjected to a vacuum again after 48 hours' immersion they were found still to release a few air bubbles from sealed cells. These experiments made it evident that even in open cells of combs immersed for 48 hours at ordinary atmospheric pressure a considerable por-

tion of the cell space is often filled with air. Of course the solution, particularly an alcoholic solution when used with open cells, forms a film on the surface of the cell around the bubble, as has been described by Demuth (*9*), and thus comes in contact with the larval remains. In the case of capped cells, however, under normal atmospheric pressure this bubble can not get out, and only a small quantity of liquid can gain access, in some cases probably not enough to form this moist film.

It was thought at first that this vacuum treatment might be a satisfactory method of disinfecting foulbrood combs, but aside from the cost of apparatus it was found that after this procedure it was practically impossible to remove the disinfectant from the sealed cells, even by means of centrifugal force, particularly from those whose cappings had not been broken, until after removal of the cappings. Liquid in the perfectly capped cells evaporated slowly, and a deposit of solid paraformaldehyde would probably remain in them. This residue has been found objectionable if not positively detrimental to the bees when combs with such a residue are given to a colony. Cultures made from a few combs so treated and allowed to dry for a long time gave completely satisfactory results, no growth being obtained from any cells, either open or sealed. For combs from which all cappings have been completely removed this method of filling all the cells with liquid, thus assuring actual contact of all cell surfaces and cell contents with the water disinfectant, shoud be satisfactory, provided a simple and inexpensive vacuum apparatus can be devised.

PERFORATION OF CAPPINGS

It seemed evident that because of the greater impermeability of many of the cappings the diseased material in some of the sealed cells of the immersed combs was prevented from coming in contact with sufficient disinfectant to kill the spores of *Bacillus larvae* in the infected remains. A preliminary method of perforating brood cappings was tried. By means of a blunt needle holes variable in size and intended to resemble perforations in cappings made by the bees, were made in all cappings, through which the solution might be able to enter the cells more readily. Series of samples of combs with cappings perforated in this manner were treated in both alcohol-formalin and water-formalin solutions, some for 24 and others for 48 hours, as well as in two lots of soap solution for 48 hours. In the 24-hour tests with alcohol-formalin solution, of the 20 scales cultured (Table 8) 5 gave positive growths of *B. larvae*, whereas in the case of the water-formalin solution only 1 scale from a perforated cell, of 20 cultured (Table 10), showed a few germinated spores. In the 48-hour tests with alcohol-formalin solution only 1 scale showed growth out of 20 cultured from perforated cells (Table 7). In the 48-hour tests with water-formalin solution 2 scales, of the 20 cultured from perforated cells, showed a few germinated spores (Table 9). In tests with soap-formalin solution and perforated cappings (Table 1) the contents of all such cells were apparently sterilized as far as this particular experiment was carried. These results indicate that in the case of both solutions the action was aided by perforating the cappings, but in a few instances the sterilizing action

was apparently still incomplete, probably because a trapped air bubble had prevented sufficient solution from passing through the opening.

TABLE 7.—*Cultural results of various tests with samples of comb treated for 48 hours in commercial alcohol-formalin solution*

	Number of consecutive pieces of comb immersed in same lot of solution	Open cells			First comb in series containing open cells showing growth of *B. larvae* in cultures	Sealed cells			First comb in series containing sealed cells showing growth of *B. larvae* in cultures
		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth		Number of scales cultured	Cultures showing growth of *B larvae*	Cultures showing no growth	
Preliminary tests	4	4	0	4	----------	4	[1] 4	0	First.
	3	3	0	3	----------	3	1	2	Second.
	3	3	0	3	----------	3	1	2	First.
	7	7	0	7	----------	7	[1] 2	5	Fifth
	7	7	0	7	----------	7	1	6	Third.
	8	8	0	8	----------	8	2	6	First.
	5	5	0	5	----------	5	[2] 1	4	Third.
	7	7	0	7	----------	7	5	2	Second.
	7	7	0	7	----------	7	3	4	First.
	10	10	0	10	----------	10	2	8	Ninth.
Open (uncapped) cells compared with sealed cells; used solution.	10	50	0	50	----------	40	[1] 10	30	Third.
	10	50	0	50	----------	40	[3] 18	22	Do.
Open (uncapped) cells compared with sealed cells; fresh solution.	10	50	0	50	----------	50	[4] 25	25	First.
Fresh solution; control for samples washed after treatment.	10	50	0	50	----------	----	----	----	
Open cells, washed in water after treatment	10	50	[2] 3	47	First	----	----	----	
Cells with perforated cappings compared with open cells.	10	10	0	10	----------	10	0	10	
	10	10	0	10	----------	10	1	9	Tenth.

[1] One showed few spores germinated.
[2] Few spores germinated.
[3] Three showed few spores germinated.
[4] Eight showed few spores germinated.

TABLE 8.—*Cultural results of various tests with samples of comb treated for 24 hours in commercial alcohol-formalin solution*

	Number of consecutive pieces of comb immersed in same lot of solution	Open cells			First comb in series containing open cells showing growth of *B. larvae* in cultures	Sealed cells			First comb in series containing sealed cells showing growth of *B. larvae* in cultures
		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	
Preliminary tests	5	5	0	5	----------	5	2	3	First.
Uncapped cells only; used solution	10	50	[1] 2	48	First	----	----	----	
	10	50	0	50	----------	----	----	----	
Uncapped cells only; fresh solution	10	50	[1] 1	49	Tenth	----	----	----	
Fresh solution; control for samples washed after treatment; uncapped cells only.	10	50	[2] 3	47	Sixth	----	----	----	
Open cells, washed in water after treatment	10	50	[3] 4	46	First	----	----	----	
Cells with perforated cappings compared with open cells.	10	10	0	10	----------	10	3	7	Eighth.
	10	10	0	10	----------	10	[2] 2	8	Fourth.

[1] Few spores germinated. [2] One showed few spores germinated. [3] Two showed few spores germinated.

TABLE 9.—*Cultural results of various tests with samples of comb treated for 48 hours in water-formalin solution*

	Number of consecutive pieces of comb immersed in same lot of solution	Open cells				Sealed cells			
		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	First comb in series containing open cells showing growth of *B. larvae* in cultures	Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	First comb in series containing sealed cells showing growth of *B. larvae* in cultures
Preliminary tests	6	6	0	6		6	[1] 4	2	Third.
	6	6	0	6		6	[2] 3	3	Do.
	6	6	0	6		6	1	5	Fifth.
Open (uncapped) cells compared with sealed cells; used solution.	10	50	0	50		40	[1] 12	28	Fourth.
	10	50	0	50		40	[2] 10	30	Do.
Open (uncapped) cells compared with sealed cells; fresh solution.	10	50	0	50		50	[3] 15	35	Third.
Fresh solution; control for samples washed after treatment.	10	50	0	50					
Open cells, washed in water after treatment	10	50	0	50					
Cells with perforated cappings compared with open cells.	10	10	0	10		10	[1] 2	8	Fourth
	10	10	0	10		10	0	10	

[1] Two showed few spores germinated.
[2] One showed few spores germinated.
[3] Seven showed few spores germinated.

TABLE 10.—*Cultural results of various tests with samples of comb treated for 24 hours in water-formalin solution*

	Number of consecutive pieces of comb immersed in same lot of solution	Open cells				Sealed cells			
		Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	First comb in series containing open cells showing growth of *B. larvae* in cultures	Number of scales cultured	Cultures showing growth of *B. larvae*	Cultures showing no growth	First comb in series containing sealed cells showing growth of *B. larvae* in cultures
Open cells only; used solution	10	50	0	50					
	10	50	0	50					
Open cells only; fresh solution	10	50	0	50					
Fresh solution; control for samples washed after treatment.	10	50	0	50					
Open cells washed in water after treatment	10	50	[1] 3	47	First				
Cells with perforated cappings compared with open cells.	10	10	0	10		10	[2] 1	9	Seventh.
	10	10	0	10		10	0	10	

[1] Germination of few spores; doubtful in the case of two cultures.
[2] Showed few spores germinated.

CAPPINGS REMOVED WITH KNIFE

Considerable experimenting with various methods of opening cappings sufficiently to allow easy access of solutions into the cells demonstrated that the only satisfactory way is to use an uncapping knife and actually to cut away the cappings, as is done when uncapping honey, before the combs are placed in the disinfectant solution. If the knife is sharp and well heated, brood cells may be as easily uncapped as honey cells, leaving a sharp, complete opening and adding but little to the labor factor in handling the combs. If the knife is

dull, it will tear the comb, closing many of the cells, so that the solution can not enter. All honey cells that previously have not been extracted can be uncapped at the same time as the brood cells.[2]

Several series of combs uncapped in this manner were treated with both the alcohol-formalin and water-formalin solutions for 24 and for 48 hours. In some of the 48-hour tests the cappings were cut from only one side of the combs, so that cultures for comparison might be made from sealed cells at the same time and to obtain definite figures for the percentage of sterilization of the sealed cells. To reduce the probable error arising from the missing of occasional cells that would give growth, 5 open cells and 5 sealed cells from each comb, instead of only 1, were cultured. In the 24-hour tests and in the last 48-hour series only open cells were cultured, since from the previous experiments it was found that 24 hours and in many cases even 48 hours was not invariably sufficient to sterilize all sealed cells. In tabulating these results the number of combs in each series of samples and the total number of cells cultured is recorded.

As indicated in Table 7, a total of 281 scales from open cells of combs treated for 48 hours in alcohol-formalin solution, including the cells originally open and those opened by uncapping but excluding those washed after treatment, were cultured, none of which gave any growth of *Bacillus larvae*. A total of 191 scales from 191 sealed cells of the same combs (omitting 20 combs having perforations in the "sealed" cells and other combs from which cultures were made from open cells only) gave 115 cultures showing no growth of *B. larvae*, 61 showing good growth, and 15 showing a few germinated spores, or 75 positive cultures; 39.8 per cent of these cells were therefore not sterilized. In the 24-hour tests of scales from open cells, including all considered in Table 8 except those washed after treatment, a total of 225 scales cultured gave 219 tubes showing no growth of *B. larvae*, 2 showing good growth, and 4 showing a few germinated spores, or 6 positive cultures, a percentage of 2.7 for open cells not sterilized. Only one short (preliminary) series of scales from sealed cells of combs treated for 24 hours was tested, with 2 out of 5 cultures showing growth of *B. larvae*.

As indicated in Table 9, a total of 238 scales from open cells from samples of combs treated for 48 hours in water-formalin solution, including cells originally open and cells uncapped, but no cells washed after treatment, were cultured, none of which gave a growth of *Bacillus larvae*. A total of 148 scales from sealed cells from the same combs when cultured gave 103 showing no growth of *B. larvae*, 32 showing good growth, and 13 showing a few germinated spores, or 45 positive cultures, a percentage of 30.4 for sealed cells not sterilized. In the 24-hour tests of scales from similar open cells cultured (Table 10), 220 scales were cultured, all the cultures showing no growth of *B. larvae*. No 24-hour sealed cells were tested with

[2] In a recent article Vincens (*24*), who has charge of the station for apicultural research of the Institute of Agricultural Research at Cagnes, France, describes his method for removing cappings and soaking the contents of the cells previous to immersion in a water-formalin solution. A jet of water reduced to a spray is shot against the surface of the comb at an angle with considerable force. This destroys or perforates the cappings and fills the cells. After 12 hours the combs are again treated in like manner. Then after passage through an extractor the combs, which are now thoroughly wet, are immersed in a water-formalin solution containing only 10 per cent formalin for 24 hours, after which the solution is removed and the combs allowed to dry. Vincens reports success so far with this method used on a small scale.

water-formalin solution. A shorter period of immersion than 24 hours was not tried during these investigations.

WASHING COMBS

In one or two instances combs have been treated with alcohol-formalin solution in cold weather and allowed to dry slowly at a comparatively low temperature. These, when returned to the bees, have caused considerable trouble, and even loss of colonies by desertion of the bees. Because of the retarded rate of evaporation at lower temperatures, the formaldehyde, instead of evaporating, undergoes a transformation to the solid and much less volatile paraformaldehyde. This is probably more often true of the water-formalin solution, which evaporates more slowly. When evaporation takes place slowly, a residue sufficient to be poisonous or obnoxious to bees may remain in the cells for a considerable time. Washing combs in water after treatment was tried by Jones (*15*) to remove the odor of formaldehyde, and has been recommended by Corkins (*7*) as a preventive against this difficulty. No observations in the apiary or results of cultural tests after such treatment have been recorded. Several series of combs were tested, using pieces from which the cappings had been cut, treating some for 24 and some for 48 hours, and comparing the combs allowed to dry without washing with those washed in water.

As indicated in Table 7, 50 scales were cultured from open cells of combs washed in water after treatment for 48 hours in alcohol-formalin solution. Of these, 3 cultures showed only a few germinated spores of *Bacillus larvae*. The control series of combs with open cells (Table 7) not washed in water gave no cultures showing any growth. In the 24-hour tests 50 scales were cultured from open cells of combs washed in water after treatment for 24 hours in alcohol-formalin solution (Table 8). Of these, 2 cultures showed good growth of *B. larvae* and 2 a few germinated spores. The control series of combs (Table 8) not washed gave 2 cultures showing good growth, and 1 showing a few germinated spores.

As shown in Table 9, 50 scales were cultured from open cells of combs washed in water after treatment for 48 hours in water-formalin solution. None of the cultures gave any growth of *Bacillus larvae*. The control series of combs (Table 9) that were not washed gave from open cells no cultures showing any growth. In the 24-hour tests 50 scales were cultured from open cells of combs washed in water after treatment for 24 hours in water-formalin solution (Table 10); of these, 1 culture showed good growth of *B. larvae* and 2 showed a few doubtful germinated spores. The control series of combs with open cells (Table 10) not washed in water gave no cultures showing any growth of *B. larvae*.

DISCUSSION

Throughout these preliminary laboratory experiments on the germicidal efficiency of the various types of disinfectants tested, no solution was found which sterilized the contents of all sealed cells uniformly in all of the several series of combs immersed in it. This fact is probably of importance in the sterilization of brood

combs infected with American foulbrood by means of various formaldehyde disinfectant solutions as used by beekeepers. It was found to be true even with solutions of low surface tension and supposedly good penetration, such as alcohol-formalin solution, as well as with solutions having high wetting and spreading powers with regard to waxy surfaces, such as soap-formalin solutions. In all of the several series of combs treated, even after a treatment of 48 hours, cultures from varying numbers of scales from capped cells gave growths of *Bacillus larvae*. A comparison of the percentage of sealed cells not sterilized indicates that the efficiency of the alcohol-formalin solution is relatively no greater in this regard than that of water-formalin solution.

The explanation of this failure of the formaldehyde disinfectants to sterilize all the sealed brood may be found in the varying permeability of the brood cappings, particularly many of those covering remains of American foulbrood. In some cases no solution was able to pass through the cappings, low surface tension of the liquid and solvent action on the wax seeming to be ineffective factors. In these cases the glue-like remains of diseased dead larvæ have often become smeared over the inner surface of the cappings during the routine handling of the combs, drying there and clogging the pores of the cappings. As a result, no solution could penetrate them, at least within a length of time practicable for treating combs without subjecting them to action causing undesirable deterioration. Even on the same comb there is wide variation in the structure of the cappings themselves. As can be noted in the various tables, there is great irregularity as to which comb of a series shows the first sealed cells giving a growth of *Bacillus larvae*, indicating incomplete sterilization. The experiments with glass cells indicate the possibility that the complete sterilization achieved in many of the sealed cells is brought about by the penetration into the cells of formaldehyde gas and water vapor liberated from the disinfectant solution, rather than by actual liquid sufficient to cause sterilization. Experiments with the use of a vacuum in treating combs indicated that relatively little solution enters the majority of sealed cells at usual atmospheric pressure, but that the method should be effective with all uncapped cells. These facts may explain the few cases of recurrence of disease so far noted with the method now in use, and serve as the basis of a prediction of more cases in the future. Why more such recurrences have not been observed is not easy to explain, but it is to be remembered that this method of disinfecting combs has been in use for only about two years and much concerning it is yet to be learned.

A certain proportion of the cultures of scales from sealed cells (25 per cent of those treated with alcohol-formalin solution and about 33 per cent of those treated with water-formalin solution) showed comparatively few germinated spores. In some of these cases no growth was visible on the surface of the culture medium. Some few of these cultures on subculturing failed to show further growth or germination. The question here arises whether contact with formaldehyde insufficient to kill may lower the vitality of these organisms so that they fail to continue growth, or may lower their virulence, thereby inhibiting infection of healthy colonies from such sources. It is also possible that an insufficient number of spores

remain alive to start the disease, as experience in cultivating *Bacillus larvae* from diseased material on artificial culture media has demonstrated that a certain quantity of inoculum is necessary to start growth, whereas growth will not begin when there are only a few spores. These are among the problems awaiting solution.

Another important fact brought out by the various tests of solutions containing 20 per cent of formalin is that in the case of all combs that were treated for 48 hours no cultures made from scales taken from open cells or from cells the cappings of which had been removed gave any growth of *Bacillus larvae*. For this length of treatment complete sterilization was accomplished by the water-formalin solution as well as by the alcohol-formalin solution. In the case of the 24-hour tests a few of the scales from open cells of combs treated with alcohol-formalin solution failed to be completely sterilized, 6 of the 220 such scales cultured giving growths of *B. larvae*. Apparently a 24-hour treatment is somewhat below the minimum time in which complete sterilization of the scales in open cells may be expected. On the other hand, none of the cultures from 220 scales from open cells of comb treated 24 hours in water-formalin solution showed any growth of *B. larvae*. These results indicate that the water-formalin solution is more efficient as a germicide for American foulbrood than is the alcohol-formalin solution, provided the solutions come in actual contact with the scales for at least 24 hours.

All cappings, those over brood cells as well as honey cappings, should be carefully removed to insure sterilization of combs infected with American foulbrood. Even when this is done, apparently a 48-hour treatment is still necessary when an alcohol-formalin solution is used. From the fact that all cultures were negative which were made from scales in open cells of combs treated 24 hours in a water solution containing 20 per cent of formalin, a treatment of 24 hours in such a solution appears to be sufficient. When, after combs have been soaked in the disinfectant solution, the excess liquid has been removed in an extractor, and the combs are allowed to dry without further treatment, there is still some disinfectant left which continues to act while they are drying, until it is entirely evaporated. If circumstances, such as the necessity for drying treated combs at a comparatively low temperature, require the washing of the treated combs in water in order to prevent the formation of an undesirable residue caused by the retarded evaporation, the combs should be treated 48 hours, whether in water-formalin or in alcohol-formalin solution; an additional 12 hours in the case of alcohol-formalin solution would give a greater margin of safety. If this is not done, the discontinuance of the germicidal action may permit a few scales to emerge with spores still capable of causing disease, while a period of treatment shorter than 48 hours before washing in water would be entirely insufficient for complete germicidal action.

The uncapping of all brood cells removes the necessity of using a solution capable of penetrating the wax. With the cappings removed, the dissolving action, or penetration of the wax of the comb, seems of questionable value. It is noteworthy that not one case has definitely been recorded of disease resulting from the use in healthy colonies of tons of comb foundation that for years has been made from wax rendered from combs once containing American foulbrood.

The uncapping of all brood cells eliminates the necessity for a solution with low surface tension. The much less expensive water-formalin solution will be found to enter the open cells sufficiently to soften, loosen, and sterilize the scales as effectively as an alcoholic solution, although the entrance may not be quite so rapid. The slower entrance into the cells is more than counterbalanced by the greater germicidal efficiency of formalin in the presence of water as compared with that of the same disinfectant in an alcoholic solution. The water-formalin solution readily softens and penetrates the masses of pollen in cells, since stored pollen is held together by small amounts of honey, readily soluble in water. Aside from the countless spores contained in the diseased remains and those in the infected honey or pollen, practically the only other spores that might in any way get to healthy larvæ and cause disease are not in masses, but are scattered individually over the surfaces of the comb, having accidentally been carried there by the bees in their work.

White (*27, p. 32*) has shown that spores suspended in a 20 per cent formalin solution would be killed inside of a few hours; it follows that when individual spores on combs come in direct contact with the disinfectant they must be killed in a comparatively short time. If, as was found to be the case in the laboratory experiments, soaking for 24 hours of scales of American foulbrood in open cells kills all spores embedded in them, probably immersion for only a few hours is necessary to kill these spores on the comb surfaces, as would be the case with dry extracting combs that have been in diseased colonies but have contained no dead brood. The few spores that might become embedded in propolis are as negligible as those in the wax.

The uncapping of all brood cells, as well as of any sealed cells of honey in diseased brood combs, naturally adds somewhat to the labor cost of treating these combs; but if results in the apiary compare at all with the results in the laboratory, the lowering of the cost by elimination of the alcohol, using only formalin in water, will more than offset the slight additional labor cost. However, before treatment it is almost always necessary to uncap some sealed honey in combs from diseased colonies; if the brood is uncapped at the same time, comparatively little extra effort or time will be required. The washing of combs, when necessary after treatment for 48 hours in the water-formalin solution, also adds to the labor costs; but in this case again the extra labor should be repaid by the results.

The data presented indicate that a 20 per cent solution of formalin in water is the most efficient as well as the most economical disinfectant so far found for the sterilization of combs infected with American foulbrood, provided the cappings are all completely removed. It is hoped that apiary tests will be made to determine the practicability of these results. Naturally, even with the most reliable process, carelessness in handling the combs, and more particularly carelessness in the treatment of the diseased colonies from which the combs are taken, will be fatal to success in gaining control over this disease which has thus far caused such great losses. The must approved methods for the treatment of American foulbrood have been discussed elsewhere.[3]

[3] Methods of treatment and control of American foulbrood are discussed in United States Department of Agriculture Farmers' Bulletin 1084.

CONCLUSIONS

A number of soap solutions were found unsatisfactory as carriers for 20 per cent of their volume of formalin to be used as a disinfectant for treating American foulbrood combs; (1) because of the difficulty of controlling the reaction of the solution and of preventing the precipitation of the soap and (2) because of the failure of the soap solution to penetrate all cappings of sealed cells sufficiently to kill all spores of *Bacillus larvae* contained in the diseased material therein, and to do this within a period of 48 hours.

Certain liquids of low surface tension other than ethyl alcohol, such as acetone and iso-propyl alcohol, are somewhat more efficient as carriers for formalin than most of the solutions tried, including ethyl alcohol, as indicated by the comparatively few sealed cells which failed to be completely sterilized by them within a period of 48 hours. On the other hand, these liquids are too expensive for practical use, even if satisfactory as carriers for the formalin. Less expensive liquids tried, such as a commercial methyl-ethyl ketone, are unsatisfactory because of failure to sterilize scales in sealed cells in 48 hours.

Miscellaneous disinfectants such as dilute hydrochloric acid, various dilutions of iodine, acetic acid added to water-formalin solution to increase its penetrating power, and such substances as gelatine, added to water-formalin solution to increase the wetting and spreading powers, are all unsatisfactory as disinfectants for sterilizing American foulbrood combs, since none of them completely sterilizes all sealed cells in 48 hours. The hydrochloric acid solutions and all but quite concentrated iodine solutions even fail to sterilize all the open cells. Formaldehyde, used as formalin solution, when employed in sufficient proportions is the most efficient and practical germicidal agent thus far used for the purpose of disinfecting American foulbrood combs. A solution containing less than 20 per cent of formalin is found to be unreliable.

The results obtained with various dilutions of alcohol and of alcohol-formalin solution as the carrier for 20 per cent of their volume of formalin are not sufficiently complete to warrant conclusions as to their relative efficiency. All of these solutions are unsatisfactory, since they do not completely sterilize all sealed cells in 48 hours. A 20 per cent solution of formalin in water, without alcohol, is slightly less efficient than the alcoholic solutions in sterilizing in 48 hours the contents of sealed cells, because of its failure to penetrate many of the cappings; but it sterilizes all open cells in that period.

The commercial alcohol-formalin solution, like all the other solutions tested, fails to sterilize completely the scales from all sealed cells with a treatment of 48 hours.

The variation in the permeability of many of the brood-cell cappings accounts for the failure to sterilize many of the sealed cells within a period of 48 hours. In some cases cappings completely resist the passage of liquid or vapors into the cells, thereby making low surface tension and solvent action unavailing within the period of time practicable for the satisfactory treatment of combs.

The results of experiments with glass observation cells, as well as of the application of a vacuum to the solutions containing combs

under treatment, indicate that little if any actual liquid enters cells where the capping is intact and more or less impermeable. It is probable that when such sealed cells are sterilized the disinfection is brought about by the passage through the capping of gas and vapor liberated from the solution.

The perforation of brood cappings adds to the efficiency of the disinfectant solutions, both alcoholic and water; but, aside from the difficulty of doing this in a practical way, the sterilization of such cells is not always uniformly complete, even after a treatment of 48 hours.

To obtain uniformly complete sterilization of all infected cells in American foulbrood combs, no matter what solution is used, all cappings, covering both brood and honey, should be carefully removed before the combs are immersed in the solution. This can be easily done if care is used, with a hot, sharp, uncapping knife, and adds little to the labor costs.

The sterilization of combs infected with American foulbrood, when all the cells are uncapped and alcohol-formalin solution is used, requires, if complete from the standpoint of the cultural results obtained, more than a 24-hour treatment. A treatment of 48 hours would give a margin of safety.

The sterilization of combs infected with American foulbrood, when all the cells are uncapped and a 20 per cent solution of formalin in water is used, if complete from the standpoint of the cultural results obtained, requires treatment for 24 hours at least. A somewhat longer period would give a greater margin of safety.

The washing of combs in water, to prevent the formation of a residue of paraformaldehyde after treatment with disinfectants containing 20 per cent of formalin, should not be attempted unless the combs have been treated at least 48 hours in the solution. A somewhat longer period would give a greater margin of safety, particularly with the alcohol-formalin solution.

To reduce the chance of missing any cells that have not been completely sterilized, when testing the efficiency of any disinfectant solution, a sufficiently large number of cells should be cultured. The proportion should be approximately from 3 to 5 per cent of the cells of each class, open and closed, so distributed as to represent fairly all the cells treated.

In view of the cultural results obtained, a 20 per cent solution of formalin in water was found the most satisfactory disinfectant for use in sterilizing combs infected with American foulbrood, with regard to both germicidal action and low cost, provided the proper precautions are taken. All honey should be extracted, all brood cappings should be completely removed, and the combs should be treated at least 24 hours, or 48 hours if it is found desirable to wash them in water after treatment. Before such a procedure can be recommended unreservedly exhaustive tests must be carried out under apiary conditions.

Care must be taken not only with the process of disinfecting combs infected with American foulbrood, no matter what solution or method is used, but equal or greater care must be exercised in the treatment of the diseased colonies themselves to eliminate the danger of recurrence of disease from that source. The successful sterilization of the combs will otherwise be of little avail.

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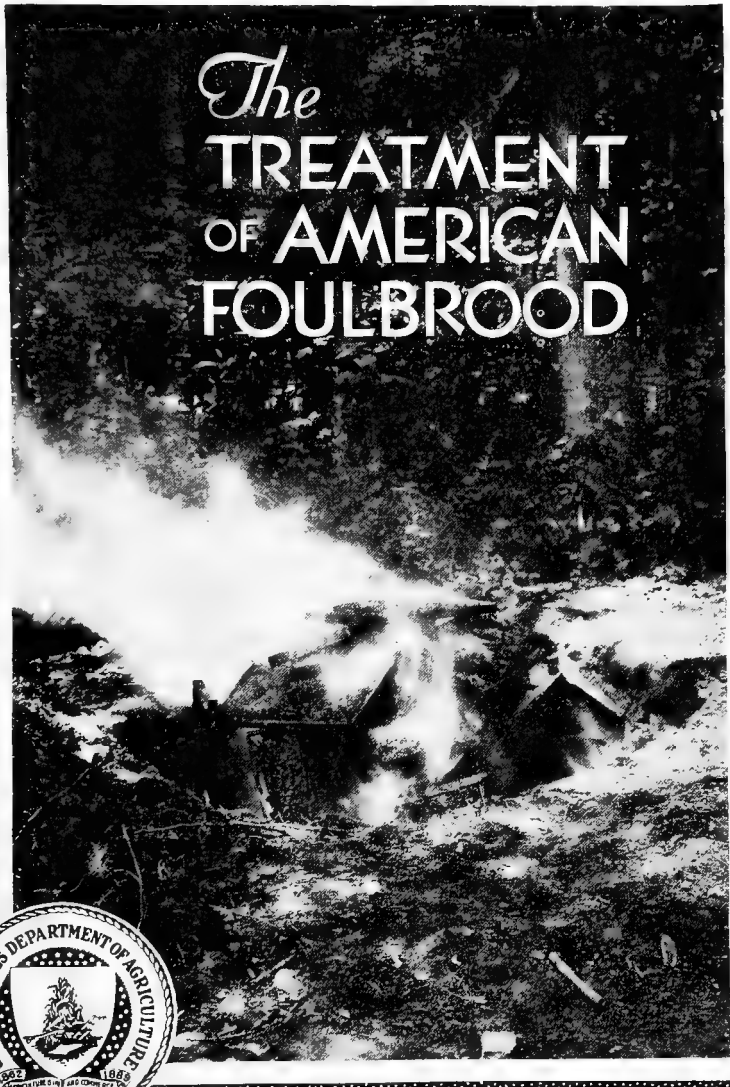
U. S. DEPARTMENT OF AGRICULTURE
FARMERS' BULLETIN No. 1713

The TREATMENT OF AMERICAN FOULBROOD



AMERICAN FOULBROOD is a disease of the brood of bees which causes serious losses to beekeepers. Its occurrence is practically world-wide, it attacks all races of bees, and strong colonies are as liable to infection as weak ones.

It is important that the beekeeper recognize the symptoms of the disease and be familiar with the manner in which it is spread, in order that he may take precautions to keep it out of his apiary and to prevent it from spreading from one colony to another in case any of his colonies become infected. He should also know how to treat the disease in the most effective way. Such information is given in this bulletin. It is impossible to manipulate colonies so that they cannot contract the disease, but much worry and financial loss can be avoided by dealing promptly and effectively with the disease as soon as it appears.

Samples of brood suspected of being diseased are diagnosed free of charge by the Division of Bee Culture, Bureau of Entomology, United States Department of Agriculture, Washington, D.C.

This bulletin supersedes Farmers' Bulletin 1084, entitled "Control of American Foulbrood."

Washington, D.C. Issued October 1933

THE TREATMENT OF AMERICAN FOULBROOD

By JAS. I. HAMBLETON, *senior apiculturist, in charge, Division of Bee Culture, Bureau of Entomology*

CONTENTS

AMERICAN FOULBROOD is a disease of the brood of bees which causes series losses to beekeepers. Not only does it take a heavy toll in the actual destruction of colonies and increase the cost of operating an apiary, but, what is perhaps of equal importance, the presence of disease in an apiary, or in the vicinity of one, causes such mental anxiety to some beekeepers that it unquestionably prevents them from succeeding in the bee industry.

The disease is practically world-wide in its distribution and is of common occurrence in the United States. All races of bees are susceptible. There have been statements in the beekeeping literature that lead one to believe that the brood of certain strains of bees is immune, but there is no evidence upon which to base this assumption. There may be various strains of the disease, differing in virulence, and individual colonies may react differently. Not enough variation has yet been detected, however, to warrant giving such suspected cases special treatment. Worker larvae are particularly susceptible to the disease, although queen and drone larvae are occasionally affected. Adult bees are immune to it.

CAUSE OF THE DISEASE

American foulbrood is caused by a species of spore-bearing bacteria known as *Bacillus larvae*. The living honeybee larva is its only known host and the disease is transmitted primarily by means of the spores. The bees that work within the hive become contaminated with these spores in attempting to remove the diseased brood, and carry them from one cell to another. Once the disease has spread generally throughout the brood nest, the bees cease trying to remove the dead brood, and it accumulates until the colony dies owing to the absence of emerging bees.

In performing such tasks as feeding the larvae, building the cells, ripening the nectar, and transferring it from one part of the hive to another, the bees may contaminate not only honey that is in the brood nest, but also that in the supers above the queen excluder. This does not mean, however, that all the honey in the brood nest or supers necessarily becomes contaminated.

Spores may come in contact with larvae of any age, but the larvae rarely die until they have developed to the point where they lie lengthwise in the cells or the cells are being sealed preparatory to transformation to pupae. During the early coiled stages the sugar content of the larva is usually high and, since the germ of American foulbrood will not grow in highly concentrated sugar or honey, it is only after much of the body sugar has been utilized that the spores can develop. Underfed larvae have a low sugar content, and in contact with such larvae the spores are able to germinate and to kill the larvae while they are still coiled.

The spores of American foulbrood are invisible to the naked eye, and they are extremely resistant to sunlight, drying, heat, and commonly used chemical disinfectants. The maximum time that the spores retain their virulence has not been determined, but they are known to remain alive for years in honey and brood combs.

SPREAD OF THE DISEASE

It has always been considered that the disease is spread from colony to colony most commonly by the robbing of hives containing disease-weakened colonies or bees that have died of American foulbrood, by bees from healthy colonies. Robbing unquestionably scatters American foulbrood. A disease-weakened colony does not defend itself well; therefore it is possible for robber bees from healthy colonies to help themselves to contaminated honey. On the other hand, the beekeeper himself often unwittingly spreads the disease within an apiary when he moves combs of brood and honey from one hive to another, or unites weak colonies, which may be diseased, with strong, healthy ones. These are probably the most common means of spread. The disease may also be carried from one colony to another when bees enter the wrong hive, a practice generally referred to as drifting.

The dissemination of the disease beyond the range of flight of the bee can be accounted for by the transportation of infected material, including honey, into a disease-free area, where it is later made accessible to healthy bees. Experimental evidence indicates that the commercial shipment of honey is not such an important means of spread as many persons suppose. The sale of used, contaminated equipment is, however, one of the principal avenues through which this disease is spread from one locality to another.

A beekeeper who does not know anything about American foulbrood, or how to check its spread in his apiary, will not be able to maintain his colonies with profit if they become weakened by the disease. Finding this to be the case, he may sell his empty hives, combs, and other accessories, perhaps to another beekeeper who knows no more about the bee diseases than he does. Purchasers of

used beekeeping equipment should make sure that it is free from disease material. Some States wisely restrict the sale of used beekeeping equipment to that which is known to be uncontaminated.

SYMPTOMS

In the apiary American foulbrood can be detected only by the presence of brood remains. The spores of the disease organism can be recovered and identified only by bacteriological technic.

The disease may be recognized by the sunken and perforated cappings and the isolated sealed cells in the midst of recently emerged brood. The dead larvae have a melted-down appearance and are usually extended lengthwise in the cells (fig. 1). Occasionally the bees die while in the coiled stage, and in this condition the brood may resemble that dead of European foulbrood. Dead larvae are slightly yellowish or dirty white in color at first, but become chocolate brown or black upon further decay. Shortly after death of the larvae, and until the contents of the cells become too thick, the brood remains can be drawn out with a toothpick into fine silklike threads, and are quite ropy and gluelike. Upon drying, the brood remains, called scales (fig. 1, F, G), become tough or brittle and adhere so tightly to the floor and base of the cells that the bees cannot remove them. The scales are very thin and in old, dark brood combs are easily overlooked.

Pupae that die of the disease undergo similar changes in color and consistency and in the final formation of a scale (fig. 2). Occasionally the tongue of a dead pupa adheres to the roof of the cell. This is a significant, but not an infallible, symptom.

Bees remove many of the cappings from cells containing dead brood, and this makes it appear that the larvae or pupae died before being sealed.

American foulbrood has a characteristic odor, which is pronounced when the disease is in an advanced stage.

Although adult bees are not attacked, loss of brood causes an infected colony to become gradually weaker, and usually to die during the second year of the disease.

The constancy and the uniformity of the symptoms characterize this disease more than does any one symptom. Isolated sealed, sunken, or perforated cells in the midst of healthy emerging brood should be examined whenever disease is suspected. It is not difficult to make a reliable diagnosis in the apiary except perhaps when only 1 or 2 recently dead larvae or pupae are present. In such cases a portion of the comb containing the suspected brood should be sent to a competent inspector. In the meantime the entrance of the suspected hive should be contracted and the colony left undisturbed until the nature of the trouble has been determined.

OTHER BROOD DISEASES SOMETIMES MISTAKEN FOR AMERICAN FOULBROOD

Many colonies have been destroyed or treated in the erroneous belief that they were infected with American foulbrood. On the other hand, the disease has been spread when American foulbrood

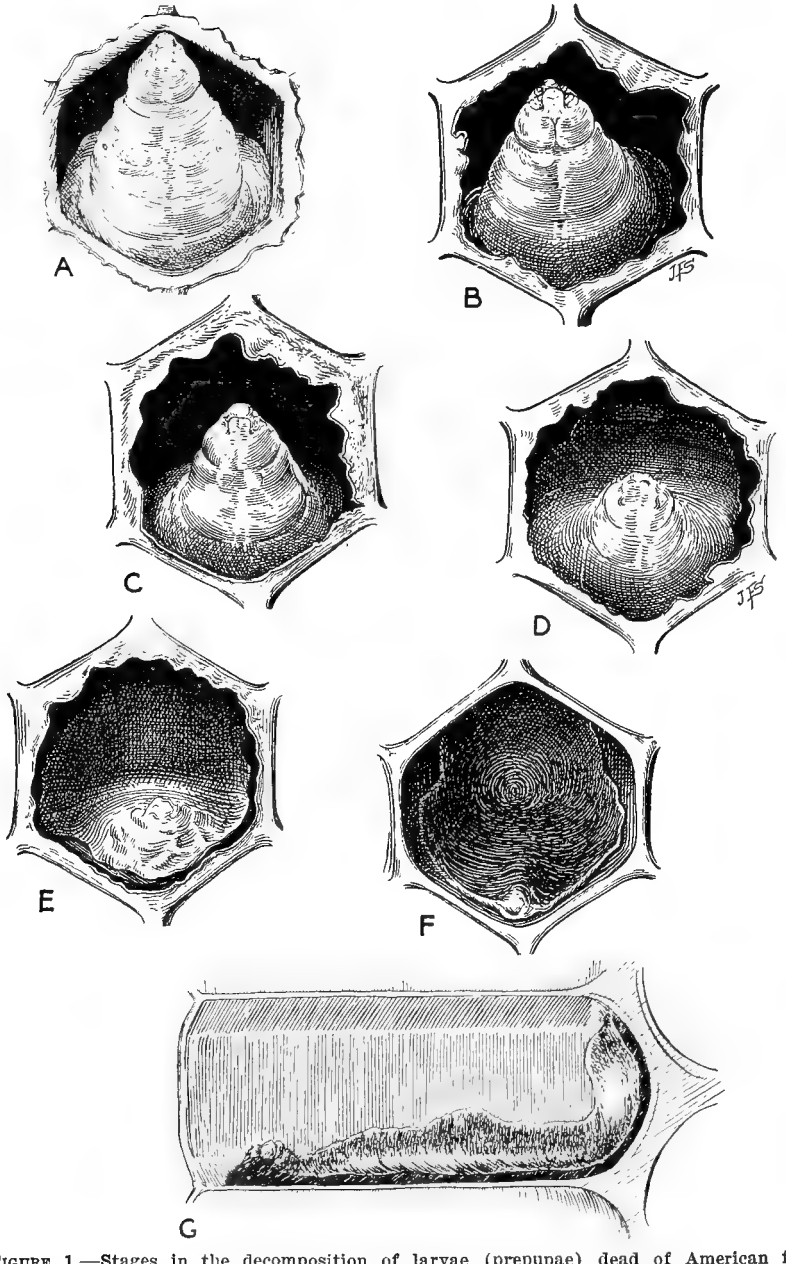


FIGURE 1.—Stages in the decomposition of larvae (prepupae) dead of American foulbrood: *A*, Healthy larva at the age when most of the brood dies of American foulbrood. *B*, *C*, *D*, *E*, Progressive stages in the decomposition of dead larvae. These stages can usually be detected only by removing the cappings. *F*, Scale of American foulbrood. Except in new combs the scale is difficult to see by looking straight into the cell. The comb should be held so that the line of sight falls on the long floor of the cells. This can be done by grasping the comb by the top bar and holding it 8 or 10 inches below the eyes and tipping the bottom bar slightly away from you. *G*, Longitudinal view of an American foulbrood scale.

has been mistaken for some of the less serious brood diseases, which require different treatment. It is therefore of the utmost importance that a correct diagnosis be made before corrective measures are applied. For this reason brief descriptions are here given of the

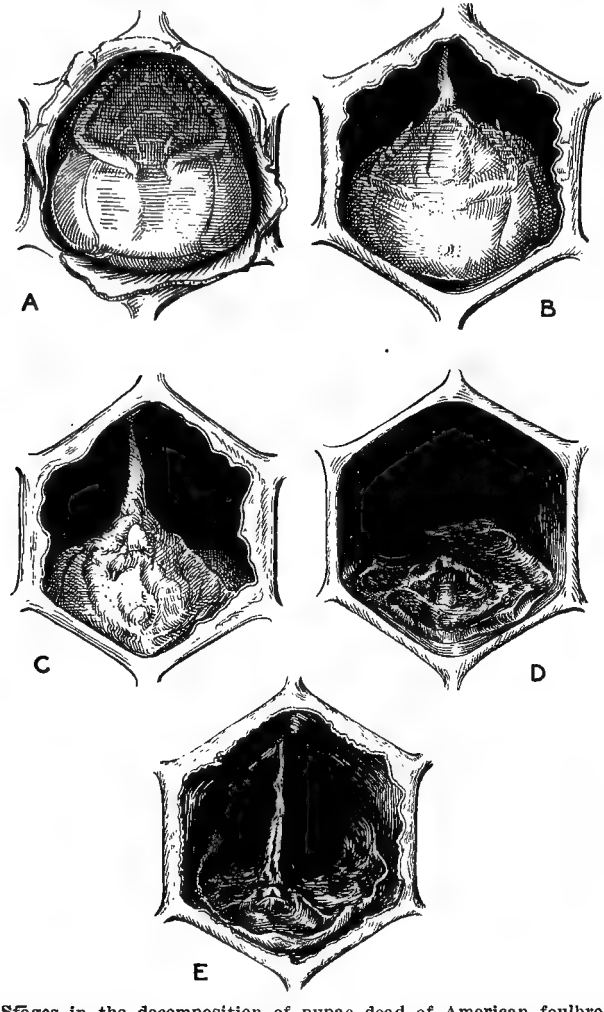


FIGURE 2.—Stages in the decomposition of pupae dead of American foulbrood: *A*, *B*, *C*, Heads of pupae showing progressive stages of melting down and decay. In *B* and *C* the tongues show prominently. *D*, Scale of American foulbrood formed from the drying down of a diseased pupa. *E*, Scale of American foulbrood formed from the drying down of a diseased pupa, with a vestige of the tongue adhering to the roof of the cell.

two other common brood disease of the apiary, European foulbrood and sacbrood.

European foulbrood usually kills the larvae in the coiled stages (fig. 3). The dead larvae are slightly yellowish white in color. The brood remains are watery, pastelike, or granular, the appearance varying according to the age at which the larvae die. The scales

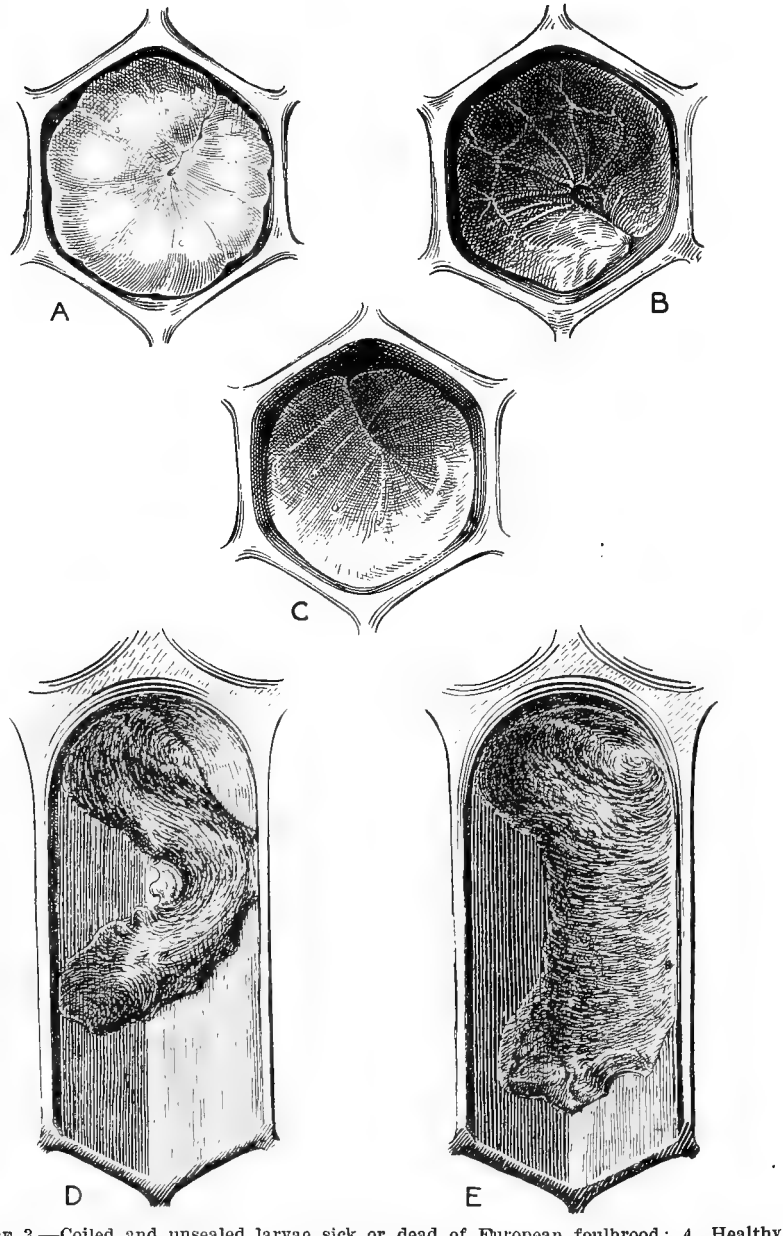


FIGURE 3.—Coiled and unsealed larvae sick or dead of European foulbrood: *A*, Healthy coiled larva at the earliest stage at which larvae die of European foulbrood; *B*, scale formed by a dried-down larva; *C*, one of several positions assumed by a sick larva prior to death; *D*, *E*, longitudinal views of scales formed from larvae that had assumed a nearly lengthwise position at the time of death, quite different from the scale shown in *B*.

do not adhere tightly to the cells and are removed by the bees in a strong colony. Occasionally larvae dead of European foulbrood become brown and ropy and present other symptoms similar to those

dead of American foulbrood (fig. 4). In such cases a correct diagnosis can usually be made only after a microscopic examination.

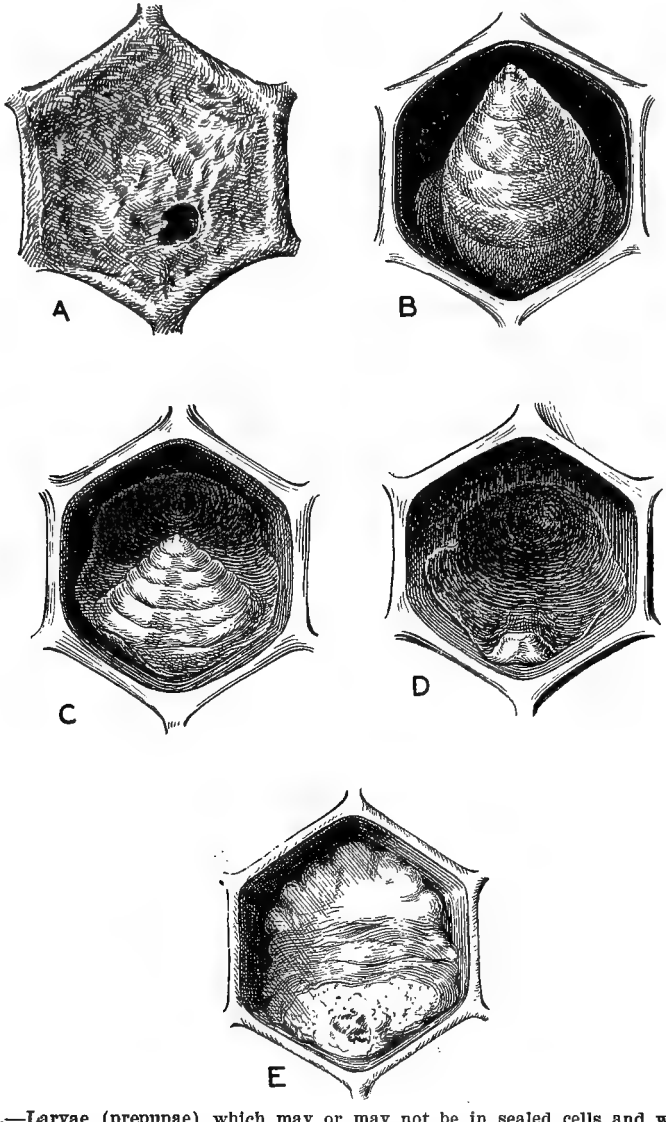


FIGURE 4.—Larvae (prepupae) which may or may not be in sealed cells and which are lying lengthwise at the time of death from European foulbrood. Stages similar in appearance to those illustrated here are encountered in American foulbrood. *A*, Sunken and perforated capping of a cell containing a larva dead of European foulbrood; *B*, larva lying lengthwise in the cell and recently dead of European foulbrood; *C*, same as *B* except in a more advanced stage of decomposition; *D*, scale formed by dried-down larva dead of European foulbrood; *E*, the remains of a larva dead of European foulbrood, part of which has been removed by the bees.

Sacbrood kills the larvae while they are extended in the cell (fig. 5), and the cappings become sunken and perforated, as in American foulbrood. The dead larvae are yellowish at first, but become

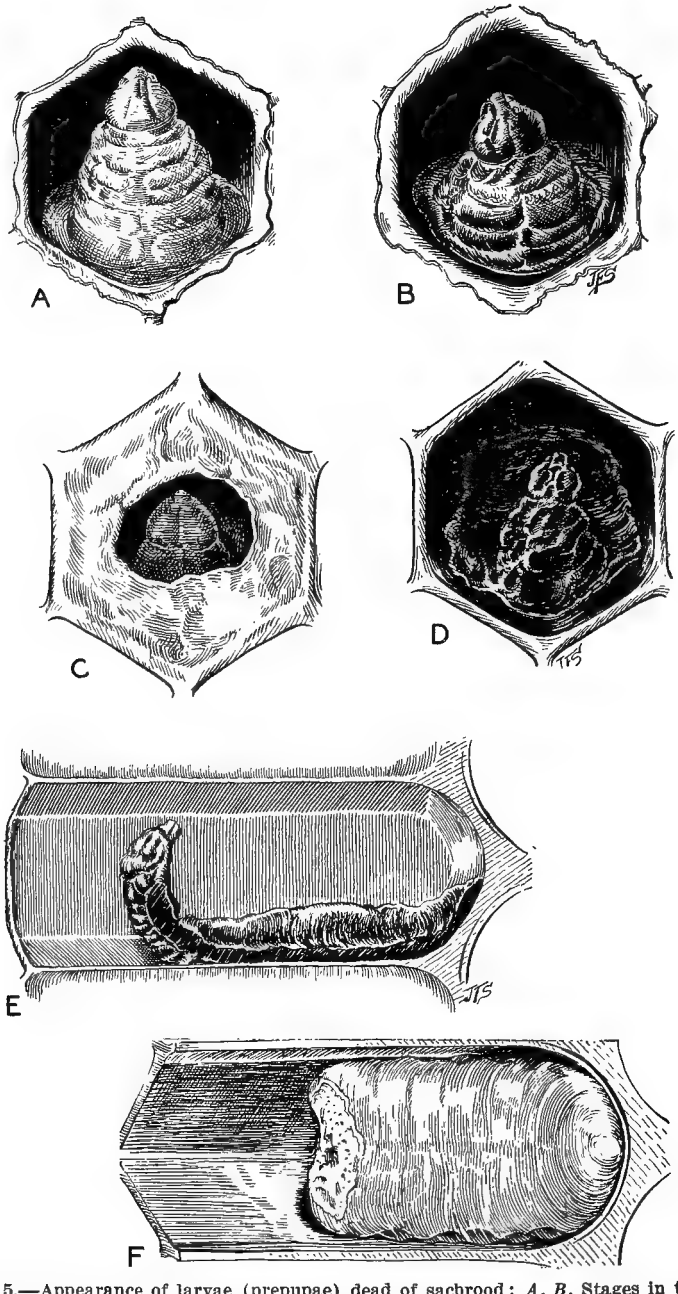


FIGURE 5.—Appearance of larvae (prepupae) dead of sacbrood: *A*, *B*, Stages in the course of the disease; *C*, the erect head end of a dead larva showing through an opening that the bees have made in the capping; *D*, *E*, two views showing the scale of sacbrood; *F*, the head portion of this larva has been gnawed away by the bees. Note how the head remains erect in all stages.

brown or black as decay advances. The heads remain erect during the process of decay. The larval skin becomes tough and saclike, thus giving the disease its name, and the material inside is watery and granular. The brood remains do not adhere to the cells; therefore, the bees are able to remove them and the disease does little damage.

TREATMENT

BURNING DISEASED COLONIES

It is now commonly recognized that the safest, and in the end the most economical, means of stamping out American foulbrood is to burn the diseased colonies. While this procedure may seem wasteful to those who believe that less drastic measures afford ample protection, it is the only method that leaves no opportunity for the disease to recur, thus relieving the mind of the beekeeper.

FIGURE 6.—Killing the bees of a diseased colony with calcium cyanide.

Diseased colonies should be burned as soon as possible after the infection is discovered. Before this is done, however, the bees must be killed. A tablespoonful of calcium cyanide, an extremely poisonous chemical which must be handled with great care, spread on a sheet of paper or cardboard and slipped into the entrance of the hive (fig. 6), which should be left open, will kill the bees in a few minutes. As as an extra precaution additional cyanide may be thrown into the top of the hive, since occasionally the bees fall onto the poison placed in the entrance so rapidly as to prevent the fumes from penetrating all parts of the hive. All field bees that try to gain entrance to the hive will also be killed.

Gasoline is sometimes used to kill the bees. In such cases the entrance to the hive is closed, a pint or more of gasoline is then poured over the top frames, and the hive is closed tightly.

After the bees have been killed, the contents of the hive should be burned with the least possible delay in order to avoid trouble

from robber bees, as both calcium cyanide and gasoline act as repellents for only a short time.

Before the bees are killed, a pit 18 inches or more deep, and wide enough to hold all the material to be burned, should be dug in a place not likely to be plowed or otherwise disturbed. A hot fire should then be kindled, with plenty of scrap material and with cross members stout enough to support the weight of the frames and placed so as to permit plenty of ventilation underneath. A brisk hot fire is necessary thoroughly to burn the brood and honey.

The hives containing the dead bees should be carried intact close to the pit and the bees and frames fed to the fire as fast as circumstances permit. The bottom board, hive bodies, inner covers, and tops are not burned. By placing the hives on pieces of burlap or stout paper it will be easy to gather up and burn the bits of comb honey or dead bees which may be dropped during the operation. After everything has been completely burned, the topsoil surrounding the fire should be raked into the pit to prevent bees from healthy colonies from having access to any dead bees or honey. The pit should then be filled.

If the killing of the bees and the burning are done at night, the danger of interference from robber bees will be lessened. It is essential, of course, to have everything well planned and all necessary material at hand.

No beekeeper should wait for an inspector to discover and burn his infected colonies, but should, himself, periodically inspect all colonies and promptly destroy every diseased one.

It should be understood that the burning of all diseased colonies in an apiary gets rid only of the colonies in which American foulbrood in an active form is plainly manifest. If there has been any equalization of the brood, if supers or combs have been transferred from one colony to another, or if diseased colonies have been robbed out, it is highly probable that the disease will show up later in other colonies. So even where burning is done carefully and thoroughly, it is usually at least 3 or 4 years before the disease can be stamped out of an apiary.

DISINFECTING EMPTY HIVES AFTER BURNING

After the burning, the hive bodies, bottom boards, inner covers, and tops should be taken into the honey house, thoroughly scraped to remove all propolis and wax, and then scrubbed, both inside and out, with a hot soap or lye solution and a stiff brush. The scrapings should be burned and the wash water disposed of in such a manner that it is not accessible to the bees.

Washing with soap and water is also the best way to remove spores from the hands, clothing, tools, and extracting equipment. Disinfectants strong enough to kill the spores are injurious to the hands.

If it is not feasible to wash the hive bodies, they may be stacked 7 or 8 high to form a chimney, the inside walls sprinkled with kerosene, and ignited. A little ventilation and fuel at the bottom of the stack will produce a hotter fire. Gasoline can also be used for this operation, but extreme precaution is necessary. As soon as the inside is scorched, the fire should be smothered by placing a board

over the top super. The outside of the hive bodies should then be thoroughly washed to remove all traces of honey. A gasoline blowtorch is a handy tool for scorching, but its use is rather slow.

SHAKING NOT RECOMMENDED

For many years the Department of Agriculture recommended the treating of infected colonies by the shaking method, whereby the bees in a diseased colony are shaken from the old combs into a clean hive on clean frames. This procedure reduces the losses due to the disease, and a careful operator, who thoroughly understands the disease, may be able to maintain his apiaries in this way. The disease is rarely eradicated by this method, however, and it usually has to be adopted as a routine manipulation. Treated colonies have to be nursed along, and the very act of shaking, if not done with meticulous care, is apt to spread the disease. Moreover, there is always a doubt as to whether the shaking is successful. A treated colony or a colony on disinfected combs cannot be pronounced clean for 2 years. Now, after many years during which colonies have been shaken to get rid of the disease and at the same time save as much as possible in the way of bees and equipment, the disease situation in the United States has not materially improved.

OBJECTIONS TO THE USE OF DISINFECTING SOLUTIONS

Disinfecting solutions are of only limited value in the treatment of American foulbrood. In the first place, their use for treating brood combs does not obviate the shaking treatment, as the bees must be removed before the combs are disinfected. Moreover, the careless handling of combs during the disinfecting operation may result in failure. When disease reappears after a colony has been shaken and later placed on treated combs, it is impossible to tell whether the method of shaking was at fault, the disinfection inadequate, or the colony reinfected.

Although there are several disinfectants which, when properly used, will kill the spores of American foulbrood without destroying the comb, none has yet been found to sterilize the spores in sealed honey without destroying the comb and making the honey poisonous to bees and brood. Individual sealed cells are easily overlooked and it is probable that many, if not most, of the cells of honey in the brood chamber of a diseased colony are contaminated.

Another disadvantage in the use of disinfectants is that bees are loath to accept treated combs, and as a result the size of the honey crop is reduced.

DISINFECTING SUPER COMBS

Disinfectants can be used effectively in treating super combs that have never contained brood. Super combs are ordinarily used on whatever colony needs them and are not set aside for designated colonies. After all the diseased colonies in an apiary have been disposed of, it is often not possible to know whether any of the general supply of super combs have become contaminated by being used on colonies with foulbrood. Therefore, it is safest to disinfect all the super combs in an outfit in which there has been appreciable

amount of American foulbrood. This is the only use recommended for disinfecting solutions in connection with the treatment of this disease.

Super combs can be disinfected with a 20 percent formalin-water solution—that is, 20 parts of formalin[1] to 80 parts of water, liquid measure. The combs to be disinfected should be free of honey. They should be kept immersed in the solution at a temperature of not less than 70° F. for at least 24 hours. At lower temperatures sterilization proceeds much more slowly. In order that the solution may come in contact with all parts of the cells, after being placed in the solution the combs should be agitated to dispel as many air bubbles as possible. This can also be accomplished by pouring the solution into the tank so that it rises slowly enough to fill each cell completely.

The 20 percent formalin-water solution may be used repeatedly without much deterioration in strength. It is advisable, however, to add formalin occasionally to maintain full strength of the solution.

Formalin is unpleasant to work with, although not dangerous. It is well for the operator to protect his hands with rubber gloves.

Formalin-alcohol solution, formaldehyde gas, and chlorine also kill the spores of American foulbrood without necessarily destroying the combs. Formalin-alcohol solution, a patented article, is slightly less effective than the 20 percent formalin-water solution and is more expensive. Formaldehyde in gaseous form cannot be recommended for treating American foulbrood combs. The use of chlorine, although still in the experimental stage, has produced disappointing results and, moreover, it is extremely dangerous to handle.

TREATMENT BY STATE INSPECTORS

Under most conditions inspectors are justified in burning every diseased colony immediately, because such a colony constitutes a menace to all healthy colonies in the vicinity. The maintenance of such a serious nuisance as a colony containing American foulbrood should not be tolerated. The best interests of the industry demand the prompt disposal of all such colonies.

When a State inspection force is applying the area-clean-up method for the first time, however, and when the incidence of the disease is high, the use of the shaking treatment is sometimes justifiable. The advisability of using it depends not only upon the amount of disease in a particular apiary or area, but upon the character of the beekeeping, the kind of equipment employed, and the facilities and experience of the beekeepers for doing the job.

Where shaking is used in the first steps of an eradication program, the State inspectors should give every consideration to the protection of healthy colonies. The establishment of temporary yards where diseased colonies can be treated without endangering healthy colonies is strongly recommended in this connection. These temporary yards are not to be confused with hospital yards, which were recommended at one time and with which many beekeepers are

[1] Formalin or formaldehyde solution is an aqueous solution containing from 37 to 40 percent of formaldehyde gas.

familiar. Temporary yards are used only to shake and reestablish the bees in a place where healthy colonies are not endangered. The contents of the hives other than bees should be burned with the least possible delay.

HANDLING AND DISPOSING OF HONEY FROM AFFECTED COLONIES

The honey from a diseased colony, if it constitutes a super or more, may be saved and marketed. The handling of this honey, however, requires special attention. At no time should it be accessible to the bees. Therefore, since no honey house is, strictly speaking, bee-tight, the honey should be bottled or canned as soon as possible, every vestige of honey washed from the outside of containers and from the extracting equipment and honey house, and the empty combs burned. No attempt should be made to recover honey from diseased colonies unless there is a distinct economic saving.

Since it is often impossible to ascertain the source of honey purchased on the open market, such honey should not be fed to colonies of bees if it can be avoided. If such honey has to be used, it should first be diluted with an equal volume of water and boiled for an hour in a closed vessel. Boiled honey, however, should not be fed for winter stores.

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DIAGNOSING BEE DISEASES IN THE APIARY

By C. E. BURNSIDE, *assistant apiculturist*, and A. P. STURTEVANT, *associate apiculturist, Division of Bee Culture, Bureau of Entomology and Plant Quarantine*

CONTENTS

FOREWORD

Bees, like all other living creatures, are subject to diseases, and their manner of living in crowded hives makes it almost inevitable that any contagious disorder will spread within the hive or to other colonies unless it is detected and the appropriate treatment given.

Other publications of the Department furnish information on the methods of treatment. This circular tells where to look and what to notice in the examination of colonies for possible or suspected disease.

More than one disease may be present in a colony, therefore the beekeeper should not discontinue the search on finding the symptoms of one disease. Especially is it important that American foulbrood be detected if it is present in the apiary.

If the nature of the disease is not apparent, samples of brood comb or the adult bees should be sent to the State apiary inspector or the Bee Culture Laboratory of the Bureau of Entomology and Plant Quarantine, National Agricultural Research Center, Beltsville, Md., as directed on page 34.

IMPORTANCE OF BEE DISEASES AND THEIR RECOGNITION

Bee diseases are found throughout the United States wherever bees are kept. These diseases cause large annual losses in bees, honey, and equipment and very materially add to the cost of honey

production. Unless bee diseases are recognized and controlled, individual colonies or even those of entire apiaries may be seriously weakened or destroyed.

It is important that beekeepers recognize bee diseases in their early stages so that they can apply proper methods of treatment, since practically all the diseases are more or less contagious and can spread from diseased to healthy colonies. Some of the diseases cause only slight losses, and can, to a certain extent, be disregarded. Others, however, are serious, and prompt treatment is required to prevent their spread. Consequently it is necessary that the beekeeper be able to recognize even the less serious diseases so as not to confuse them with the serious ones. The symptoms of sacbrood and European foulbrood, for instance, are often confused with those of American foulbrood. Furthermore, American foulbrood may be mistaken for European foulbrood, and if the usual treatment for the latter is applied, the disease not only will not be arrested but is likely to spread to healthy colonies.

In recent years new bee diseases have been discovered. One of these, parafoulbrood, a serious brood disease, at present appears to exist only in limited sections of the South. It is highly desirable to prevent the further spread of these newly discovered diseases and, consequently, beekeepers should learn to differentiate them from the other more widely distributed diseases.

There are also a number of abnormal conditions of bees that at times cause heavy losses and can easily be confused with some of the diseases. It has recently been found that nectar or pollen, or both, from certain plants may cause the poisoning of brood and adult bees. Then, too, the symptoms of poisoning or other abnormal conditions of bees such as chilling, starvation, or the presence of brood of infertile queens or laying workers, can easily be confused with the symptoms of some of the diseases.

No attempt has been made in this circular to describe methods of treatment, since these are available in other publications of the Department of Agriculture and in State bulletins and other beekeeping literature. Abnormal conditions of bees that are often difficult to distinguish from diseases are not discussed in detail, since it is planned to describe them in another publication.

BROOD DISEASES

WHAT TO OBSERVE WHEN LOOKING FOR BROOD DISEASES

To identify the brood diseases, any dead brood found in the cells should be examined carefully. The appearance of the combs may indicate which brood disease is present, but final diagnosis should always depend upon the symptoms shown by the dead brood. Dead brood in open cells can be seen clearly if a comb is held so inclined that the direct light of the sun falls on the lower side and bottom of the cells (fig. 1). If there is no dead brood in the open cells, any sunken, discolored, or punctured cappings should be removed and these cells examined for dead brood.

When dead brood is found, the following important points should be determined: (1) Age of the brood when death occurred, (2) po-

sition of the dead brood in the cells, (3) color of the dead brood, (4) consistency of the dead brood in different stages of decay, (5) odor coming from the combs, and (6) odor of dead larvae in different stages of decay.

A chart or guide for use in diagnosing diseases of the brood of bees is given in table 1 (p. 22).

It should always be kept in mind that more than one brood disease may be present in a colony. Of first importance at all times is

FIGURE 1.—Inspecting combs for brood diseases. A convenient way to hold the comb while looking for dead brood. The arrow indicates the direction of the sun's rays, which should fall on the lower side and bottom of the cells.

the early discovery of American foulbrood. When a less serious brood disease is found, it should be determined whether or not American foulbrood also is present.

AMERICAN FOULBROOD

CAUSE

American foulbrood is an infectious disease of the brood of bees caused by a bacterium known as *Bacillus larvae*. It is the most destructive of the brood diseases, is very infectious, and diseased colonies practically always die. This bacterium causes the death of larvae and pupae by its growth and multiplication within the stomach. It also causes a typical decay of the dead brood. *B. larvae* resists drying, the action of chemicals, both high and low temperatures, and the dehydrating action of honey; consequently American foulbrood cannot be treated successfully except by burning the infected combs and bees.

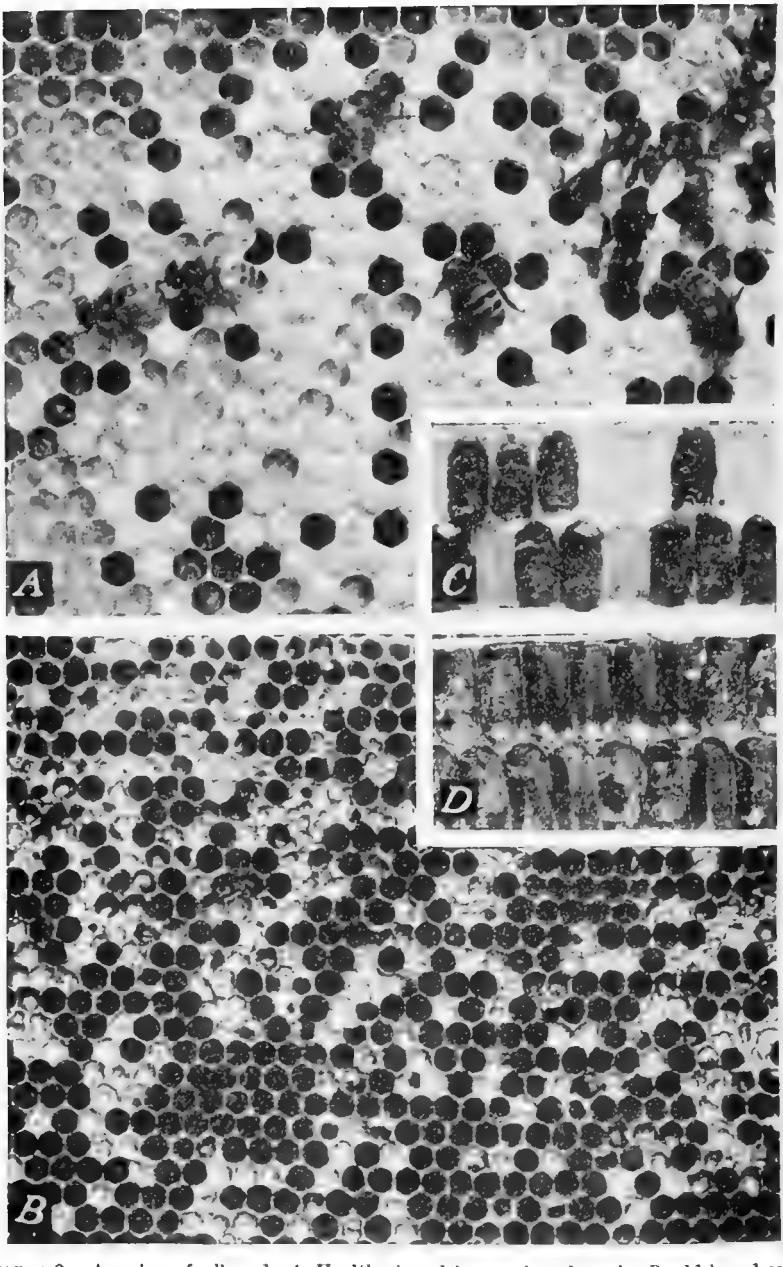

FIGURE 2.—American foulbrood: *A*, Healthy brood in new brood comb; *B*, old brood comb with an advanced case of American foulbrood; *C*, scales of larvae dead of American foulbrood on a cross section of a new comb; *D*, scales of larvae dead of American foulbrood in an old brood comb.

EFFECT UPON THE COLONY

The strength of a recently infected colony will not be noticeably affected, and there will be only one or a few dead larvae or pupae in sealed cells with slightly discolored or sunken cappings. The

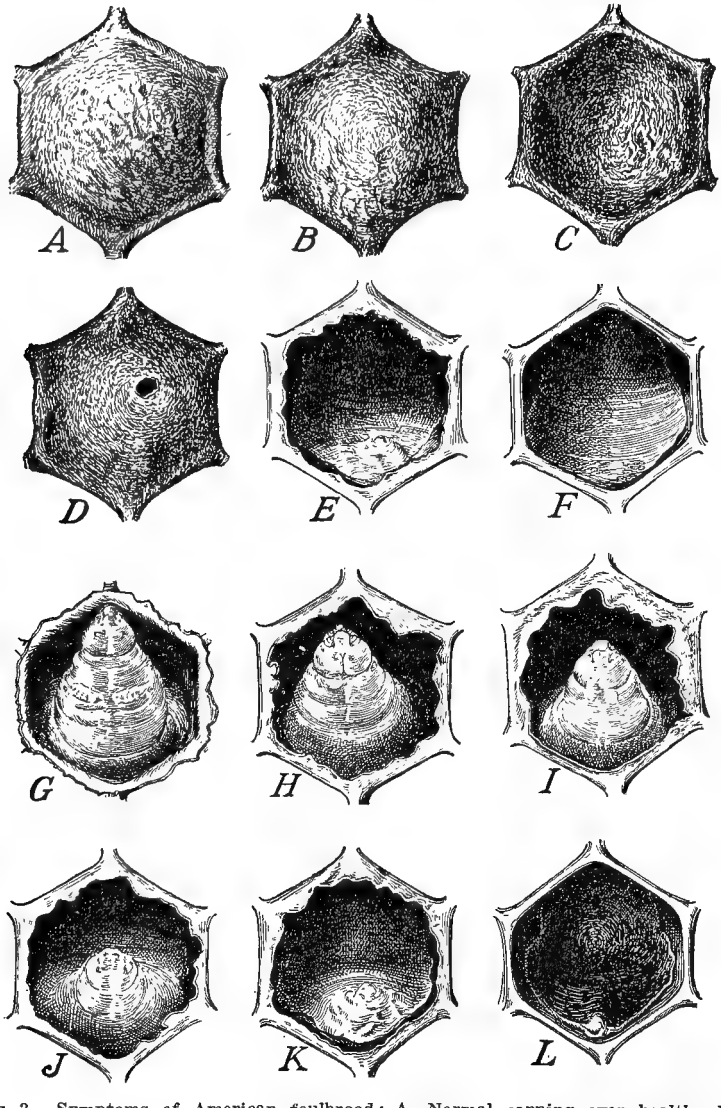

FIGURE 3.—Symptoms of American foulbrood: A, Normal capping over healthy larva; *B–F*, stages in the discoloration and removal of cappings; *G*, capping removed to show healthy larva; *H–L*, stages in the decay and drying of larvae killed by American foulbrood; oral views.

disease may not develop to a critical stage and seriously weaken the colony until the following year. At other times, however, the disease may advance more rapidly and seriously weaken or kill the colony the first season. If the disease has been present and active

for a considerable period, the colony will be noticeably weakened, and a large proportion of the cells (75 percent or more) will contain dead brood. *All weak, infected colonies found during any time of*

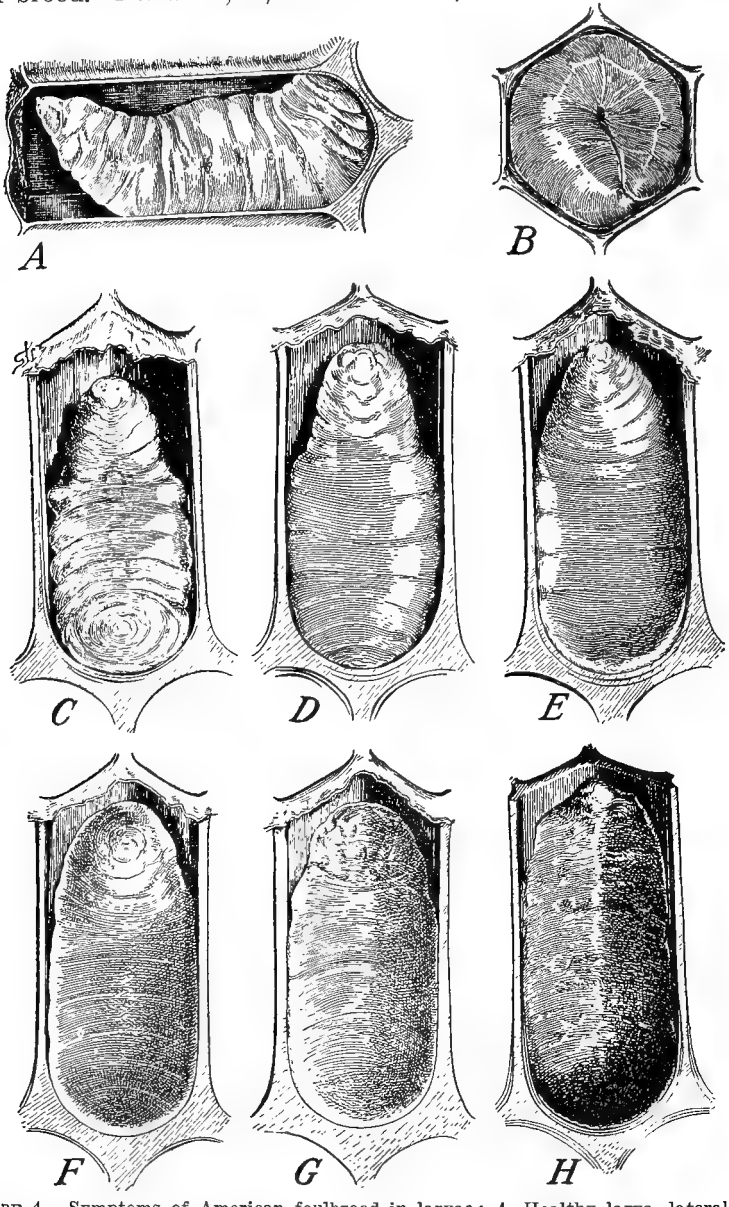

FIGURE 4.—Symptoms of American foulbrood in larvae: *A*, Healthy larva, lateral view; *B*, coiled larva recently dead; *C*, healthy larva, ventral view; *D–H*, stages in decay and drying of larvae, ventral views.

year should be burned at once to prevent spread of the disease through robbing. Under no circumstances should colonies be permitted to remain in the apiary until they have become seriously weakened by or die of American foulbrood.

APPEARANCE OF THE COMBS AND CAPPINGS

In healthy brood combs, where a normal queen has been laying, there is a certain regularity in the arrangement of areas containing eggs, larvae, pupae, and emerging bees (fig. 2, *A*), and the cappings are convex and uniform in appearance (fig. 3, *A*). In a colony in-

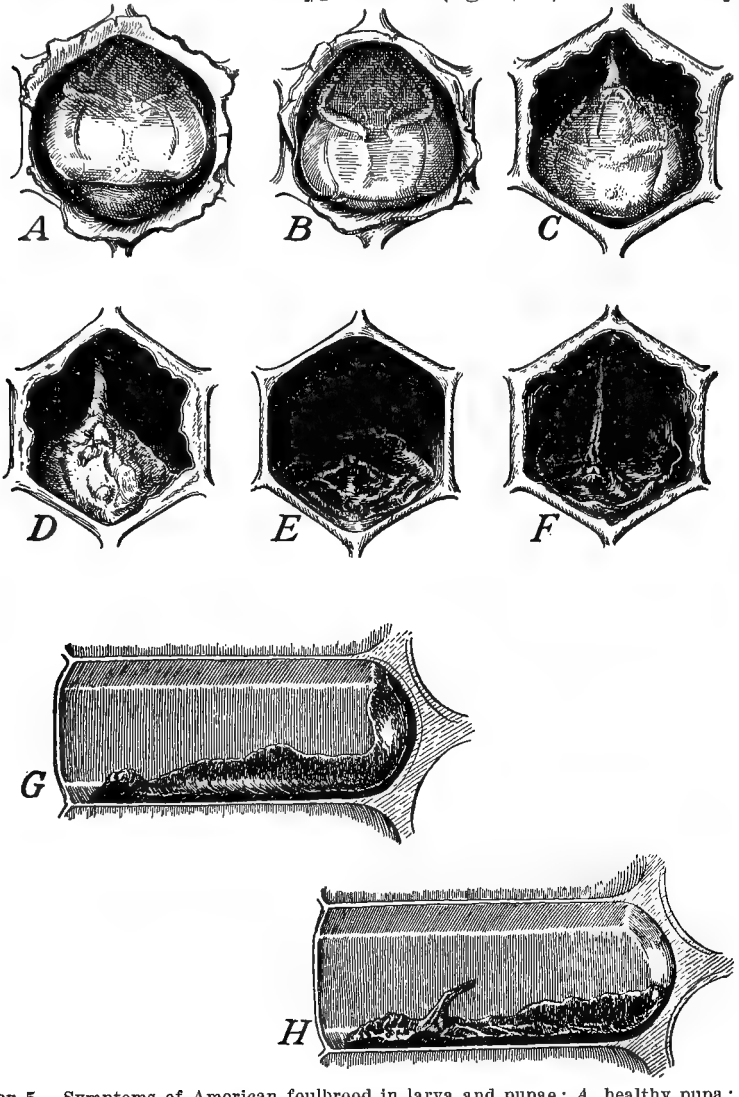

FIGURE 5.—Symptoms of American foulbrood in larva and pupae: *A*, healthy pupa; *B–F*, stages in the decay and drying of pupae; *G*, scale of dead larva, lateral view; *H*, scale of dead pupa, lateral view.

fected with American foulbrood the brood is more or less irregularly arranged, depending on the degree of infection. Great irregularity, due to the intermingling of cells of healthy brood with uncapped and capped cells of dead brood and cells with punctured and sunken cappings, is sometimes spoken of as the "pepperbox" appearance (fig.

2, *B*). Dead brood in cells with discolored, sunken, or punctured cappings (fig. 3, *B*, *C*, *D*) should always be studied carefully to determined whether death was caused by American foulbrood.

In advanced stages of the disease many of the cappings are punctured (fig. 2, *B*). Cappings may also be broken away at the edge and settled down on the dead brood, appearing dark brown and shining. Cappings over dead brood are often removed by adult bees, and in advanced cases many dried scales, as the remains of dead larvae and pupae are then called, can be seen in uncapped cells (fig. 3, *E* and *F*).

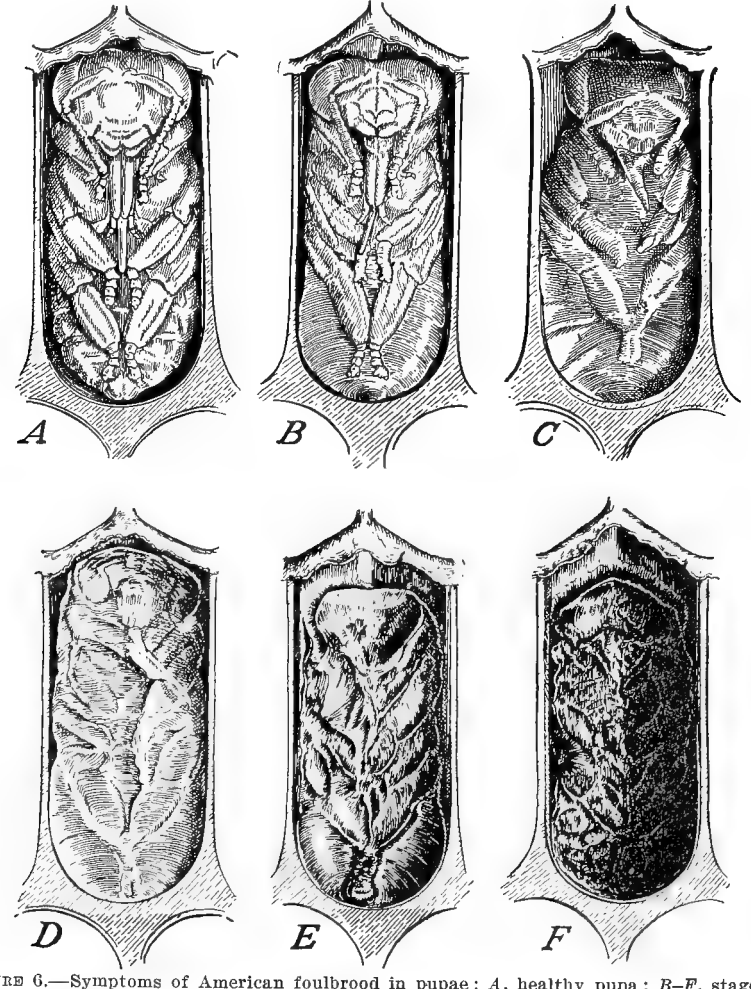

FIGURE 6.—Symptoms of American foulbrood in pupae: *A*, healthy pupa; *B–F*, stages in the decay and drying of pupae, ventral views.

SYMPTOMS SHOWN BY THE DEAD BROOD

KIND AND AGE OF AFFECTED BROOD

Usually only worker brood is affected, but occasionally drone and queen brood are also killed. Adult bees are never affected by this disease.

Death occurs quite uniformly after the larvae have been capped over, have spun their cocoons, and are fully extended on the floor of the cells, as shown by the healthy larvae in figures 3, *G*, and 4, *A*. Occasionally death occurs after the pupa has formed but before the body (except the eyes) is pigmented. (See healthy pupae in figs. 5, *A*, and 6, *A*.) In advanced cases a few larvae may die while coiled on the bottom of the cells (fig. 4, *B*), but only rarely does death occur when larvae are irregularly twisted on the side walls. Larvae killed by American foulbrood that are coiled or irregularly twisted often show symptoms similar to those of European foulbrood, and a laboratory examination may be necessary to determine whether the latter disease also is present.

COLOR AND CONSISTENCY OF THE DEAD BROOD

Soon after death the glistening white color of healthy larvae and pupae changes to dull white. About 2 weeks after death the color is very light brown, and the well-rounded appearance is lost. The dead brood gradually sink in the cells during decay and become darker (figs. 3 to 6), changing from a light coffee color to dark chocolate brown by the end of the fourth week. Scales are very dark brown or nearly black. The decay and drying of dead brood ordinarily require a month or more. Scales are difficult to distinguish in old brood comb, since they are about the same color as the comb (fig. 2, *D*); but in new comb they are readily distinguished (fig. 2, *C*).

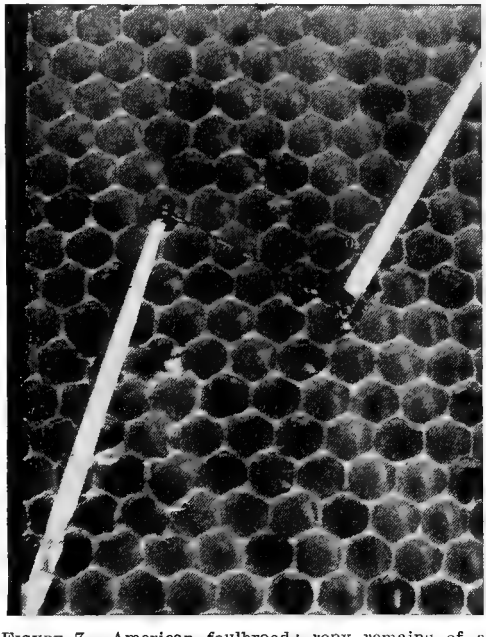

FIGURE 7.—American foulbrood; ropy remains of a decayed larva.

During the early stages of decay the body wall is easily ruptured, and the tissues are soft and watery. Occasionally the body divisions of the dead larva are more clearly marked than are those in healthy ones the consistency of dead brood becomes characteristically glue-like about 3 weeks after death. When a toothpick or match is thrust into a decayed larva and withdrawn, the decaying mass adheres and can be drawn out an inch or more in a glulike thread (fig. 7). Decayed larvae finally become dry and brittle.

APPEARANCE OF THE DEAD BROOD

The appearance and position in the cells of brood killed by American foulbrood are remarkably uniform. The dead larvae lie extended along the lower side wall with their posterior ends curved

part way up onto the bottom of the cells (fig. 5, *G*). There may be a small raised swelling near the head end of the scale, but this rarely is prominent. In advanced cases rows of cells contain dead larvae uniformly in this position.

When scales are numerous the disease can be diagnosed from their appearance alone. Scales can be seen extended along the lower side walls when the comb is held inclined so that a bright light falls on the lower side walls and bottoms of the cells (fig. 1). Occasionally cross markings which represent the segmentation of the larvae can be seen on the scales. When completely dried the scales are brittle and adhere so tightly to the cell walls that it is difficult to remove a scale without breaking it. When death occurs after pupation has started, the form of the pupa can be recognized in the scale (figs. 5, *H*, and 6, *F*). *The mouth parts of the dead pupa may protrude from the head of the scale and appear as a fine thread slanting slightly backwards into the cell and at times adhering to the upper wall* (fig. 5, *F*). The appearance of protruding or "stuck up" tongues is one of the most dependable symptoms of American foulbrood.

ODOR OF THE DEAD BROOD

In the first stages of decay, while the remains are still white, practically no odor is detectable. When the remains begin to turn brown and become ropy, however, an odor develops that is different from the typical gluepot odor characteristic of the advanced stages of this disease. In later stages, when the dead brood is brown and decidedly ropy, the familiar gluepot odor is always present, but it practically disappears when the scales are completely dry. In advanced cases, when much decaying brood is present, the gluepot odor can be detected even a foot or more from the combs. Since the odor of American foulbrood is characteristic, the use of the odor test is of considerable value in the diagnosis of doubtful cases. The odor can best be judged by holding some of the decayed remains on a toothpick at the entrance to the nostril and breathing deeply.

EUROPEAN FOULBROOD

CAUSE

European foulbrood is an infectious bacterial disease of the brood of honeybees. The bacteria grow within the stomach of infected worker, queen, and drone larvae and cause their death, but pupae are rarely attacked. Adult bees are not affected by this disease.

The earliest studies on European foulbrood seemed to indicate that it was caused by a rod-shaped bacterium, *Bacillus alvei*, which is commonly found in decayed brood. Later it was observed that lancet-shaped bacteria, different in shape and size from the rods and spores of *B. alvei* found in decayed brood, are usually present in large numbers in sick and recently dead larvae. This lancet-shaped bacterium, which was given the name *Bacillus pluton*, is now commonly considered to be the cause of European foulbrood. It has been found recently, however, that the rod-shaped *B. alvei* is capable of changing its form to a lancet-shaped bacterium resembling *B.*

pluton or other forms of bacteria found in larvae affected by European foulbrood. It seems probable, therefore, that *B. alvei* and *B. pluton* are only different forms of the same bacterium.

RACES OF BEES AFFECTED AND CONDITION OF COLONIES

Common black and Italian-black hybrid bees are more frequently affected by European foulbrood than are Italians, and weak colonies are usually more seriously affected than are strong ones. This disease frequently appears year after year in colonies of black or hybrid bees, and heavy losses may be suffered, but among Italian bees losses are usually unimportant. At times, however, European foulbrood spreads within strong colonies as well as within weak ones, and occasionally Italian bees are seriously affected.

EFFECT UPON THE COLONY

European foulbrood is most common in the spring, when brood rearing is at its height. Usually the earliest reared brood is not affected. Sometimes this disease appears suddenly and spreads rapidly within infected colonies. At other times it spreads slowly and does little damage. As a rule it subsides by midsummer, but occasionally it continues to be active during summer and fall, or it may reappear in the fall. A good honey flow seems to hasten recovery. In severe cases colonies are seriously weakened or killed. Usually the worker bees remove dead brood promptly (fig. 8, *M*); but in some colonies, particularly weak ones, it is allowed to accumulate.

SYMPTOMS

APPEARANCE OF THE COMBS

In mild cases and in early stages of European foulbrood the arrangement of the brood in the combs is not noticeably irregular. The degree of irregularity increases with severity of the disease and the length of time it has been present. In advanced cases open cells, which may be empty or contain eggs or healthy or affected brood, are irregularly scattered among cells of capped brood (fig. 9). Cells with discolored, sunken, or punctured cappings (fig. 8, *N* and *O*) may be present, but these are less common than in American foulbrood. Irregular arrangement of the brood is not a dependable symptom of European foulbrood, however, and final diagnosis should depend upon symptoms shown by the dead individuals.

APPEARANCE OF SICK LARVAE AND TIME OF DEATH

Sick larvae lose the plumpness and glistening white color of healthy larvae and become flat white. A faint yellow color, which is an important symptom, may also appear before death. Sick larvae may show abnormal movements and occupy an unnatural position in the cells.

The greater number of larvae die while coiled on the bottom of open cells (fig. 8, *A–I*). Many larvae also die at the age when they

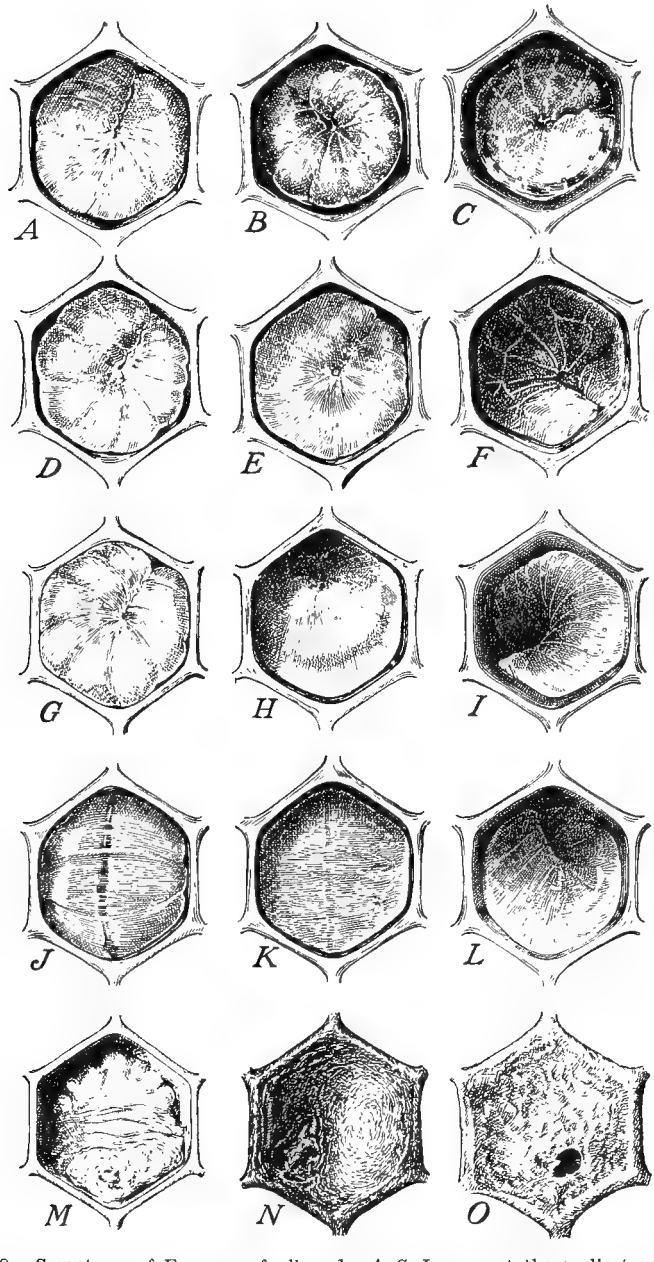

FIGURE 8.—Symptoms of European foulbrood. *A–C*, Larvae at the earliest age at which they may be attacked by the disease; *A*, earliest symptoms, *B*, more advanced symptoms, *C*, scale of a larva that died at this age. *D*, Healthy larva of slightly older age. *E*, Sick larva of this age. *F*, Scale of larva of this age. *G*, Healthy larva at the oldest age that larvae normally remain coiled on the bottom of the cells. *H*, *I*, Larvae of this age dead of European foulbrood. *J*, Healthy larva just before the cell is capped. *K*, *L*, Larvae of this age dead of European foulbrood. *M*, Dead larva that has been partly removed by the bees. *N*, Discolored and sunken capping over a dead larva. *O*, Punctured capping over a dead larva.

would normally be spinning their coccoons (figs. 8, *J–L*, and 10, *D–F*). Comparatively few larvae die while fully extended (fig. 10, *A*, *B*, *C*, *G*, *H*, *I*). Pupae are rarely affected by this disease.

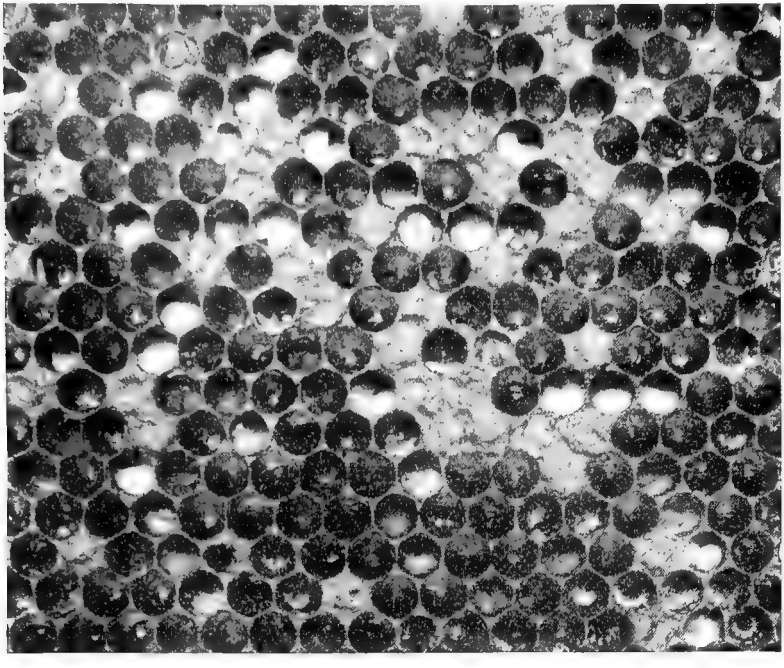

FIGURE 9.—European foulbrood; heavily infected comb showing larvae in various stages of disease and decay.

Larvae dead of European foulbrood, therefore, are usually coiled on the bottom of the cells but may be irregularly twisted or fully extended.

COLOR OF THE DEAD BROOD

Soon after death larvae become dull and grayish or yellowish-white. During decay the color deepens and may become brown or almost black. The tracheae, or breathing tubes, in dead larvae usually show more clearly than in healthy ones (fig. 8, *C* and *F*). They appear as radiating white lines in the dead coiled larvae and as narrow white lines across larvae that die while extended. A white line which crosses the radiating white lines can frequently be seen on the side of dead larvae. The prominence of the tracheae is a valuable symptom of European foulbrood but is not strictly dependable.

An elongated, dull grayish-white or yellowish-white mass can be seen through the skin along the back of sick and recently dead larvae. This mass is within the chyle stomach and consists of a turbid fluid that contains many bacteria. In healthy larvae, pollen in the stomach can often be seen through the skin along the back (fig. 8, *J*), but the color is usually of a brighter and deeper shade

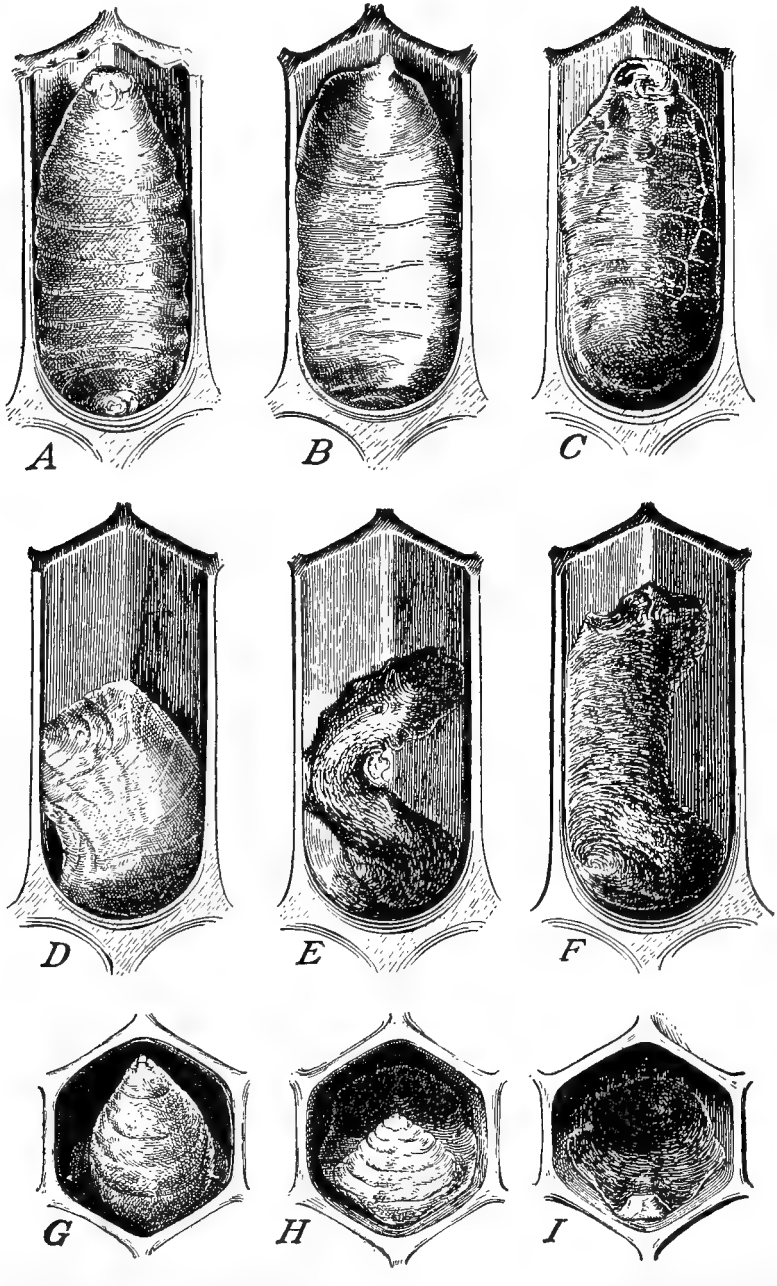

FIGURE 10.—Symptoms of European foulbrood: *A*, Ventral view of an extended larva recently dead of European foulbrood; *B*, extended larva partly decayed; *C*, scale of an extended larva; *D*, recently dead larva, and *E* and *F*, scales of dead larvae irregularly twisted; *G*, oral view of recently dead extended larva; *H*, partially decayed larva; *I*, scale of larva that died after straightening out.

of yellow than in affected larvae. Dissecting sick or recently dead larvae and examining the contents of the digestive tract helps in making a diagnosis after experience has been gained.

CHANGES CAUSED BY DECAY AND DRYING

The appearance of the dead larvae changes gradually during decay and drying. The gray and the yellow colors deepen during decay, but the depth of the color in scales varies considerably. Larvae that die before the cells are sealed dry rapidly, and decay is soon stopped; hence these scales are usually light colored. Larvae that die after the cells are sealed usually become dark brown or nearly black. Diagnosis of European foulbrood is more difficult after the dead brood is decayed and dry.

For a short time after death, larvae can be removed from the cells without tearing the skin. Within a few days the skin and other tissues become soft; and the larvae settle against the lower wall of the cells, and appear moist, melting, and flattened. At this stage in decay, larvae are somewhat translucent and watery and cannot be removed entire. Upon drying they become pasty, sometimes ropy, and finally rubbery or brittle. Scales of European foulbrood usually do not cling closely to the cell walls and are easy to remove.

Larvae that die of European foulbrood in sealed cells may become quite ropy and resemble larvae dead of American foulbrood. Since the bees remove dead brood from open cells first, it sometimes happens after disease ceases to be active that the brood which died in sealed cells is all that remains in the combs. When this happens it may be difficult to tell whether American foulbrood, or European foulbrood, or both of these diseases are present.

ODOR OF DEAD BROOD

The odors of European foulbrood cannot be accurately described but must be learned by smelling of the dead brood. When there are many decaying larvae in the combs an odor that is characteristic of this disease can sometimes be detected. Usually the odor of recently dead larvae is slight. A sour odor is sometimes present in partially decayed larvae. Some larvae, particularly those that die after they have straightened out and the cells are sealed, develop a putrid odor resembling the odor of decayed meat. This odor is nearly always present in larvae killed by European foulbrood which in other respects resemble larvae killed by American foulbrood. After the odors have been learned, the odor test helps considerably in distinguishing between European and American foulbrood when other symptoms overlap.

PARAFOULBROOD

CAUSE

Parafoulbrood is caused by bacteria which resemble the bacteria of European foulbrood. Worker, queen, and drone larvae and sometimes pupae are killed by the bacteria, which grow within the digestive tract, but adult bees are not affected by this disease. The spore stage of the bacterium found in affected brood is known as *Bacillus para-alvei.*

DISTRIBUTION AND RACES OF BEES AFFECTED

This disease has been found only in limited sections of North Carolina, South Carolina, Georgia, and Florida. All the races of bees common in North America are susceptible, but Italians appear to be more resistant than are common blacks and hybrids. Weak colonies are usually more seriously affected than strong ones, but heavy losses of brood may also occur in strong colonies.

EFFECT UPON COLONIES

Parafoulbrood progresses rapidly within some colonies and seriously weakens or kills them. In others it progresses slowly, the colonies are not noticeably weakened, and the disease disappears of its own accord. Some colonies clean out the dead brood promptly, while in others it is allowed to accumulate. In some apiaries only a few colonies will be diseased, while in others every colony will be

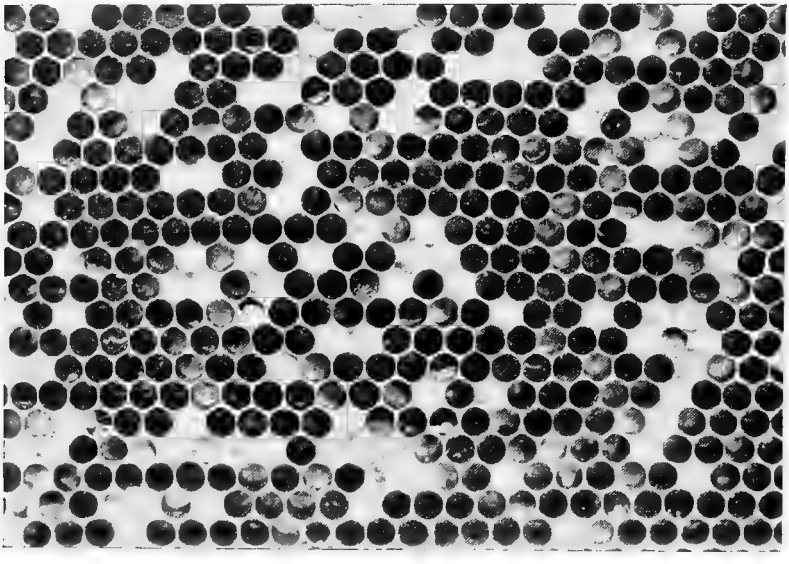

FIGURE 11.—Parafoulbrood; a heavily infected comb showing larvae in various stages of disease and decay after death.

affected. Loss caused by parafoulbrood may vary from the weakening of a few colonies to the loss of entire apiaries. This disease usually appears in the spring and disappears by midsummer, but occasionally colonies exhibit symptoms of the disease throughout the year, or there may be a slight increase of infection in the autumn. The first brood reared in the spring is not affected.

SYMPTOMS

APPEARANCE OF THE COMBS

Infected combs resemble combs with European foulbrood. The brood is more or less irregular, depending upon the amount of infection and the length of time the disease has been active (fig. 11).

Dead brood in open cells is removed by the bees sooner than that in sealed cells. Occasionally the bees increase the thickness of the cappings over dead brood in sealed cells. Such cappings appear dark, sunken, and greasy, and are sharply depressed in the center. Dead larvae may remain in these cells for months, or even over winter.

APPEARANCE OF SICK BROOD AND TIME OF DEATH

Sick larvae change from glistening white to dull or flat white, and a slight loss of plumpness may be noticed. They move uneasily in their cells and are often found in abnormal positions. A yellow discoloration occasionally appears before the larvae die.

Death from parafoulbrood usually occurs when the larvae are coiled or irregularly twisted in the cells, but many extended larvae and a few pupae are killed. The average age at the time of death is usually somewhat greater than in case of European foulbrood.

APPEARANCE OF DEAD BROOD

Larvae dead of parafoulbrood are coiled, irregularly twisted, or fully extended in the cells, depending largely on the age when death occurs. Usually the number of larvae and pupae that die in sealed cells is somewhat greater and the number of larvae that die while coiled is less than is the case in European foulbrood.

Larvae that die in open cells dry rapidly and usually form light-colored scales, although some become light brown, reddish brown, or dark brown. Larvae that die in sealed cells dry more slowly, and decay continues for a longer time. Many of these become reddish brown during decay and form dark-colored scales. In an occasional decayed larva or scale the tracheae show clearly. In sick or recently dead larvae the stomach can be seen through the skin along the back. The content of the stomach consists of a turbid grayish or yellow-gray fluid that contains many bacteria.

CONSISTENCY OF DEAD BROOD

Dead larvae soon become soft and watery. In capped cells some become decidedly ropy during decay and form dark reddish-brown or brown scales of a leathery consistency. In open cells the larvae usually become pasty and later form light-colored brittle scales. In some dead larvae ropiness develops rapidly, while in others it develops slowly or is entirely absent. Ropiness in parafoulbrood often resembles this symptom in American foulbrood. When this occurs a distinction can usually be made by noting the color and odor of the dead brood.

The scales can be removed easily from the cells.

ODOR OF DEAD BROOD

Only a slight odor can be detected in recently dead brood, and most larvae have but slight odor during decay. Many dead larvae in sealed cells and also some in open cells, however, develop an intense putrid odor similar to that of European foulbrood but fre-

quently much more intense. It can sometimes be detected as soon as a decayed larva is removed from the comb, and can also be detected in the dry scales.

A reliable symptom of this disease is a reddish-brown color and ropy consistency of decayed brood, particularly when accompanied by a pronounced putrid odor.

SACBROOD

Sacbrood is caused by a filterable virus, an organism so small that it will pass through a porcelain filter and cannot be seen under the most powerful microscope. Infection in the case of sacbrood takes place by way of the alimentary canal. Both worker and drone brood may be affected. It has not been definitely determined

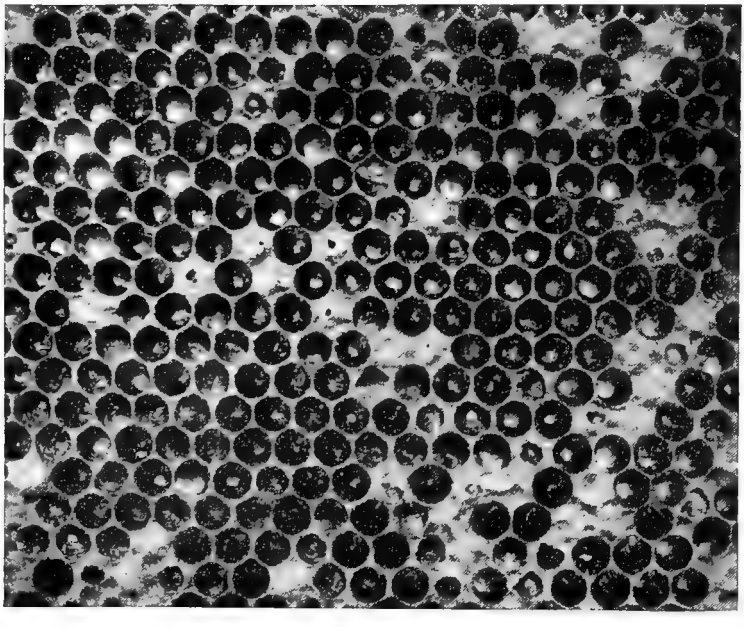

FIGURE 12.—A brood comb heavily infected with sacbrood, showing numerous dead larvae.

whether or not queen larvae are killed. Pupae are killed occasionally, but adult bees are not affected.

IMPORTANCE

Sacbrood is a widely distributed disease, but it usually does not cause serious losses. It is important, however, for beekeepers to recognize sacbrood so that it will not be confused with the foulbrood diseases.

Sacbrood may appear at any time during the brood-rearing season, but it is most common during the first half of the season, and practically always subsides after the main honey flow has started. In ordinary cases the colonies are not noticeably weakened by sacbrood, but in exceptional cases, when 50 percent or more of the brood is affected, they may be considerably weakened.

SYMPTOMS

APPEARANCE OF THE COMBS

In colonies with sacbrood the brood is slightly irregular. Scattered here and there among the healthy brood are cells containing dead brood (fig. 12). The cappings over dead brood are first punctured and later removed by the bees. The holes vary in size, and occasionally there is more than one. Sometimes the size and uniform shape of the hole indicate that the cell has never been completely capped. Dead larvae usually lie fully extended on the floor of the cell (fig. 13, *B–F*), showing the dark-brown heads through the openings (fig. 12). When these conditions are present the dead larvae should be studied carefully.

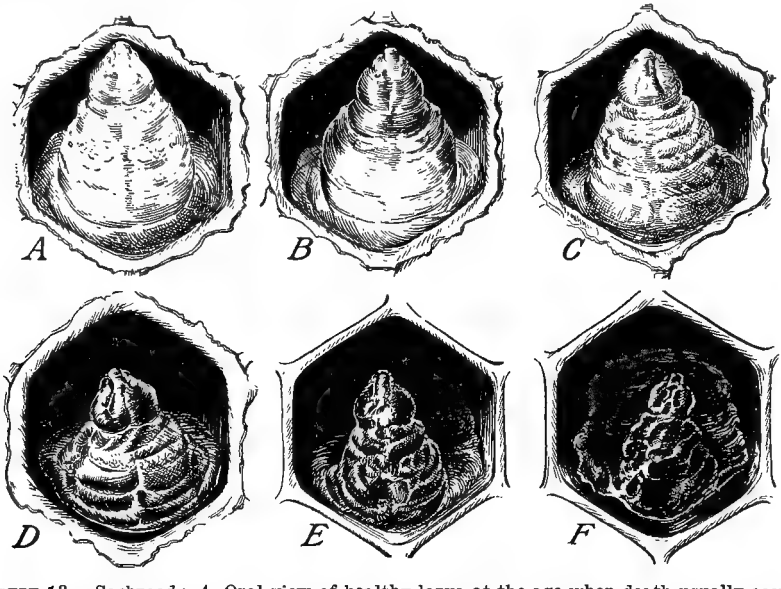

FIGURE 13.—Sacbrood: *A*, Oral view of healthy larva at the age when death usually occurs from sacbrood; *B–F*, stages in decay and drying of larvae dead of sacbrood.

AGE OF AFFECTED LARVAE

Death from sacbrood almost always occurs after the cell is capped and the larva has spun its cocoon and is motionless. At this stage the larva is fully extended on the floor of the cell. In heavily infected colonies a few coiled larvae may be killed.

COLOR AND ODOR OF THE DEAD BROOD

Shortly after death caused by sacbrood the color of the larva changes from the pearly white to a slightly yellowish color. This gradually becomes darker, beginning with the head and front third of the larva, which soon changes to a brown or grayish brown and later a dark brown. Scales are almost black for the entire length, the head end usually being darkest.

There is little, if any, distinctive odor associated with sacbrood, although watery, saclike larvae in the later stages may have a slightly sour odor.

CONSISTENCY OF DEAD BROOD

The skins of dead larvae remain tough, and are easily removed from the cells intact. The internal tissues at the same time become watery, but rarely show any indication of ropiness. Suspended in the waterlike liquid are numerous fine brown granules. When a dead larva is removed from the cell, liquid collects beneath the skin, which resembles a sac; hence the name sacbrood. As the larva dries, the skin becomes wrinkled, usually most noticeable in the front third (fig. 13, *C–F*). After thorough drying it forms a scale.

POSITION OF THE DEAD BROOD IN THE CELLS

Larvae killed by sacbrood almost invariably lie extended lengthwise with their backs on the floor of the cells (fig. 14, *G*). In contrast with American foulbrood (fig. 5, *G*), the head and front third of a larva dead of sacbrood is elevated while the tail end, as drying progresses, slumps partly down off the bottom of the cell. The raised head is a distinctive symptom of sacbrood. Since adult bees often remove recently dead larvae by biting off a piece at a time, occasional cells will be found in which only part of the dead larva remains.

THE SCALES

Scales of larvae dead from sacbrood can be removed from the cells with ease. They are dark grayish brown, or nearly black, and are hard and brittle with the head end turned sharply upward. The outline may be somewhat wavy. The back or lower surface is smooth and polished, while the upper surface is rough and somewhat concave. The lower surface takes the form of the cell walls and gives the entire scale a boatlike appearance often referred to as gondola-shaped or like a Chinese shoe.

INFECTION WITH TWO OR MORE BROOD DISEASES

In localities where two or more brood diseases are prevalent, more than one brood disease will occasionally be found in the same colony or even in the same comb. So far as is known a single larva is never affected by more than one disease. When American foulbrood is found in the same comb with European foulbrood or sacbrood, usually one of the diseases will be more prominent, at least in the active stages, which may cause the mixed infection to be overlooked, the beekeeper seeing only the most prominent symptoms. In cases where there is doubt or a suspicion that more than one disease may be present in the same colony, a laboratory diagnosis is desirable to prevent improper treatment. *Since American foulbrood is the most serious, a careful search for this disease should always be made even when another disease is known to be present.*

Table 1 gives in summary form the characters differentiating the principal brood diseases.

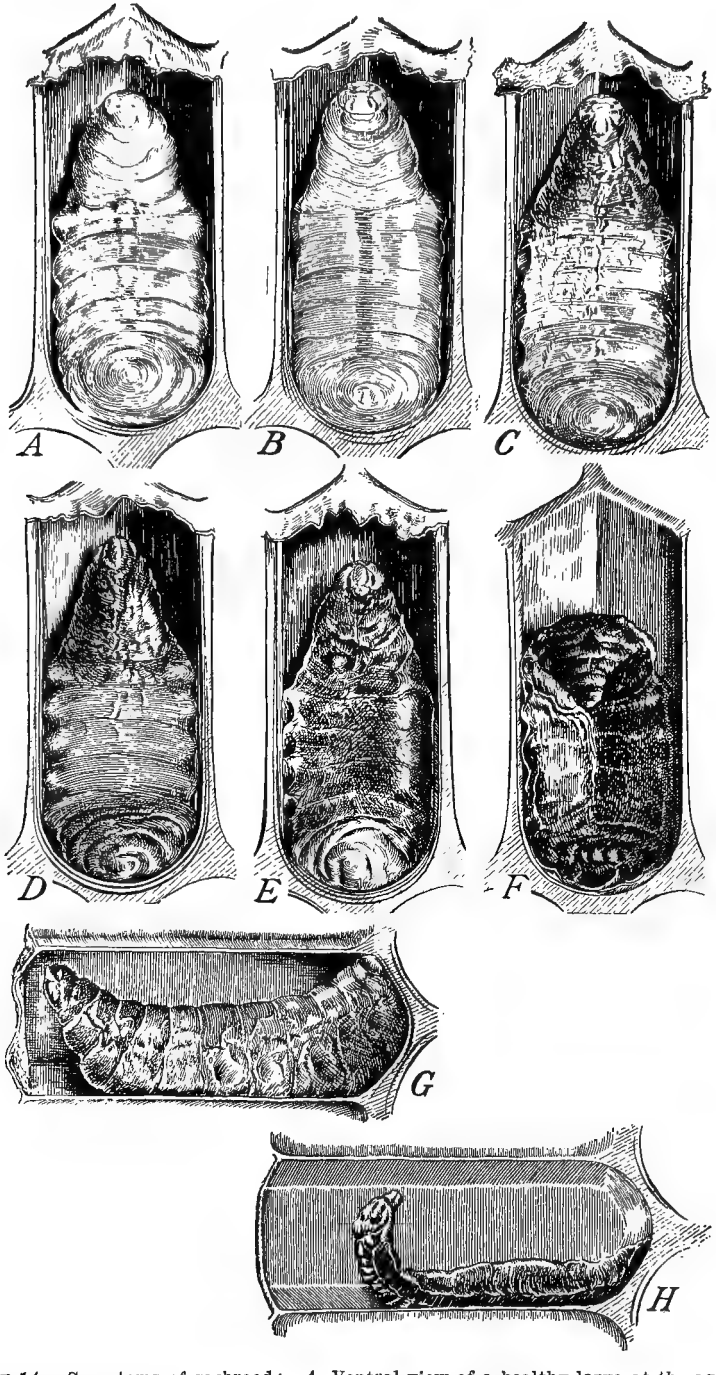

FIGURE 14.—Symptoms of sacbrood: *A*, Ventral view of a healthy larva at the age when death usually occurs from sacbrood; *B–F*, stages in the decay and drying of larvae dead of sacbrood, ventral views; *G*, lateral view of larva recently dead of sacbrood; *H*, lateral view of scale.

TABLE 1.—*Differentiating characters in the diagnosis of brood diseases*

Characters to observe	American foulbrood	European foulbrood	Parafoulbrood	Sacbrood
General appearance of brood combs.	Brood irregular; intermingling of capped, open, and punctured cells; much dead brood in capped cells, cells with punctured cappings, and cells uncapped by the bees.	Brood irregular; dead brood mostly in open cells.	Brood irregular; most of dead larvae in open cells; varying amount of dead brood in sealed cells.	Brood slightly irregular; dead brood mostly in cells with punctured cappings or in uncapped cells.
Appearance of cappings over dead brood.	Many punctured, sunken, and discolored.	Few cappings sunken, punctured, or discolored.	Cappings over dead brood punctured, discolored, sunken, or thickened and sharply depressed in the center.	Usually punctured.
Proportion of brood dead.	Varying from 1 or a few to 75 percent or more.	Varying from a few coiled larvae to most of the larvae in open cells, also a few larvae in capped cells.	Varying from few larvae to practically all the brood.	Small amount of brood dead; in severe cases, 50 percent or more.
Age at time of death.	Late larval and early pupal stage; rarely coiled stage.	Coiled stage; occasionally late larval stage.	Coiled larval stage; occasionally late larval and early pupal stage.	Late larval stage; occasionally coiled larval or pupal stage.
Position of dead brood.	Fully extended on floor of cell; tail turned up on bottom; head lying flat; great regularity.	Coiled on bottom or twisted on side walls; few larvae fully extended on floor of cell; very irregular.	Coiled on bottom, twisted on side walls, or fully extended on the floor of the cell; great irregularity.	Fully extended on floor; heads prominently raised; great regularity.
Color of dead brood.	At first dull white; then light brown; later coffee brown, dark brown, or almost black.	At first dull white, grayish white, or yellowish white; often becoming brown, dark brown, or nearly black.	At first dull white or grayish white, becoming light brown, brown, reddish brown, or dark brown.	Grayish to straw-colored, becoming brown, grayish black or black; head end usually darker.
Kind of brood affected.	Mostly worker; occasionally drone; rarely queen.	Worker, drone, and queen	Worker, drone, and queen	Usually only worker but sometimes drone.
Consistency of dead brood.	At first watery or slightly viscid, becoming ropy; finally brittle.	At first soft and watery; afterwards pasty, rarely viscid and ropy; scales tough, rubbery, or brittle.	At first soft and watery; in open cells becoming pasty and brittle; in capped cells frequently becoming ropy, finally tough or leathery.	Skin fairly tough; contents watery and granular; scales tough, brittle when completely dry.
Scales	Uniformly extended on lower side wall; tail curved up; dead pupae with tongue extended upward, often attached to upper cell wall; difficult to remove from cells.	Usually coiled on bottom of cells; often irregularly twisted; sometimes fully extended; tracheae often clearly visible; tough and rubbery; easily removed from cells.	Coiled on bottom, irregularly twisted on side walls, or fully extended in the cell; tracheae sometimes visible; easily removed from cells.	Uniformly extended on lower side wall; head prominently raised; outline wavy; grayish brown to nearly black; head darker; easily removed from cells.
Odor	No odor at first; distinctive odor different from gluepot odor in early stage of decay; gluepot odor in brown ropy remains and scales; odors constant.	Usually no specific odor in dead, coiled larvae; sour odor sometimes present in partly decayed remains; decayed-meat odor often present in decaying brood in sealed cells, finally penetrating and acidlike; odors variable.	Variable, resembling odor in European foulbrood but much more intense in ropy remains.	Absent or slightly sour.

FUNGOUS DISEASES OF BROOD

CAUSES

In addition to the diseases previously described, diseases of the brood of bees are caused by several different fungi. The most common of these are species of *Aspergillus*. In this country *A. flavus* attacks brood more frequently than other fungi. In Europe a fungus known as *Pericystis apis* causes a disease of brood known as "chalk brood." This fungus does not occur in North America.

IMPORTANCE

Normally only slight losses of brood are caused by fungous diseases. The small amount of brood that is killed is removed promptly by the worker bees and is rarely noticed by the beekeeper. Brood is most likely to become infected when moisture collects in the hive late in the winter and early in the spring, permitting fungi to grow over the combs.

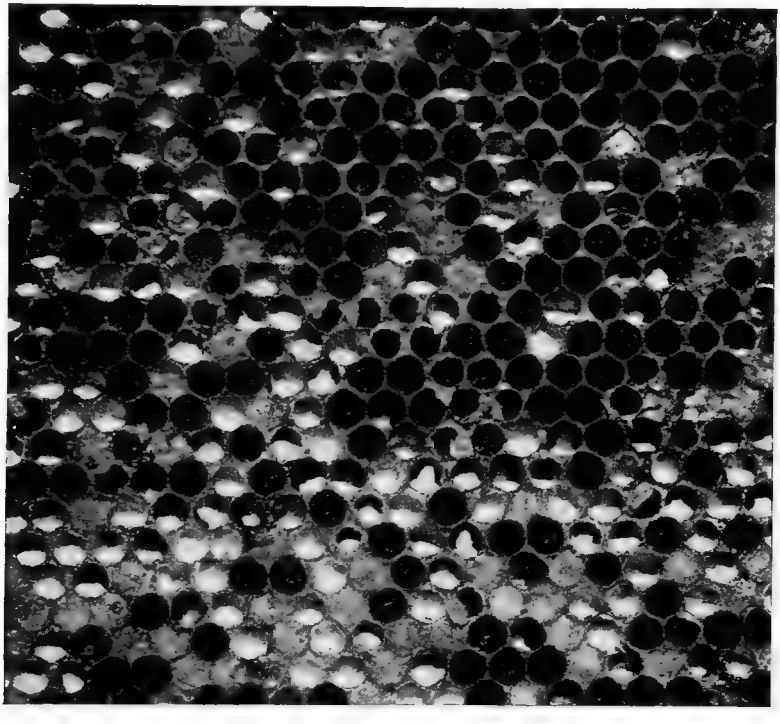

FIGURE 15.—Brood comb artificially inoculated with *Aspergillus flavus*, a fungus that kills the brood of bees. Dead larvae of different ages can be seen in the cells.

AGE OF BROOD AND RACES OF BEES AFFECTED

Brood of all ages, and also adult bees, are susceptible to fungous diseases. After the feeding period is passed, however, and the cells have been capped, brood is less likely to become infected. All the races of bees common in this country are susceptible.

APPEARANCE OF THE DEAD BROOD

A larva killed by a fungus becomes noticeably harder soon after it dies, and the glistening white changes to a dull creamy white. Later the dead larva becomes shrunken and wrinkled. The head end of a larva that dies after it has straightened out in the cell dries most rapidly and often curves upward at first but later tends to straighten out again (fig. 15). The fungus soon grows through the skin in a ring just back of the head and forms a sort of white collar. Within 1 or 2 days the fungus grows over the entire larva and forms a false skin which clings closely to the true skin. The color at this stage is chalky white. The fungus produces spores on the outer surface of the dead larva, and the white changes to a shade of green, black, or other color, corresponding to the color of the spores. Spores form earliest and most abundantly near the head end of

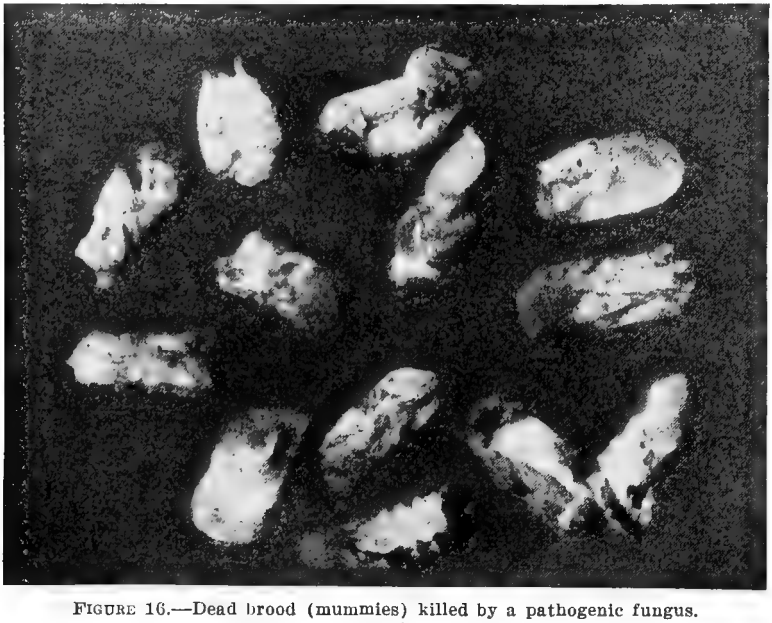

FIGURE 16.—Dead brood (mummies) killed by a pathogenic fungus.

dead larvae. The color of the spores deepens as they mature and fades as they become old and dry. After dead larvae and pupae have become dry they are known as mummies (fig. 16). In Europe the disease of bees caused by *Aspergillus flavus* is called "stone brood" on account of the hard texture of the dead brood.

DISEASES OF ADULT BEES

WHAT TO OBSERVE WHEN LOOKING FOR DISEASES OF ADULT BEES

No general rules can be given for the diagnosis of diseases of adult bees. Such diagnosis is made more difficult by the fact that at any time of the year many bees may die as a result of old age or abnormal conditions. Symptoms of the different diseases over-

lap, and usually a diagnosis cannot be made in the apiary. There are a few dependable symptoms of diseases of adult bees, however, which can be recognized without a miscroscope, and with good samples it is sometimes possible to make a diagnosis in the apiary.

NOSEMA DISEASE

CAUSE

Nosema disease is caused by a minute, single-celled animal parasite known as *Nosema apis*. Adult workers, drones, and queens are affected. Spores of *N. apis* enter the body of the adult bee with food or water. They germinate within the stomach and attack the tissues which line the stomach or mid-intestine, with varying harmful effects.

IMPORTANCE

Nosema disease is wide-spread and under conditions favorable for its spread causes extensive losses of adult bees. When accompanied by dysentery brought on by long winter confinement, the disease may spread rapidly within infected colonies and result in the death of the colonies late in the winter or in the spring; or heavy losses from Nosema disease may continue for weeks after the bees have been flying freely and dysentery has subsided. Infected bees usually perform their normal duties until they are too weak to continue. The shortened life of infected bees weakens or kills the colony.

SYMPTOMS SHOWN BY THE COLONY

The first noticeable symptoms shown by a colony heavily infected by *Nosema apis* are increasing restlessness of the bees and a weakening of the colony. When only a small number of bees are infected, the loss may be so gradual that it is not noticed. At other times the death rate among adult bees is very high, and the colony dwindles rapidly. The queen usually is among the last handful of bees to die. Nosema disease may appear annually at about the same time. During any time of year, however, colonies with bees infected by *N. apis* may be found that show no noticeable loss.

SYMPTOMS SHOWN BY INFECTED BEES

In the individual bee the symptom most commonly observed is inability to fly more than a few yards without alighting. Many bees will be seen crawling on the ground, on the bottom board, at the entrance, and on the top of frames when the cover is removed. Sometimes infected bees crawl actively long distances from the hive, or they may crawl up blades of grass in an effort to fly. At times they collect in small groups on the ground in front of the hive.

It is mostly the older workers that are killed, although drones, queens, and young workers may be attacked. At times the disease seems to be aggravated by periods of cold, damp weather, particularly in the spring when the bees cannot fly freely.

The legs of affected bees may be dragged along in crawling, as if paralyzed; and the rear wings may be unhooked from the front

wings and held at abnormal angles. Such bees are capable of only feeble fanning with the wings. The abdomen is often distended with feces and may appear shining or greasy.

APPEARANCE OF THE INTESTINAL TRACT

The intestinal tract of bees infected by *Nosema apis* is frequently swollen and discolored. When favorable specimens are at hand this symptom can be used for diagnosis in the apiary. If the bees are alive, or have just died, the entire intestinal tract can be removed as follows: Remove the head and hold the thorax with the thumb and forefinger, then grasp the tip of the abdomen with a pair of forceps and pull gently. By this procedure the entire intestinal tract can frequently be withdrawn from the abdomen.

In healthy bees the long, cylindrical mid-intestine is usually of a brownish-red, yellowish, or grayish-white color. Circular constrictions show for nearly the entire length of the intestine (fig. 17, *A*), and the tissues are fairly tough and of a healthy appearance. When Nosema disease is present the mid-intestine swells (fig. 17, *B*) but finally shrinks to about normal size. Heavily infected intestines are usually of a dull grayish white, and some or all of the circular constrictions disappear (fig. 17, *B*). The tissues become soft and watery and are more easily crushed than are the tissues of healthy intestines. The fluid that flows from heavily infected intestines when they are crushed is whiter and more turbid than is the fluid from healthy intestines. After experience has been gained, it is often possible, when favorable specimens can be obtained, to make a diagnosis of Nosema disease in the apiary. There is considerable variation in the appearance of the mid-intestine of healthy as well as infected bees, however, and in many cases, particularly after the bees are dead, a microscopical examination is necessary for a diagnosis of Nosema disease.

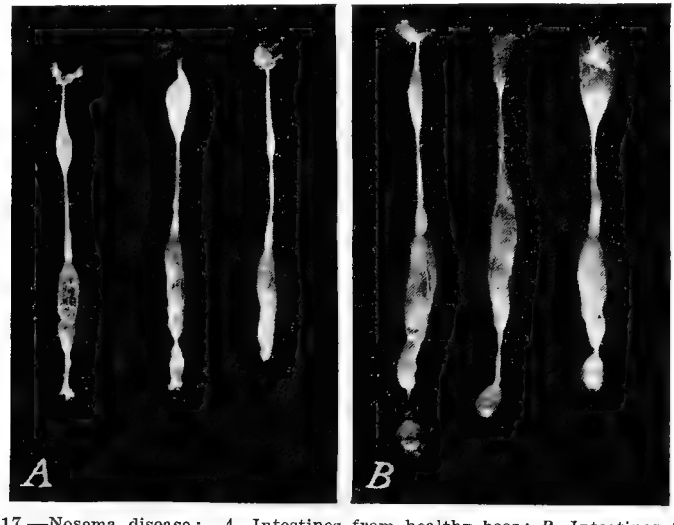

FIGURE 17.—Nosema disease: *A*, Intestines from healthy bees; *B*, Intestines from bees infected with Nosema disease.

ACARINE DISEASE

CAUSE

Acarine disease of adult honeybees is caused by a very small mite, *Acarapis woodi* Rennie. This mite lives as a parasite in the anterior thoracic tracheae (breathing organs), where it feeds directly upon the tissues of the bees. Bees are not noticeably injured by one or a few mites, but the mites breed and multiply within the trachea until they become very numerous. Heavily infested bees are unable to fly and soon die.

DISTRIBUTION

This disease of adult bees is not present in North America, but serious losses occur from it in Europe. Queens imported from Europe are sent directly upon arrival in the United States to the Government Bee Culture Laboratory at Washington. The attendant bees are examined for mites and other bee diseases. The imported queens are placed in new cages with young worker bees from the Government apiary before they are sent to the beekeeper who purchased them, in accordance with an act of Congress of 1922.[1] The rules and regulations and special rules incident to this act can be obtained by writing to the Bee Culture Laboratory, Bureau of Entomology and Plant Quarantine, National Agricultural Research Center, Beltsville, Md.

TRANSMISSION

The mites enter the tracheae at their openings (spiracles). When a few bees, or even one, of a colony become infested with fertile female mites, acarine disease may be transmitted to other bees within the colony. The mites mate within the tracheae, and later some of the females crawl out and enter the tracheae of other bees within the hive, thereby transmitting the disease. Acarine disease is thought to be transmitted from diseased to healthy colonies by the drifting of infested workers, or drones, or by robber bees. This disease may also be transmitted by requeening a colony with an infested queen.

SYMPTOMS

Infested bees are unable to breathe normally, and the walls of the tracheae and other tissues are injured. Bees that contain large numbers of mites are unable to fly and are known as crawlers. Crawlers usually leave the hive, when the weather is favorable, and

[1] The act of Aug. 31, 1922 (Public, No. 293—67th Cong.), entitled "An Act To regulate foreign commerce in the importation into the United States of the adult honeybee (Apis mellifica)," provides as follows:

"* * * That, in order to prevent the introduction and spread of diseases dangerous to the adult honeybee, the importation into the United States of the honeybee (Apis mellifica) in its adult stage is hereby prohibited, and all adult honeybees offered for import into the United States shall be destroyed if not immediately exported: *Provided,* That such adult honeybees may be imported into the United States for experimental or scientific purposes by the United States Department of Agriculture: *And provided further,* That such adult honeybees may be imported into the United States from countries in which the Secretary of Agriculture shall determine that no diseases dangerous to adult honeybees exist, under rules and regulations prescribed by the Secretary of the Treasury and the Secretary of Agriculture.

"Sec. 2. That any person who shall violate any of the provisions of this Act shall be deemed guilty of a misdemeanor and shall, upon conviction thereof, be punished by a fine not exceeding $500 or by imprisonment not exceeding one year, or both such fine and imprisonment in the discretion of the court."

die outside. When large numbers of infested bees crawl from the hive at about the same time, the condition is known as mass crawling.

Bees often continue to work for weeks after they have become infested by mites, and acarine disease may be well advanced in a colony before symptoms are noticeable. The most commonly recognized symptoms are crawling and the loss of ability to fly. Crawling may come on gradually when the disease spreads slowly within the colony, or it may develop rapidly and result in mass crawling. After mass crawling has occurred, the colony is freed of most of the diseased bees and may appear to recover temporarily. Mass crawling often follows a period of unfavorable weather. Crawling is frequently accompanied by retention of feces, swollen abdomens, and unjointed wings.

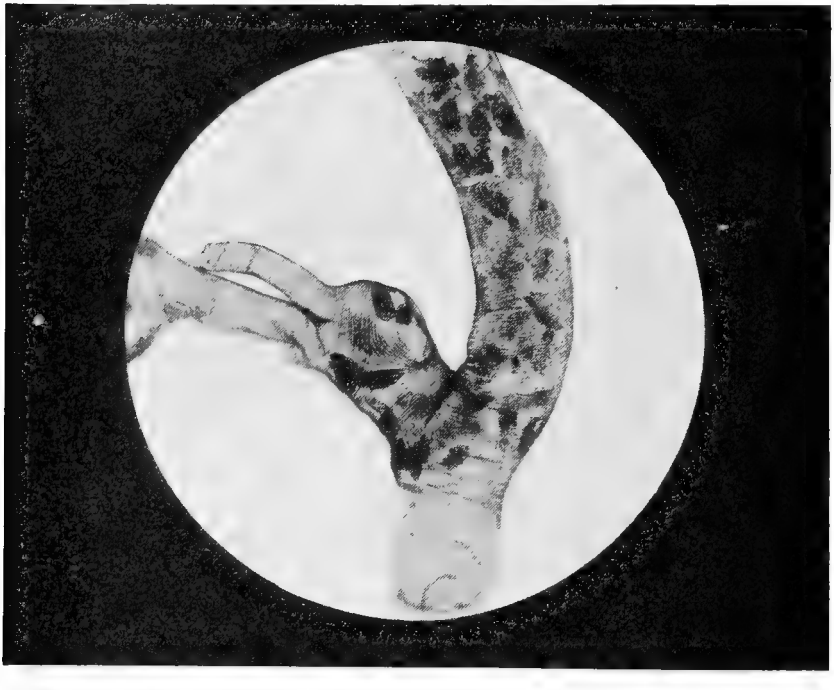

FIGURE 18.—Acarine disease. Discolored trachea taken from the thorax of an infested bee. The mites that cause acarine disease can be seen through the tracheal wall. Magnified 75 times. (Photograph by J. Rennie.)

DIAGNOSIS IN THE APIARY

In healthy bees the tracheae are always pure white. In heavily infested bees the tracheae become bronzed or blackened in irregular spots. The presence of these spots is used as a symptom in diagnosis. With the aid of a lens that magnifies 6 or 8 times, the dark-colored spots can be distinguished. They may be few in number, or there may be so many that the trachea appears black (fig. 18).

In making examinations for acarine disease in the apiary it is best to use crawlers. The tracheae of bees killed by other disorders often

become black after a few days, while infested bees that are able to fly may not show the discolored spots on the tracheae. The head and front part of the thorax (prothorax) with the first pair of legs should be cut away and discarded. This will bring into view the first pair of breathing tubes, which are the ones most likely to be discolored if acarine disease is present.

SEPTICEMIA

CAUSE

Septicemia is a slightly infectious disease of adult honeybees. It is caused by growth in the blood of infected bees of a bacterium known as *Bacillus apisepticus*. This bacterium may be present in colonies, in the soil near infected colonies, or in water that has been in contact with bees killed by septicemia. Bees that become wet

FIGURE 19.—Septicemia. Bees dead of septicemia dismembered by slight handling.

with soil water from about the hives may become infected. This is probably the most common way by which the disease spreads. The bacteria seem to enter the blood of bees by way of the breathing tubes. The presence of large numbers of bacteria in the food seems not to injure bees; but if a drop of water that contains the bacteria is spread over the entrance to the breathing organs (spiracles), disease and death from septicemia usually result. The disease does not spread readily from sick or dead bees to healthy ones unless plenty of moisture is present. The bacteria are soon killed by drying, and the disease rarely occurs under dry conditions. Several other species of bacteria and yeasts cause septicemia when they are placed in the blood of bees by puncturing the body covering, but they seem to be unable to gain entrance to the blood of uninjured bees.

SYMPTOMS

Bees die within a few hours after they have shown the first symptoms of septicemia. Sick bees leave the hive or are carried out by healthy workers. Sick bees resemble bees that are chilled, and their movements gradually become slower. Before death, the blood loses the normal clear, pale-brown color and becomes turbid and milky, owing to the presence of many bacteria. This symptom can sometimes be used in diagnosis in the apiary. By pulling off the head and abdomen of a dying or recently dead bee and pinching the thorax between the fingers a drop of blood oozes out that can be examined. Dead bees decay rapidly, the muscles of the thorax soon becoming soft and pasty, and the bodies have a characteristic putrid odor that is of some assistance in the diagnosis of this disease. Within 1 or 2 days the body, legs, wings, and antennae usually fall apart at the joints when the bees are handled (fig. 19).

EFFECT UPON THE COLONY

Only rarely are colonies noticeably weakened by septicemia, but many individual bees may be killed. Septicemia is less serious than Nosema disease or acarine disease.

AMOEBA DISEASE

CAUSE

Amoeba disease of bees, caused by a one-celled animal parasite, *Vahlkampfia* (*Malpighamoeba*) *mellifica*, was discovered a few years ago in Europe. This parasite grows in the excretory organs of adult bees. In 1927 it was found in two colonies of bees in the apiary of the Bee Culture Laboratory at Somerset, Md. In 1929 it was recognized in a sample of bees sent to this laboratory from California and in 1935 in a sample from Illinois. In Europe, amoeba disease was found only in colonies with Nosema disease, and it was suspected that the two diseases were in some way related. Nosema disease was not found, however, in one of the two colonies with amoeba disease in the apiary of the Bee Culture Laboratory, nor in the sample from California.

IMPORTANCE

Very little is known about the disease, but the infected bees are undoubtedly injured. Its economic importance is probably negligible, although this point has not been definitely determined.

SYMPTOMS

The disease cannot be recognized in the apiary by any symptoms. Dead bees that contain the parasites do not differ in appearance from bees dead of other causes. Field bees that appear entirely normal may also be infected. The parasites are found, often in large numbers, in the excretory organs (malpighian tubules) of the bees.

FUNGOUS DISEASES OF ADULT BEES

CAUSES

It has been known for many years that in Europe a disease of adult honeybees is caused by a common fungus, *Aspergillus flavus*. In North America it has recently been found that this same fungus and several others attack adult bees. *A. flavus* has already been noted as attacking brood (p. 23). When recently emerged bees are kept at a temperature about 12° or 14° below that of the brood nest they may be attacked and killed by *Mucor hiemalis*, a fungus closely related to the common black bread mold. Old bees are not affected by this fungus.

Spores of pathogenic fungi get into the digestive tract of bees with food or with water. If a bee comes in contact with fungus spores, some of them may cling to the mouth parts and be swallowed later. Nonpathogenic fungi are unable to grow within the stomach of bees, and the fungi themselves may be killed. Pathogenic fungi, on the other hand, grow readily. At first the fungus grows within the stomach, but later the muscles and other soft tissues are penetrated by numerous fungus branches, and death results. When dead bees are kept under moist conditions, the fungus may grow through the body wall and form spores on the outer surface (fig. 20).

FIGURE 20.—Adult workers and a drone bee killed by *Aspergillus flavus*. Spores of the fungus are seen on the bodies of the bees.

IMPORTANCE

Losses of adult bees caused by fungi are usually of little economic importance. When pathogenic fungi grow within the hive on combs, frames, dead bees, etc., late in the winter or early in spring, fungous diseases are most likely to cause significant losses. This can be

largely prevented, however, by providing good wintering conditions for the bees.

SYMPTOMS

The first noticeable symptoms are restlessness and weakness. Weakness increases until death occurs. A few sick bees may die in or near the hive, but they usually fly or crawl from the hive and seem intent upon getting as far away as possible before they die. For this reason mycosis of adult bees is likely to be overlooked, particularly when only a few bees at a time are affected.

By pressing the abdomen between the fingers, an increased firmness can sometimes be noticed at the time of death, but it is most noticeable a few hours later. It is unsafe to depend upon this symptom longer than about 1 or 2 days after death, since nonpathogenic fungi may produce similar symptoms in bees killed by other disorders.

"PARALYSIS"

CAUSE

The so-called paralysis of adult honeybees appears to be a slightly infectious disease that causes weakness, trembling, and death of the affected bees. The cause of paralysis has not been definitely determined, although various theories regarding the cause have been advanced from time to time. Recent work at the Government Bee Culture Laboratory indicates that paralysis is infectious.

IMPORTANCE

Paralysis of honeybees is a widely distributed disorder, but it causes greater losses in warm than in cold climates. Affected colonies usually recover after a short time, but in some cases the disorder continues throughout the season. In the Northern States it usually disappears or remains confined to one or a few colonies within an apiary, but in the South it sometimes spreads and causes considerable loss. The losses range from a few bees in mild cases to most of the bees of the affected colonies in malignant cases.

SYMPTOMS

Owing to the fact that the appearance of the sick and dead bees is not always the same, there seems to be a difference of opinion regarding the symptoms of paralysis. Other disturbances of adult bees may also have been mistaken for paralysis.

During the early stages of paralysis, affected bees remain on the combs and cannot be distinguished readily, except that the healthy bees often tug and pull at them excitedly. The sick bees make but little effort to defend themselves. Sometimes they offer food or attempt to escape by crawling away. Finally, they leave the hive and die outside or crawl into a corner of the hive or onto the top bars, where they remain until death occurs or until they are carried out of the hive by the healthy bees. Some affected bees die within a day or two after the symptoms have become noticeable, others linger for more than a week, while still others recover. The abdo-

mens of the sick bees are usually of normal size but often appear swollen or, less frequently, shrunken. Some of the sick bees retain their hairs until they die, whereas others become partially or entirely hairless, probably because their hairs are pulled out by healthy bees. Loss of hairs is accompanied by a darkening of the abdomen and thorax and a shiny or greasy appearance.

The most characteristic symptom of paralysis is weakness and a trembling or shaking movement of the body and wings, frequently accompanied by hairlessness and sprawled legs and wings. Sick bees that are motionless will sometimes show the trembling movements when disturbed. Some of the symptoms given here for paralysis are also present in other disorders of adult bees and cannot be depended upon alone for diagnosis. Trembling, weakness, and hairlessness, particularly when accompanied by dark, shining abdomens and sprawled legs and wings, seem to be the most dependable symptoms of paralysis. Bees in this condition tend to collect on top of the frames. A diagnosis can sometimes be made by carefully opening the hive, disturbing the colony as little as possible, and examining the bees on the top bars of the brood nest.

TRANSMISSION

It has been found by beekeepers that combs of brood from colonies with paralysis can be given to healthy colonies without spreading the disturbance and that the bees emerging from these combs remain healthy. In experiments at the Bee Culture Laboratory, combs of honey and pollen from affected colonies were placed in a healthy colony without paralysis being transmitted. When all the combs of an affected Italian colony were replaced with combs of brood from a healthy Caucasian colony, paralysis appeared among the young Caucasian bees within 2 weeks after the first of them emerged. Paralysis appeared to be transmitted when sick bees and young healthy ones were confined in the same cage. When young healthy bees were wet with water containing the macerated remains of affected bees, paralysis also appeared to be transmitted. The results of these experiments seem to indicate that paralysis is slightly infectious and spreads directly from sick or dead bees to healthy ones.

SENDING SAMPLES FOR LABORATORY EXAMINATION

If only a small amount of brood or a few bees are affected, or the symptoms are unusual, it is sometimes difficult to make a definite diagnosis in the apiary. Examination by laboratory methods is then necessary. It is also desirable at times to have diagnoses made in the apiary verified in the laboratory.

HOW TO PREPARE SAMPLES OF BROOD

In sending samples for laboratory examination, the following instructions should be followed: (1) Cut a sample of comb at least 4 by 5 inches in size. (2) Be sure that the sample contains as much of the dead or discolored brood as possible. (3) *No honey should be present*, and the comb should not be crushed. (4) Mail the

sample in a wooden (fig. 21) or strong cardboard box. *Do not use tin, glass, or waxed paper.*

Smears of dead brood and small crushed pieces of comb are frequently unsatisfactory for diagnosis but will be examined in case the foregoing instructions cannot be followed.

HOW TO SEND SAMPLES OF ADULT BEES

(1) Select, if possible, bees that are sick or recently dead; bees that have been dead for some time are not satisfactory for examination. (2) Send at least 50 bees in a sample; if poisoning by arsenicals is suspected, 200 or more bees will be needed for analysis. (3) Send bees in a wooden or strong cardboard box and *not in tin or glass.*

FIGURE 21.—How to send a sample of brood for laboratory examination.

HOW TO SEND SAMPLES OF TREATED COMB

(1) Send a sample not less than 4 by 5 inches in size if infection is heavy, or an entire brood comb if infection is slight. (2) Brood remains should be present in abundance. (3) Pack the comb in a clean wooden box as soon after treatment as possible. (4) *Do not send samples that contain honey.*

HOW TO ADDRESS SAMPLES

All samples should be addressed to the BEE CULTURE LABORATORY, BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, NATIONAL AGRICULTURAL RESEARCH CENTER, BELTSVILLE, MD.

Your name and address should be plainly written on the box. If the sample is forwarded by an inspector, his name and address should also appear on the box.

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THE OCCURRENCE OF DISEASES OF ADULT BEES, II.

E. F. PHILLIPS,
Apiculturist, Bureau of Entomology

CONTENTS.

INTRODUCTION.

IN VIEW of the wide interest of American beekeepers in the Isle of Wight disease and in the effort to prevent its introduction into the United States, it seems well to summarize the reports that have come to the attention of the Bureau of Entomology regarding its distribution throughout the world. In this paper the situation in Europe is chiefly discussed, partly because the disease is found there, but especially because importations of queenbees are desired from few, if any, countries other than those of continental Europe. There seems to have been no effort elsewhere to make such a compilation, yet this information must be needed by beekeepers of many countries. Under the law enacted by Congress in August, 1922, prohibiting importation of adult bees into the United States, the Secretary of Agriculture is authorized to lift the prohibition from countries in which it is determined that no disease dangerous to adult bees exists, and in the formulation of the regulations under this law information on the distribution of the Isle of Wight disease throughout the world is vitally important. It is also desirable to record the data obtained from the additional search for this disease made within the United States in 1922, since the first publication on this subject. Obviously no claim of originality is made for this

paper, since no cases of this disease are known to occur within the United States and the writer has not had opportunity to study it abroad. All quotations from writers in foreign languages are translations for which the present writer is responsible.

The writer would respectfully request the cooperation of beekeepers in the United States and in foreign countries in furnishing information regarding the Isle of Wight disease in any part of the world, based on the finding of the mite which causes it. American beekeepers are again urged to send for examination samples of all adult bees which show any abnormality. Beekeepers of other countries may *during the season of 1924* send such material to the Bee Culture Laboratory, Bureau of Entomology, Washington, D. C., U. S. A., from any country where provisions are not made for such investigations. For the benefit of foreign beekeepers not familiar with the work of the bureau, it may be stated that it is not the policy of the Bureau of Entomology to publish the names of those sending beekeeping materials for examination. There is no charge for these examinations. Correspondence is preferred in English, French, or German.

NATURE OF THE ISLE OF WIGHT DISEASE.

In a previous publication of the bureau (*46*),[1] in which the Isle of Wight disease was discussed, the nature of the disease was not described, since it was not anticipated at that time that the interest in this subject would be so great. It seems desirable now to summarize the observations on this disease and to correct certain errors that have appeared in American beekeeping literature concerning it, without any attempt to add any new facts concerning the disease. No adequate discussion of this subject has been available to American beekeepers generally, and many inquiries have come to the Bureau of Entomology concerning it.

NAME OF THE DISEASE.

The Isle of Wight disease, as it is commonly called, is also sometimes known in Great Britain as acarine disease, from the order name (Acarina) of the mite which causes it. The latter name was given to the disease by Rennie (*54*) and is preferred by him. In German these two names are translated as "Insel-Wight-Krankheit" and "Milbenkrankheit," while in French, in addition to the translations of the common names, acariose is sometimes used. The disease was first recognized in 1904 in the Isle of Wight, hence the usual common name, and in succeeding years was reported to have spread with great rapidity throughout Great Britain. It is probable, however, that it existed in England previous to its discovery on the Isle of Wight (*29*), and the Isle of Wight can not be considered as its place of origin. The name of "Isle of Wight disease" is used in this paper because it is better known to American beekeepers than "acarine disease." It is preferable, in the opinion of the writer, since it is not descriptive. Descriptive names for brood diseases have proved confusing and it was solely to avoid this confusion that the author proposed the present generally accepted names for the two serious brood diseases in 1906.

[1] Reference is made by number (italic) to "Literature cited," p. 27.

GENERIC NAME OF THE MITE.

Dr. John Rennie, of the University of Aberdeen, Scotland, with his associates, P. Bruce White and Miss Elsie J. Harvey, found in 1920 that this scourge is caused by a microscopically small parasitic mite,[2] which was named *Tarsonemus woodi* (*50*). In this paper it will be noted that in referring to reports from various authors the generic names Acarapis and Tarsonemus are both used for this mite. The name *Tarsonemus woodi* was given this mite by Rennie, and Tarsonemus is still accepted by him as the correct generic name (*17*). Hirst (*31*, *32*) believes that because of certain important structural modifications, probably attributable to its parasitic habit, a new genus, Acarapis, should be erected for it. It is coming to be rather generally accepted that Hirst is correct, and his standing as a specialist in this field entitles his opinion to great weight. The fact that the generic name Acarapis is composed of two appropriate words, "acarus" (a mite) and "apis" (the honeybee), makes it a fitting name for this parasitic species, but in taxonomic nomenclature fitness of the name argues neither for nor against its acceptance (*25*). The name *Acarapis woodi* is preferred by Dr. H. E. Ewing (*25*), of this bureau, specialist in mites, and by the writer (*48*).

An important paper on this subject by Vitzthum (*70*), the well-known mite specialist of Germany, has recently appeared, in which it is shown that the mite causing the Isle of Wight disease certainly does not belong to the genus Tarsonemus, as claimed by Rennie, who discovered it, but that it properly belongs to the new genus, Acarapis, erected by Hirst. Vitzthum states that while Acarapis is related to the genus Tarsonemus, in that both genera belong to the Heterostigmata, it is more nearly related to certain other genera containing mites parasitic on other insects. He points out that, in spite of the fact that Acarapis contains the only known species of mites which are internal parasites, the body form has not been materially modified by the parasitic habit. This taxonomic analysis by Vitzthum seems to settle conclusively the disputed question as to the correct generic name for this species.

ORIGIN OF THE PARASITIC HABIT.

Since the recognition of the Isle of Wight disease there has been much speculation among beekeepers as to how the parasite arose and when and by what means it assumed its parasitic habit. There is, of course, not the slightest reason to believe that this mite first invaded the thoraxes of bees on the Isle of Wight in 1904, or that its parasitic habit is a new one. From its specialized structure, one must conclude that the parasite has existed as such for untold centuries (*cf.* *50*). It is an interesting speculation whether this mite took on

[2] Mites do not belong to the class of insects (Hexapoda), but are members of the class of Arachnida, to which also belong spiders and scorpions. A prominent difference between the two classes is the presence of four pairs of legs in the adult arachnids, instead of three pairs as in insects. Mites (Acarina) are small animals some species of which are found in great abundance. They generally have a saclike, unsegmented body, usually fused with the cephalothorax, and the mouth parts form a beak. The abdomen and cephalothorax are, however, distinct and the abdomen is segmented in the Tarsonemidae, to which *Acarapis woodi* belongs. The larva on hatching from the egg usually has only three pairs of legs, but after molting has four pairs as in most adult mites. Most species are free-living, as predacious forms or scavengers, but some species are parasitic and cause certain plant diseases. Some animal diseases are properly attributed to this group. Certain species are found in human ailments, such as itch. *Acarapis woodi* seems to be the only described tarsonemid mite which is pathogenic on animals.

its present parasitic habit as an invader of colonial honeybees, or whether, as Bouvier (*14*) suggests, it was first a parasite of solitary bees. It has not so far been found in solitary bees. The only practical bearing which this question might have is that if it were actually found that the mite exists in solitary bees at this time, the control of the Isle of Wight disease might be much more difficult (*50*). Rennie reports (*50*) that he and his associates have examined a considerable number of other insect species but have found the tracheæ always clear of mites. The parasite may also have been a plant feeder, as some have suggested, but there seems to be no evidence of it (*50*), except that other tarsonemid mites have this habit. Migratory nymph stages of related species of tarsonemid mites have been found in tracheæ of other insects, but it has usually been assumed that this is a mere accidental and fatal situation for these mites, and that they have entered the spiracles after attaching themselves to the insects, as migratory nymphs of many mite species do. A modification of some such behavior may account for the acquisition of a parasitic and pathogenic habit for *Acarapis woodi*. The course of evolution of this mite is a question which appears to have little practical significance.

METHOD OF ATTACK.

Acarapis woodi apparently enters the body of the honeybee only through the first thoracic spiracles on either side of the thorax, these being larger openings than any other spiracles along the side of the body. Queens, workers, and drones seem equally susceptible to attack, and the mites may enter either one or both sides of the thorax. The structure of the parts of the bee concerned in this invasion has been studied by Snodgrass (*56*), so that it is useless to go into detail here. All developmental stages of the mite have now been found by Rennie (*50*) and Ewing (*24*), indicating that it is able to pass its entire life in this situation. No migratory nymph stage has been described for this species, such as is described for some related species. This species has not so far been found except within the thoracic tracheæ or as migrants from them, or on the outside of the bee, as described by Morgenthaler (*41*, *44*). It appears, therefore, that it is a highly specialized animal, both in structure and in habit, adapted to a parasitic life, and that the disease is strictly a contagious one. The word "infestation" should be used for this disease, not "infection."

The means by which these imprisoned mites feed is not wholly clear, but it is assumed, without doubt correctly, that they draw their nourishment from the blood of their host. They spot the tracheal trunks with feces and thus color the normally white walls brown or black in a characteristic fashion, so that their presence is easily detected on dissection with slight magnification. Efforts to make artificially controlled inoculations of these mites have not been especially successful (*30*), and there is some uncertainty as to the manner in which they pass from one bee to another and succeed in entering the thorax, but there can be little doubt that they do this merely by crawling out of one bee and attaching themselves to another, later to enter the spiracles. The adult and nymph stages of both sexes are capable of locomotion, but, according to Rennie (*52*), only the adult fertilized female mite migrates effectively.

Rennie (*52*) has repeatedly seen the female mites on the outside of infested bees, where they have apparently migrated after mating. The mites live but a short time away from their hosts or after the death of the bees, usually not more than 24 hours.

CAUSE OF SYMPTOMS.

The manner in which these parasites injure their hosts and cause the Isle of Wight disease is also not entirely clear (*59*, *34*). Inability to fly, so common in advanced cases of the Isle of Wight disease, may be produced by artificial stopping of the spiracles of the thorax (*59*), so that "crawlers," so frequently found in badly infested colonies, may result from mere mechanical shutting off of the air which passes into the tracheal trunks. Mites are often so numerous as to make the passage of air virtually impossible. The muscles of flight are located immediately adjacent to the tracheæ which are invaded, and an infinite number of small tracheal branches permeate this musculature (*56*), so that it may be assumed that the stoppage of these trunks would affect the aeration of the flight muscles, and possibly also that of the head, before any other portion of the bee's body would be affected by this mechanical stoppage. This would seem adequate explanation of the lack of power to fly so frequently seen in advanced stages of the Isle of Wight disease, but since the same symptom is seen in bees affected with other diseases of adult bees, this makes the mechanical explanation less certain. The so-called bee paralysis and Nosema disease, both found in the United States, are not caused by this mite and the tracheæ of bees suffering from either of these diseases are clean. In addition to the puncturing of the tracheal walls to suck the blood of the host and the stopping of air circulation, there is believed to be (*59*) some change in adjacent tissues. It is also assumed that these parasites may produce some material which has a toxic effect on the host, but this does not appear certain. The way in which the symptoms of this disease are produced seems to be a question of theoretical interest only, since, regardless of its method, this mite obviously causes the Isle of Wight disease, and therefore the control of the disease must rest on the elimination of the mites.

SYMPTOMS.

The symptom most commonly observed in a colony suffering from a severe attack of the Isle of Wight disease is that many bees crawl inactively on the ground in front of the hive, unable to fly. They are frequently seen falling from the alighting board to the ground or in cool weather gathered in little clusters on the ground in front of the hive. Such bees usually fail to return to the hive and die of hunger or exposure, probably usually of hunger, since active bees in summer live without food for only a few hours. The wings are sometimes carried in a position abnormal for walking bees, with the front and hind wings neither hooked together nor lying flat on the dorsal surface, often described as "dislocated." The badly infested bees in the spring are usually heavily laden with feces, probably because of their inability to fly during winter. Doubtless because of this accumulation of feces, attention was first given the alimentary tract as the probable location of the cause of the disease (*29*, *46*).

It should be recognized, however, that if for any reason bees are unable to fly during the period of the year when brood is being reared, feces rapidly accumulate, so that there is no necessary significance in their accumulation. The presence of "crawlers" seems to be the only fairly constant symptom observed in the severe cases; in fact, there are no positive symptoms and there may be no external sign of any sort, so that the only way to be sure of the presence of the disease at present is through the finding of mites by microscopic examination. The crawling bees are often found some distance from the hive. Rennie (*51*, *52*, *53*) emphasizes the fact that crawling is an advanced symptom and that the disease may exist in a colony or apiary for months before this symptom is seen.

The crawling of bees in front of the hive has also been recorded in almost all cases of the so-called bee paralysis observed by American beekeepers and for virtually all of the known affections of adult bees. Whether this indication of abnormality is any more prevalent for the Isle of Wight disease than for most cases of paralysis can not be stated with certainty, but to one familiar with severe cases of bee paralysis but not with the Isle of Wight disease it would seem doubtful. Rennie (*54*) reports the similarity of symptoms in the various diseases of adult bees in England. The shaking of the abdomen, sometimes so prominent a symptom in bee paralysis, and the hurried climbing of grass blades in an effort to take wing are not commonly given as symptoms of the Isle of Wight disease, although they have been recorded (*54*).

CONFUSION DUE TO CHARACTER OF SYMPTOMS.

Since almost the same symptoms have been described for all known diseases of adult bees, as well as for cases of arsenical poisoning, it must be concluded that information on the identification or distribution of any of these diseases based only on diagnosis by symptoms is utterly valueless. Some American beekeepers were formerly led from descriptions of symptoms to believe that the Isle of Wight disease is present in the United States, and more recently certain European beekeepers have arrived at the same conclusion. On this unfounded assumption they reason (*19*) that the methods of treatment for adult bee diseases used in some cases in the United States should be helpful in the treatment of the Isle of Wight disease. If then, as appears to have been the case, there is only a slight manifestation of the disease for a day or two after the treatment, they feel justified in rushing into print with this method as a recommended treatment for Isle of Wight disease. Rennie has repeatedly warned beekeepers against such reasoning, but apparently without avail. The treatments that have been described for bee paralysis in the United States were used without knowledge of its cause, and such treatments have been of doubtful benefit at best, so that it can not be recommended that European beekeepers look to this country for methods of controlling the Isle of Wight disease.

REMEDIES.

It is natural that in an epidemic such as has occurred in Great Britain a great variety of remedies should be tried and recommended. Extravagant claims have been made for certain apparently worthless

proprietary compounds and drugs, and unfortunately some of these have received the indorsement of certain prominent beekeepers, although Rennie has given repeated warnings against reliance on such unfounded claims. None of the claims made for chemical treatments can as yet be accepted as valid, and apparently no specific treatment has yet been found. Doctor Rennie has devised a trap for the catching of crawling bees as they leave the hive, but to what extent it is beneficial can not be determined from a reading of the literature. From the nature of the cause of the disease a hopeful line of attack has been suggested through some gas which will penetrate the tracheæ and kill the mites but leave the bees unharmed. Whether a bee is worth saving which has mites, dead or alive, in its tracheal trunks is questionable. The most promising method of attack seems to be the elimination of the infested bees and the rearing of a large force of young bees to take their places, thus reducing the infestation, as has been urged by both Rennie (*52*) and Morgenthaler (*44*). Rennie (*53*) emphasizes the necessity for swarm control measures, since swarms are especially prone to show crawlers soon after issuing. He says (p. 74):

> I lay special emphasis on rational and intelligent management, because I am convinced that much of the loss which has occurred in the past has given to the disease a gravity which is not inherent in it, and has been due to lack of exact knowledge on the part of competent beekeepers in the one case and to unsatisfactory beekeeping in addition in the other.

Such a method of control is far more promising for the development of beekeeping in the infested regions than one based merely on the destruction of the mites. It is identical in theory with the measures for the control of European foulbrood used in the United States.

Rennie (*67*), in his presidential address before the Apis Club, April 7, 1923, discusses the treatment of affected colonies but does not give any definite directions. He seems to entertain the hope of the eradication of the disease from Great Britain.

EFFECT OF CLIMATE.

The effect of climate on the propagation of the mite *Acarapis woodi* has not been studied, and there are no data from which one dares draw conclusions. Since both severe and chronic cases of the disease exist, it may be concluded that some environmental factor may have an influence on the severity of the disease, but it is not indicated that these factors are those of climate. Severe cases of the disease have now been described in Great Britain, France, and Switzerland, and it is certain that almost any climate encountered in any of these countries may be duplicated somewhere within the borders of the United States. To assume that the disease would not do damage here if it were introduced, because of climatic differences, is extremely dangerous, since absolutely nothing has been learned of the influence of climate on the disease.

DAMAGE.

The damage resulting from the Isle of Wight disease is a subject which it is extremely difficult to discuss at this distance. Reports have varied greatly, all the way from complete destruction of single

apiaries and of all bees over considerable areas to relatively minor losses. In certain cases (*44*) to be discussed later (p. 18), the results are chiefly a reduction of the honey crop through the death of an abnormal number of field workers. It is quite natural that early reports from Great Britain should emphasize the severe cases, especially during the period when the cause of the disease was not yet known. There are evidently chronic cases of the Isle of Wight disease in England and Switzerland, as is clear from a reading of the literature. There is also reason to suspect that such cases are common in France, because of several statements that the disease is less destructive there than in England, these writers evidently having in mind only the reports of severe attacks in England. There can be no question, after reading the reports from Great Britain for a number of years, that the total losses to beekeeping have been heavy and that in many cases beekeepers have lost all their bees from this disease. Some competent beekeepers claim that the damage in England is not now so great as formerly, but such claims are always to be expected as soon as a disease begins to come under control through more knowledge, and there is no definite reason to believe that the Isle of Wight disease is becoming less capable of doing damage.

An interesting paper has appeared by Anderson (*61*), of the North of Scotland College of Agriculture, in which he discusses especially the apparently decreased virulence of the Isle of Wight disease in Great Britain in recent years, as well as the various methods which have been employed for its control. The reason for the assumed decrease in the damage from the disease is not clear, but it is stated that on entering new districts the disease is as serious as ever.

ERRORS FROM DOUBTFUL DIAGNOSIS.

A curious difficulty which one encounters in attempting to estimate the damage done in Great Britain by the Isle of Wight disease and the efficacy of suggested remedies arises from diagnoses by incompetent persons, who, even before the determination of the cause of the disease, published diagnoses from dead bees submitted for examination. Some British beekeepers have relied on these diagnoses and have then used them as a basis for experiments on treatments, and it is thus impossible to determine the value of much of this work. Many American beekeepers have discounted the reports of heavy losses in Great Britain from the Isle of Wight disease chiefly because of their lack of confidence in the diagnoses and, in fact, many were for this reason led into a sense of false security regarding the disease. Even since the discovery of the cause of the disease, such ill-advised practices seem to continue, in spite of the repeated warnings of Rennie.

THE UNITED STATES.

Immediately following the publication of the first paper (*46*) on this subject a conference called by the chairman of a committee appointed by the apiculture section of the Association of Economic Entomologists was held at the bee culture laboratory on March 9, 1922, to consider the desirability of taking steps to prevent the intro-

duction of the Isle of Wight disease into the United States. At this conference it was decided unanimously that immediate action was desirable. It was recognized, of course, that the failure to find the Isle of Wight disease in the United States during the season of 1921 was not conclusive evidence that the disease is absent from this country, but the far more important fact was to be considered that never in the history of American beekeeping has there been any damage from a disease of adult honeybees comparable to that reported from Great Britain. It seemed justifiable to assume that the Isle of Wight disease is not found in the United States, and therefore desirable that, if possible, this scourge be kept out of the country.

Immediately following the conference, and as a result of its recommendations, the Secretary of Agriculture recommended to the Postmaster General that the postal regulations be so amended as to prohibit the receipt through the mails of queenbees and their accompanying worker bees from all foreign countries except the Dominion of Canada. It was thought that with this as a temporary precautionary measure it would be possible to prevent the introduction of the disease until such time as more complete protective measures were available. The revised postal regulation was announced under date of March 21, 1922. The action of the Dominion of Canada, also taken at the advice of the committee of the apiculture section, will be discussed later. This action made it possible to admit queenbees from Canada by an exception to the postal regulation with safety.

At the conference on March 9 the drafting of a bill for presentation to Congress was also discussed, and as a result a bill was introduced into both Houses of Congress early in April to regulate foreign commerce in the importation of adult honeybees into the United States. This bill was amended at the hearing before the House of Representatives Committee on Agriculture, passed both Houses as amended, and was approved by the President August 31, 1922. The history of this bill in Congress has been recorded by Fracker, Rea, and Gooderham (*27*).

Following the passage of the law, two conferences were held regarding the regulations provided for by the law for the exemption of certain countries which were determined by the Secretary of Agriculture to be free of all diseases dangerous to adult honeybees. The first was called by the American Honey Producers' League at St. Louis, Mo., February 8, 1923, and the other by the Bureau of Entomology at Washington on March 12. The rules and regulations (see appendix) are based on the facts regarding the distribution of the Isle of Wight disease presented in this circular.

In several instances, before the cause of the Isle of Wight disease was discovered, statements appeared in bee journals of the United States to the effect that the disease is present in this country, but as these records are without value they are not specifically mentioned. The symptoms described by beekeepers of Great Britain for the Isle of Wight disease were so nearly identical with some of those observed for diseases of adult bees in the United States as to make such belief plausible. As has been explained earlier (p. 6), this can not be accepted as evidence that the disease is actually present.

The failure to find any cases of the Isle of Wight disease in 1921, together with the much stronger evidence based on the lack of any

serious disease of adult bees in the United States, justified the Department of Agriculture in indorsing the bill before Congress and in taking every possible means to prevent the introduction of the disease into the country. The results of the examinations of adult bees in 1921 have been published (*46*).

The work of the season of 1922 in the search for the mite causing the Isle of Wight disease was done chiefly by L. M. Bertholf, under the supervision of A. P. Sturtevant. Beekeepers were again invited to send to the laboratory any adult bees which showed any abnormality whatever, and as a result 183 specimens were received for examination. During the season the beekeeping literature of this country and the correspondence of the bureau failed to record any instances of any disease or abnormality of adult bees of any moment, and this probably is the reason for the relatively small number of bees submitted for examination. As was the case during the season of 1921, the specimens were from widely distributed locations in the United States, which adds greatly to their value as evidence of the absence of the disease. The following table, prepared by Dr. Sturtevant, shows the results of the examinations made in 1922:

TABLE 1.—*Results of examinations of adult bees, 1922.*

State or country.	Counties.	Towns.	Negative.	Nosema apis.	Arsenic.	Total.
Alabama	3	3	2	1		3
Arizona	2	2	1	1		2
California	10	12	13	6		19
Colorado	4	4	4		1	5
Florida	2	2	2			2
Georgia	2	2	1	1		2
Idaho	1	1	1			1
Illinois	6	7	6	1		7
Indiana	5	5	5			5
Iowa	6	6	4	3		7
Kentucky	2	2	1	1		2
Louisiana	2	2	2			2
Maryland	1	1	1			1
Michigan	5	5	4		1	5
Minnesota	4	4	4			4
Mississippi	2	2	1	1		2
Missouri	2	2	3			3
Nebraska	3	3	2	1		3
Nevada	1	1		1		1
New Hampshire	1	1	1			1
New Jersey	8	10	3	1	[1] 8 3?	15
New York	7	9	7	1	1	9
North Carolina	1	1	3			3
Ohio	7	7	10	4		14
Oklahoma	1	1	1			1
Oregon	2	2		2		2
Pennsylvania	11	13	10	2	[2] 1	13
South Carolina	1	1	1			1
South Dakota	1	1		1		1
Tennessee	3	3	3	1		4
Texas	2	2	2			2
Utah	2	2	1	2		3
Virginia	5	5	8			8
Washington	2	2	1		1?	2
West Virginia	4	4	4			4
Wisconsin	7	8	7	7		14
Canada	3	4	2	1	1	4
Foreign:						
Italy			2			2
Carniola				4		4
	131	142	123	43	[3] 17	183

[1] 2 of these arsenic and Nosema.
[2] Also Nosema.
[3] 10 arsenic; 3 arsenic and Nosema; 4 doubtful arsenic.

The bees recorded as coming from Italy consisted of two lots of worker bees which had accompanied queenbees, one lot having been shipped in 1921. The specimens from Carniola were all lots of workers from mailing cages, the shipments being made early in 1922. During 1922 specimens were received from 36 States, Canada, Italy, and Carniola, and during 1921 and 1922 specimens have been received from 44 States and from 4 foreign countries. No cases of Isle of Wight disease have been seen in this work except the cases from Scotland discussed in the earlier publication (*46*).

THE DOMINION OF CANADA.

Following the discussion of the apparent freedom of the United States and the Dominion of Canada from the Isle of Wight disease and the danger of its introduction at the meeting of the apiculture section of the Association of Economic Entomologists in Toronto in December, 1921, and the conference of March 9 called by the committee of the apiculture section, the committee recommended to the Dominion Government that immediate steps be taken to prevent the introduction of the disease into Canada. It was further recommended by this committee that the action of the Dominion and United States Governments be such as in no way to interfere with a free interchange of bees between the two countries. The Dominion apiarist was a member of the committee of the apiculture section. The action of the Dominion Government consisted of an order of the Deputy Minister of Agriculture, dated April 22, 1922, prohibiting the importation into Canada of bees, used and second-hand hives, and raw hive goods and products, except honey and wax, from the continent of Europe. A later statement from the deputy minister includes Great Britain in this prohibition.

At no time has any noteworthy disease or abnormality of adult honeybees been reported at any place in Canada, and it is believed that there is no disease within the boundaries of that country which has not been found within the limits of the United States. Bees sent to the Bureau of Entomology from various points in Canada have shown *Nosema apis*, which is not considered a disease dangerous to adult honeybees under the act of Congress. Bees examined by the Dominion apiarist have failed to show the presence of *Acarapis woodi*. The former Dominion apiarist, F. W. L. Sladen, was familiar with the Isle of Wight disease in England, and several years before his death recommended a joint action of the Dominion of Canada and the United States to prevent the introduction of this disease, which he knew from experience to be dangerous.

GREAT BRITAIN.

It is unnecessary to attempt to give the distribution of the Isle of Wight disease in Great Britain, as it may be assumed to be distributed throughout that country. Since the announcement of the cause of the disease, a large number of writers in England have discussed this disease and its treatment, but so far no definitely specific treatment for it has been announced. Doctor Rennie has continued his observations on this disease and has issued several interesting articles (*51*, *52*, *53*) on his findings. He has also published some records of the distribution of the disease.

Further study of the disease in Great Britain is bringing to light many interesting facts. The disease varies considerably in seriousness from season to season and in various locations, and a study of the conditions under which it does little or no damage would seem to be the most promising field for investigation in devising further control measures, just as has been done in the United States for the variable brood disease, European foulbrood. The extensive shipping of "driven bees," or bees without combs, to replace colonies dead of various causes has doubtless served to spread the Isle of Wight disease, as well as Nosema disease, which is also evidently prevalent there. Since the rapid breeding up of colonies in the spring seems to be very important in eliminating the disease, it is strange that more emphasis has not been placed by British beekeepers on methods of better wintering, which are vital for success at this season in the United States.

The most popular means of combating the disease in Great Britain seems to be the importation of bees from Holland and queens from Italy, and the advocates of the two races of bees have claimed for them greater power to resist the Isle of Wight disease than is possessed by bees native to Great Britain. On the contrary, other beekeepers are vehement in their denunciation of these importations and of the wide distribution of foreign races of bees, so that at a distance it is impossible to judge the merits of the effort. It seems obvious from the discussions on this subject that the only superiority which can be claimed for the two introduced races lies in their ability to breed up faster than the native bees. If this is true they may have some advantages in combating the malady. No general plan for the control or eradication of the disease under Government supervision has been announced.

FRANCE.

Just as Department Circular 218 was going to press (March, 1922) the first record reached the Bureau of Entomology of the finding of *Acarapis woodi*, the mite causing the Isle of Wight disease, on the continent of Europe. A brief footnote was added to page 7 of the circular to announce this finding. This record appeared in the January, 1922, issue of one of the French bee journals (*49*). The determination of the mite was made by a competent observer, L. Berland, assistant in the Museum d'Histoire Naturelle, Paris, under the supervision of Prof. E. L. Bouvier. The mites found in these diseased bees were identified as *Tarsonemus woodi* after comparisons with Rennie's descriptions and illustrations. The exact location of this outbreak of the disease was not recorded,[3] except that it was in the

[3] From a recent article by Ph. J. Baldensperger ["Quelle est la patrie du Tarsonemus?" La gazette apicole, v. 24, no. 220, p. 57–59], it would appear that this first outbreak was in the town of Champseur, Hautes-Alpes. He states that he took a trip of investigation through this territory with an entomologist, M. Poutiers, of Mentone, and that on this trip they were not able to find any of the mites causing the disease, although they had been definitely identified from the apiaries in which the disease was first discovered. The apparent disappearance of the mites is not explained. Other beekeepers of the region are reported to have lost their bees, but the cause of this loss could not be determined from lack of material. A recent private communication (Apr. 21, 1923) from M. R. Poutiers, Mentone, confirms the absence of mites from the bees examined on this trip. He reports a heavy loss of adult bees at Mentone last December, in the tracheæ of which he found *Acarapis woodi*, but he is not yet entirely certain that the mite causing acarine disease in France is identical with that described in England and Scotland. He states that the disease is made more severe by dampness, but that it appears not to be so severe as in Great Britain. Investigations at Mentone are still in progress.

French Alps. This finding was confirmed in the report of the proceedings of the December 21, 1921, meeting of the Société Centrale d'Apiculture de France (*55*). Berland (*12*) later discussed methods for identifying the mite.

In March, Professor Mamelle (*2*), of l'Ecole Nationale d'Agriculture de Grignon, asked French beekeepers to send him specimens of bees for examination whenever they find any evidence of disease, and it would appear that he took up the study of the Isle of Wight disease at about that time. At the meeting of the Société Centrale of April 18, 1922, he reported (*36*) that the Isle of Wight disease had made its appearance in several Departments of France, especially in Maine-et-Loire, Côte-d'Or, and Saône-et-Loire. Whether these records include the first published record was not stated, but from their location it would appear quite improbable. Two of these Departments are not many miles from the Cantons of Switzerland in which the disease was found at about the same time. It was announced (*3*) that a committee of four men holding the rank of professor in various French institutions had undertaken to study the diseases of bees, and in the same month Professor Mamelle, a member of this committee, addressed the Société Centrale (*26*) on this subject.

In June Giraud and Sevalle (*28*) gave a summary of the work of Doctor Rennie and recorded some recent instances of diseases of adult bees in France, without, however, stating definitely whether the mite was found in the diseased bees. Without this information the record lacks significance in the present discussion.

A later record of the distribution of the Isle of Wight disease in France is to be found in a notice (*37*) in the advertising section of L'Apiculteur for July, 1922, which was an announcement regarding the work of Professor Mamelle, presumably written by him or at any rate published at his request. He thanks beekeepers who have sent him several hundred specimens of adult bees and brood for examination and explains his delay in transmitting his diagnoses, after which the following statement appears: "Regarding the acarine disease (Isle of Wight disease) it can be affirmed that the disease is found somewhat throughout France, but does not seem to present the great injuriousness as in England."

In an article written in October, 1922, Bouvier (*14*), another member of the committee which is investigating bee disease in France, gives some additional records of the finding of the disease in France and adds that it is almost certain that there was an outbreak of the disease in Ardennes in 1919. He states that the disease is "found nearly throughout France," and definitely records the disease as present in four Departments not previously recorded—Basses-Pyrénées (adjacent to Spain), Lot, Ain, and Hautes-Alpes (adjacent to Italy). This makes definite records for seven Departments of that country. The definite records are all from central and southern France. Nothing has come to the Bureau of Entomology from French sources to justify a belief that the cases of Isle of Wight disease in France are directly traceable to recent importations from England, nor has any announcement of any regulatory means for the control of the disease in France reached the bureau, and seemingly no quarantines have been established as has been claimed (*5*). Bouvier is inclined to believe that *Tarsonemus woodi* may also be a

parasite of certain solitary insects, but does not record any cases of the finding of the mite in such insects.

Other articles on this disease have recently appeared in various French bee journals, but many of them have statements regarding the disease that are not based on examinations for mites and therefore are of little if any value in the present discussion. Since several American beekeepers claim to have used flowers of sulphur successfully in combating the disease of adult bees commonly known in the United States as paralysis, certain French beekeepers are advising the use of this material in combating the Isle of Wight disease, apparently on the mistaken idea that paralysis and the Isle of Wight disease are identical. Statements to this effect serve only to confuse the situation and are wholly valueless without experiments to support them. Rennie has repeatedly warned British beekeepers against the drawing of false conclusions of this type. In a recent paper Morgenthaler (*66*) has specifically warned beekeepers not to place confidence in the use of flowers of sulphur in the treatment of the Isle of Wight disease and has shown wherein they may make serious mistakes in judging the effects of treatments tried without proper scientific checks.

An effort is being made to get more detailed information regarding the distribution of the Isle of Wight disease in France, as this will help in determining how widespread the disease is on the Continent and will permit one to form some idea of the possible duration of the outbreak and the probability that the mite is also found in adjacent countries of Europe.[4]

Several of the statements that have been made regarding the disease in France assume that it is less virulent there than in England. To what extent such statements may be accepted is uncertain, and, if such a difference exists, it may be due to the condition described by Morgenthaler (*44*) and discussed on a later page of this circular (p. 18). Possibly some of this opinion is due to an overestimation of the losses in Great Britain which naturally arose from the reading of some of the articles from that country which appeared before the cause of the disease was known. Those reporting the supposed difference between France and England do not record whether they have seen cases of the disease in Great Britain.[5]

THE DUCHEMIN MITE.

In the article by Giraud (*28*) reference is made to the finding by Émile Duchemin in France in 1866 of a mite on the exterior of dead bees and also on the sunflower, *Helianthus annuus*. This mite was mentioned by Rennie (*50, p. 776*) in the first announcement of the discovery of *Acarapis woodi*. Since this finding and the references

[4] In a recent private communication (Apr. 3, 1923), M. Lucien Berland, of the Museum Nationale d'Histoire Naturelle, Paris, who made the first determinations of the mite from the French Alps (p. 12), writes that the disease exists throughout the territory of France, not continuously, but widely distributed. He states: "It appears to me probable that the disease exists throughout Europe." He calls attention to the fact that the disease is not easily spread, for all the colonies in an apiary are not attacked. He has not found the condition which Morgenthaler (*41, 44*) describes of the mite indistinguishable from *Tarsonemus woodi* on the outside of bees. He does not believe that the spread of the disease in France is of recent occurrence.

[5] A recent letter (Apr. 22, 1923) from a prominent British beekeeper states that early in that month he imported some bees from near Marseilles and that the bees of one colony began to crawl in a few days after they were received. Examination of the tracheæ showed them to be crowded with young parasites, but the tracheal trunks were not yet stained. "I am of the opinion that English bees would not crawl at this stage. The possibility of a very severe outbreak in this region [Marseilles] is great."

made to it in recent discussions have been the cause of some confusion in the minds of beekeepers in America and elsewhere, it is well to record exactly what Duchemin found and to show that this had nothing whatever to do with the Isle of Wight disease. No claim is made by Giraud or Sevalle (*28*) that *Acarapis woodi* and the Duchemin mite are the same species.

In 1866 M. Duchemin published a brief article (*21*) in which he described his finding several years before of a mite in the apiary of a poor peasant to which Duchemin attributed the rapid death of 30 colonies of bees. On near-by flowers of the sunflower, *Helianthus annuus*, to which the bees had access, he also found a mite which he considered to be identical. He concludes from observations then made and from work which he did on this subject in 1864, several years later, that the mites inhabit the sunflower and that in this way these plants are destructive to honeybees as sources of this enemy. This finding was discussed in several succeeding numbers of the same journal by the editor, Hamet, André (*7*), and again by Duchemin (*22*), who replies to cricitisms of André. The notes by Duchemin also appear in another journal (*20*). From the recent discussion it appears that this purported discovery was then discussed in other periodicals (*15*).

After the announcement of the discovery of *Acarapis woodi* as the cause of the Isle of Wight disease, several writers referred to this early finding by Duchemin. The well-known German investigator Von Buttel-Reepen (*15*) refers to his own finding of a mite on bees of *Apis indica* and raises the question whether *Tarsonemus woodi* may not be the same mite which Duchemin found in France. This article was translated in part into English and reply was made to it by the writer (*47*). Von Buttel-Reepen wrote his article before the description of *Acarapis woodi* had been published. In the reports of M. Duchemin's discovery in certain German periodicals mention had been made of an illustration which Duchemin had prepared, but unfortunately this illustration was not available to Von Buttel-Reepen when he prepared his paper. The illustration was not given in the account of this mite which Duchemin published in the Comptes rendus hebdomadaires des séances de l'Académie des sciences [Paris] (*20*), and Rennie (*50*) refers to the absence of an illustration. It appeared in L'Apiculteur, a French bee journal, for February, 1866 (perhaps also elsewhere), and the illustration was copied in the American Bee Journal for May, 1922. Ewing (*25*) later stated that the Duchemin mite, as determined from this illustration, was doubtless a nymph of a species of Trichotarsus, so there is not the slightest reason for thinking that the Duchemin record has anything to do with the Isle of Wight disease. These mites may have been injurious to the bees, as Duchemin claims, but this finding has no bearing on the present outbreak of Isle of Wight disease in France or elsewhere.

A similar record of the finding of a mite on bees is mentioned by Manger (*39*) (see also Elsaas-Lothring. Bienenzüchter, 1884 (*1*)), and Dennler (*18*), where it is recorded that Trapp, of Strassburg, found mites in considerable numbers on the head of a bee. An illustration of a ventral view of this mite by Schmidt (much better than the illustration drawn by Duchemin of his mite) is copied by Manger, which shows that there is no reason to believe it is any way

related to the Isle of Wight disease or identical with *Acarapis woodi*. This mite is identified with a considerable degree of certainty by Vitzthum (*58*) as a migratory nymph of *Trichotarsus osmiae*. Several species of mites have been found on honeybees and in and about beehives (*11*, *16*, *43*, *50*), and it is important that such mites shall not be confused, through careless identifications, with the one species known to be pathogenic to honeybees.

SWITZERLAND.

Since the announcement of the discovery of the cause of the Isle of Wight disease in 1920, the investigators of the Schweizerische Bakteriologischen Anstalt at Liebefeld, near Bern, and the apiary inspectors have been watching for *Acarapis woodi* in that country. The investigation of bee diseases for Switzerland is carried on in that laboratory, under the direction of Prof. Robert Burri, whose work on the brood diseases of bees is well and favorably known.

During the year 1921 examinations were made of adult bees that were suspected of disease or which showed any abnormality, but no specimens of *Acarapis woodi* were found (*40*). It is now known, however, that the mite was present in Switzerland before that time (*44*). On February 1, 1922, Dr. Otto Morgenthaler (*41*) of the station found dead mites, later identified by Rennie and himself as *Tarsonemus woodi*, on the outside of bees that had died during the winter, no evidence of disease having been noted previously. He then found that mites of this species, or at any rate indistinguishable by microscopic examination, were present on similar dead bees obtained by him from five towns in Switzerland ("wherever I looked for it"). This finding of the mite on the outside of dead bees which had not shown any definite signs of disease led to speculation as to the possibility that *Acarapis woodi* is in Switzerland a harmless symbiote of the honeybee; that it lives with the bees without either species being injured by the mutual adaptations. In an English review (*13*) of the above-mentioned paper this view is suggested, following a less definite suggestion of the same nature by Morgenthaler himself. In reply to this review Morgenthaler (*42*) states, however, that this does not correctly reproduce his view and that he considered the mites found on the outside of dead bees as dead animals (parasites) which had left their hosts on the death of the hosts.

In the same numbers of the two Swiss bee journals in which the report of the unsuccessful search in 1921 appeared, there was published a note by Morgenthaler (*40*) reporting that two specimens of diseased bees had been examined in cases in which definite symptoms of disease had been noted. This indicates that the Isle of Wight disease as it is found in Great Britain is actually found in Switzerland, and precludes the belief that *Acarapis woodi* is in Switzerland always a harmless symbiote. The development of the work in Switzerland on this mite and on the conditions in the colonies which it produces have added materially to our knowledge of the subject, and while there is still much to learn it is becoming more and more evident that this mite is to be considered a serious pest of the apiary in Switzerland and elsewhere, wherever it is found.

In a later article (*43*) Morgenthaler summarizes the information on *Tarsonemus woodi*, gives various reports of the finding of mites

of other species in and about beehives, and repeats the records of the finding of *Tarsonemus woodi* in Switzerland just mentioned. At the time of the writing of this article he had been unable to find living mites in the thoracic tracheæ of bees from those colonies in which they were found dead on bees which had died during the previous winter, but he specifically states that he does not question the statement that it is the cause of the Isle of Wight disease.

The most recent discussion of the situation, which covers the ground as it is so far understood in Switzerland, is contained in an interesting lecture (*44*) which Morgenthaler delivered before the Wanderversammlung des Vereins deutschschweizerischer Bienenfreunde on August 20, 1922, in Brugg. This lecture deals briefly with most of the diseases of adult bees, but the portion which is of special interest is that part dealing with his results with the mite. It may be mentioned that he finds that the protozoan parasite *Nosema apis* is in Switzerland, as in America, usually a relatively harmless parasite, but that under certain circumstances it produces a serious disease. Morgenthaler is inclined to believe, so far as can be judged from his lecture, that *Nosema apis* and *Acarapis woodi* are usually about of equal destructiveness to the colonies, but it is not quite clear as yet what the circumstances are which increase their damaging characteristics.

He reports that the mite has now been found throughout Switzerland. This is a small country, enabling him in one season to make a rather complete survey, but he records finding the mite throughout the country. The virulent cases were found in the Cantons of Geneva and Vaud (adjacent to France), while the worst case was in the middle of the Canton of Valais (adjacent to Italy). He reports five such virulent cases, and in a private communication he reports a sixth case. Regarding the more general distribution of the mite in the milder or chronic cases, he states (*44*):

> And indeed these mite-infested apiaries were found scattered through the whole country, among others also on our northern, southern, eastern, and western boundaries, so that it may be surmised that the mite is also scattered in neighboring lands and will be found as soon as it is sought. Switzerland may therefore not be considered as an especially dangerous center of infection of the acarine disease.

Regarding the worst case of infection, in middle Valais, he states:

> The acarine disease in its virulent form has been found in Switzerland in five apiaries * * * but worst in a little village in middle Valais, where all three apiaries of the place were attacked. Here the occurrence of the disease may be traced back with certainty to the year 1915, and the chief sufferer—incidentally it may be noted, an excellent beekeeper, bee inspector, and teacher of beekeeping in an agricultural school—has in this time lost 26 of his 35 colonies. Importation of bees from England has nowhere occurred.

The record for Switzerland can not be interpreted as consisting of cases directly attributable to importations,[6] and one must con-

[6] In a recent article Fr. Leuenberger (Jahresbericht über die Faulbrutversicherung des V[ereins] D[eutsch] S[chweizerischer] B[ienenfreunde] pro 1922, *in* Schweizerische Bienen-Zeitung, n. f., v. 46 (1923), no. 3, p. 115–120), who has charge of the bee disease control work in Switzerland, states the Isle of Wight disease occurs in increasing amounts in French Switzerland and that apparently it has been brought in through importation of bees from western France. No case of the disease is reported by him from German Switzerland. He expresses the hope that the disease may be localized by energetic measures before it causes greater damage, but does not report the nature of the measures adopted. Morgenthaler (*44*) reports finding the mite on the northern boundary of Switzerland (German Switzerland).

clude that the infestation is at least of several years' duration. The same thing is, of course, true of the records from France, so that we dare not assume (*5*) that the disease in continental Europe is traceable to recent introduction from Great Britain.

Since Morgenthaler has found mites on bees which were not recognized by their owners or by inspectors as diseased, this has given rise to the idea that the mite is often not a serious pest or that it is quite harmless in most cases. For some reason this interpretation has been put on his work, but not on that of Rennie, who has described the same things. This does not seem to agree with the present ideas of Morgenthaler in the slightest degree. It is quite true that he has found mites where he himself has not been able to detect the ordinary symptoms of the Isle of Wight disease, but so has Rennie. In a private communication (January 15, 1923) he states that he sent dead bees from the Liebefeld Station apiary on which he found mites to Rennie and that Rennie found that from 3 to 5 per cent of the bees showed mites in various developmental stages in their tracheæ.

> I have been unable to confirm this finding through my own investigation, although I have dissected many such bees. I find the tracheæ always clean. I am, however, now about to test this question with new and better material.

Regarding the assumed harmlessness of these mites, Morgenthaler has the following to say (*44*):

> The harmlessness of the parasite has been erroneously asserted from these findings. I may here allude to only two points which must be regarded in the examination of this phenomenon. First, the bee colony possesses a series of contrivances for defense against the parasite, so that it does not succumb without a struggle to the first attack. The most powerful of these means of defense is found in the constant renewing of the inmates of the colony through the death of the old infested bees and the emergence of young healthy ones. It is therefore made very difficult for the parasite to obtain a foothold, notwithstanding that it can probably be found for a long time in the colony. Only when it invades the colony in overwhelming numbers because of special circumstances does it get the upper hand.
>
> Secondly, however, it is very likely that the apparently healthy colonies containing parasites after all are not entirely normal. By rigorous investigations it would indeed be shown that many remain in colony strength below what one would expect of them. Exact weighings and measurements * * * would show clearly that for many apiaries which outwardly do not give an impression of disease, the loss of flight bees is too great. The question would here also have to be examined whether the lack of swarming is not also due to infection, concerning which many beekeepers have complained these last years.

Throughout this lecture, Morgenthaler deals both with *Nosema apis* and *Acarapis woodi* as parasites which under certain circumstances, not understood, become harmful, so that these remarks are not to be interpreted as applying solely to the mite. The experience with *Nosema apis* in the United States would appear to support his contentions for that parasite.

It would therefore appear from the findings in Switzerland that whereas *Acarapis woodi* may produce a chronic and damaging disease, it can also at times become dangerous to as great a degree as has been described for England. It is also clear that the outbreaks of the virulent form of the disease can not be attributed, as some (*5*) have attempted to claim, to recent importations from England, for Morgenthaler specifically states regarding these severe outbreaks that "importation of bees from England has nowhere occurred."

Because of the thoroughness of the search in Switzerland and the wide distribution of the mite, the supposition of Morgenthaler that the mite will doubtless be found in adjacent countries when it is sought must have greater weight than the statements which have appeared from these adjacent countries that the mite is absent, since in all the adjacent countries except France no effort has been reported of surveys of the kind demanded for definite statements on this point.

The Government apiary inspection service of Switzerland has taken steps to prevent the spread of the Isle of Wight disease (*35*), and the methods employed are discussed later (p. 26).

GERMANY.

The situation regarding the Isle of Wight disease in Germany is yet very indefinitely known. In a publication issued in 1922, Hirst (*32*) states that apparently *Acarapis woodi* has been found only in English bees, but he adds a footnote in which he states (p. 97): "According to Vitzthum, Doctor Ellinger, of Weimar, has reported that the disease has made its appearance in Germany also (Bayerische Bienenzeitung, April 1922)." Count Vitzthum is a well-known specialist in mites and Doctor Ellinger is the author of several papers on bee diseases. There also appeared a statement from Alfonsus, of Vienna (*6*), in which he states (p. 2): "The occurrence of *Tarsonemus woodi* has now also been established in Germany * * * (Archiv für Bienenkunde, vol. III, 6, 1921)." The number of the Archiv für Bienenkunde to which Alfonsus refers contains two articles (*33*, *39*) on the Isle of Wight disease, but in neither case is there any statement which can be interpreted as supporting the statement quoted.

The Bayerische Bienenzeitung to which Hirst refers is not regularly received in the Bureau of Entomology, but the editor, Hofmann, of Munich, kindly sent the copies of his journal which contained articles on this subject. In the article by Vitzthum (*57*) to which Hirst refers there is only the following statement on this subject: "Doctor Ellinger of Weimar communicates that the disease has also appeared in Germany." No additional information is given and it is not stated whether this statement is based on an examination of diseased bees for the mites or merely on the general symptoms observed in diseased adult bees, which are known to be quite unreliable. Since this statement appeared only a few months (November to April) after Rennie's announcement of the discovery of the cause of the disease, it appears somewhat doubtful whether any search for the mite had been made, especially since these intervening months were during the winter. In order to clear up this point, a letter was written to Doctor Ellinger, who replied under date of January 10, 1923: "Your question on the occurrence of the *Tarsonemus woodi* in Germany I answer as follows: It is not yet certainly found in Germany." Doctor Ellinger also kindly sent some advance proof sheets of an article of his (*23*) on the diseases of bees which is soon to appear in a new edition of Unsere Bienen (Ludwig, editor), in which he makes no reference to the finding of the Isle of Wight disease in Germany. It would, therefore, appear that his statement to Count Vitzthum was based solely on external symptoms, although this is not stated.

In the meantime a number of other articles (*15*, *33*, *38*, *39*) have appeared in German journals regarding the Isle of Wight disease and its newly discovered cause, and in none of those which have come to the attention of the writer is there any statement regarding the occurrence of the disease in Germany. Dr. Bartholomäus Manger of Ingolstadt in a private communication writes (December 30, 1922) that he reads all the German bee journals and has seen no such statement. Because of the geographical position of Germany in the center of various important beekeeping regions of Europe, it seemed quite desirable to determine whether the disease is actually present in that country. Probably no American beekeeper would wish to import queenbees from Germany, almost certainly not of the race of bees native to that country. Germany has long been the home of scientific research in beekeeping and there are a large number of competent investigators in beekeeping in that country. It was felt that if the disease has actually been found in Germany this fact would indicate a wider distribution than has been assumed for the mite in most of the publications on the subject. With this thought in mind, the writer addressed a considerable number of the prominent beekeeping investigators of Germany, asking whether they had any knowledge of the actual finding of the mite within the boundaries of Germany.

The replies to these inquiries were uniformly to the effect that the mite has so far not been recorded in any German beekeeping periodical and none of the men who replied had any knowledge of such findings. It appears from these letters that no search has been made specifically for this mite, but one of the men who wrote, a prominent investigator, states that he expects to undertake such a search during the summer of 1923.

Except for the surmise of Morgenthaler, based on his finding of the mite on the northern boundary of Switzerland, adjacent to southern Germany, it may therefore be stated that there is so far no evidence of the occurrence of the Isle of Wight disease in Germany. This negative statement will be of little value in comparison with such scientific investigations as will doubtless be made in the near future.

ITALY.

American beekeepers are perhaps more concerned with the situation as to diseases of adult bees in Italy than in any other country of continental Europe, since more importations of queenbees have been made and will be desired from that country than from any other. Several articles (*8*, *9*, *10*, *45*) have appeared in the Italian bee journal on the Isle of Wight disease and on its newly discovered cause, but they are usually a summary of the work of Rennie or announcements of the finding of the disease elsewhere in England. The effort of the United States Government to prevent the introduction of the disease into this country has also been mentioned on several occasions, not always favorably (*4*).

The only definite statement which has come to the attention of the writer is a brief one by Prof. Vincenzo Asprea, of Calabria. In discussing the United States postal regulation approved March 21, 1922, prohibiting the mailing of queenbees into the United States, he says (*8*):

It is strange that while the English Government imports a large quantity of Italian queens to combat the disease, and with good success, the Americans wish to keep it out by not importing. To tell the truth, the fear is justified by the fact that the disease has been observed in the French Alps and in near-by Italian apiaries, but since then it has not been seen further.

The reference to the French Alps doubtless refers to the actual finding of the mite in diseased bees from the French Alps, previously mentioned (*49*). It will be recalled that this first report from France did not give the exact location of the apiary from which the diseased bees were received, and in that notice no mention was made of bees in neighboring apiaries, either in France or Italy, being diseased. The disease is recorded by Bouvier (*14*) as occurring in Hautes-Alpes, France.

Since the publication of this brief mention of the disease in Italy, several other articles (*9*, *10*, *45*) and notices have appeared, some of them by Asprea, which would lead one to surmise that the authors believe that the Isle of Wight disease does not occur in Italy, since they usually urge that the Italian Government shall take steps to prevent the introduction of the disease into that country. The Federazione Apistica Italiana has stated in a private communication (Dec. 20, 1922) that they believe that it will be possible to furnish full assurance of the absence of the Isle of Wight disease from Italy. It may therefore be concluded that there is little reliable evidence of the presence of the Isle of Wight disease in Italy, based on examinations of diseased bees. The chief reason for suspecting that the disease may be present is the fact that it has definitely been found in neighboring apiaries in France and Switzerland.

From private sources the author has learned that the Italian Ministry of Agriculture has been requested by the Federazione Apistica Italiana to take steps to prohibit all importation of foreign bees into Italy, in order to prevent the introduction of the Isle of Wight disease into that country, and that in all probability a search will be made in the near future to determine whether the mite is actually found in that country. The Italian beekeepers are evidently and properly anxious to prevent the introduction of the disease and to keep up the standing of their stock throughout the world. The results of their efforts will be watched with keen interest in the United States. Prof. Amyandro Ghigi of the University of Bologna, reports in a private communication (April 18, 1923) that he has examined bees from apiaries in that Province and has failed to find any *Tarsonemus woodi*.

DENMARK.

In a private communication (April 30, 1923), Dr. L. Bahr reports that *Tarsonemus woodi* has not been found in Denmark through the occurrence of the disease which it causes. No extensive inquiry has been made on this subject in that country, but further investigations will be made in the near future. Doctor Bahr further reports that no record of the mite in Sweden or Norway has come to his attention.

CZECHOSLOVAKIA.

In a letter dated May 23, 1923, from Rev. Ivan F. Kitzberger, Veleslavin, Czechoslovakia, editor of one of the leading bee journals of that country, it is stated positively that the mite causing the

Isle of Wight disease has been found in that country. Identifications of the mite were made by Blattný, an assistant in the Zoological Institute at Prague, who is a specialist on mites. He has also found several other species of mites in and about the hives, but these are not associated with any disease and some of them have previously been found in hives. No statement was made regarding the occurrence of the Isle of Wight disease or whether the mite is found without manifestation of the disease, as has occurred in parts of Switzerland.

That the Isle of Wight disease may be present to an alarming degree in Czechoslovakia is indicated by an article by Altmann (*60*), in which it is stated that during the spring of 1923 reports have been received by him of the heavy death of adult bees and of entire colonies, of such a character that the loss can not be attributed to poor wintering. Altmann is in charge of the bee investigations for the Deutsch. bienenwirtschaft. Landes-Zentral Verein für Böhmen, and asks that diseased bees be sent in for examination to determine whether *Nosema apis* or *Acarapis woodi* is the cause of the heavy losses. An even more severe loss of adult bees and of colonies has been reported from the territory of Teschen (Bohemia) by Kessler (*62*), but no indication of the cause of this loss is given.

SOUTH AFRICA.

In a letter dated June 18, 1923, from Dr. Otto Morgenthaler, Liebefeld bei Bern, Switzerland, whose investigations of the Isle of Wight disease have added so materially to our knowledge of this subject, it is stated that the Isle of Wight disease has become established in South Africa and that attacked colonies have been destroyed by officials in Natal. Details of this outbreak are lacking.

OTHER EUROPEAN COUNTRIES.

So far as has come to the attention of the Bureau of Entomology, no search has been made for the Isle of Wight disease or for the mite which causes it in any country of Europe other than those already mentioned, nor has any definite statement been seen regarding its presence, based on investigations. It has been reported to the bureau by certain American beekeepers that they have assurance that the disease does not exist in certain countries, but so far it has been impossible to get any accurate first-hand information on these subjects, and in some cases the reported evidence has been found to consist merely of letters from some queen breeder who is eager to sell his queens in this country, but who has no means of examining bees for the presence of mites or any knowledge of its presence in his country.

The finding of mites so generally in France and Switzerland, as soon as steps were taken to search for them, shows conclusively that the mite is not one which has until recently been confined to Great Britain. There is, therefore, a high degree of probability, amounting almost to a certainty, that the mite is present in countries other than those in which it has been sought. The facts which Morgenthaler (*44*) brings out regarding the wide prevalence of weak colonies, lack of swarming, and other evidence of abnormality in

the bees of Switzerland due to the infestation indicates that the mite may be present for a considerable time without the beekeeper being aware of the fact, and, even from an experienced beekeeper, one may not accept the statement that there are no cases of the disease in his country, merely on the basis of lack of visible symptoms among his own bees. Various diseases or abnormalities of adult bees, known variously as paralysis, May disease, and under other names, have been recorded repeatedly from the various countries of Europe, and since these names mean little or nothing, the causes of these conditions being purely a matter of speculation, one is entirely unable to estimate the probability that some of these conditions are actually the Isle of Wight disease. It certainly can not be considered safe to accept statements of interested persons who desire to make sales of queenbees in the United States when there is no way of checking their statements.

CARNIOLA.

The Province of Carniola, in the Kingdom of the Serbs, Croats, and Slovenes, is the home of the Carniolan bee, which has for a number of years had some ardent advocates in the United States. They are excellent bees, but have not gained the popularity in this country which Italian bees enjoy. There are only a few queen breeders in the United States who have found demand enough for these bees to make their propagation profitable, and as a result many beekeepers who prefer bees of this race have been in the habit of obtaining their queens directly from Carniola. Perhaps because of this fact, there has been more demand that this Province be excluded from the operation of the law against importations and that queens from Carniola be admitted freely than has come from those who desire queens from Italy. The statement has been made by those interested in these importations that there is no Isle of Wight disease in that Province. An effort has been made by the writer through correspondence to learn if there has been any investigation to determine whether any Isle of Wight disease or any disease of adult bees exists in Carniola, but so far without success. The president of the provincial beekeepers' association, M. Humek, wrote under date of August 18, 1922, that he is not aware of any kind of infectious bee disease in his country. He states in his letter that certain bees had been sent away for examination and that one of the best-known queen breeders of that country would send to this Government a report which would show that the Isle of Wight disease is unknown in Carniola. So far the report has not been received, and from other correspondence it would appear that there has been some unavoidable delay in the examinations, the nature of which is not clear. The Royal Department of Commerce and Industry of the Kingdom has also stated that there is no case of any disease of bees in the Kingdom. An effort is being made to obtain the data on which this statement is based.

When the revised postal regulations were adopted prohibiting the mailing of queenbees through foreign mails in March, 1922, four persons who had imported queenbees from Carniola sent to the Bureau of Entomology the original cages in which imported Carniolan queenbees had been received, including the accompanying worker bees. These worker bees were not found to contain *Acarapis woodi*, but they were heavily infected with *Nosema apis*. This

parasite is found widely distributed in the United States, and under the regulations provided for the enforcement of the importation law the disease which it causes is not considered as one dangerous to adult honeybees, but the fact remains that probably never in the work of the Bureau of Entomology on this disease have bees been examined which contained a larger number of these intestinal parasites. If such a heavy infection were found in bees in this country, one would expect there would be marked symptoms of abnormality. It therefore appears strange that the beekeepers of Carniola have never noted any abnormality of adult bees. If Carniolan bees have the ability to harbor this parasite without showing any symptoms or are better able to resist it than are other bees, this has not previously been proved. The presence of *Nosema apis* in Carniola will not serve to confuse the situation with regard to importations from that Province if the much-needed examinations of adult bees are made by competent investigators.

AUSTRIA.

No information has been received by the Bureau of Entomology concerning the details of any search which may have been made in Austria to determine the presence of the Isle of Wight disease. A letter has been received by the author from Alois Alfonsus, of the Austrian Department of Agriculture, Vienna, written from within the United States, in which he states that the scientific institute of that department has so far been unable to find *Tarsonemus woodi* in that country. He does not state how extensive a search has been made or from what parts of the country bees have been examined. No published report of this work has been reported to the bureau. Austria is immediately adjacent to Switzerland, and Morgenthaler (*44*) reports finding *Tarsonemus woodi* on the contiguous border.

While it is most essential that a serious effort be made to determine especially whether Italy and Carniola are free from the dangerous diseases of adult bees, it is also of the highest importance to American beekeepers that efforts be made to determine these facts for all of Europe, just as soon as conditions are favorable for the prosecution of such investigations. The shortness of the distances between important beekeeping regions in Europe and the considerable traffic in bees which has long been customary throughout Europe make it necessary that detailed studies be made on this point for each country, and it will doubtless be several years before the facts are adequately known.

Although beekeepers of all countries are at present interested in the Isle of Wight disease, the investigation of its presence in many countries is difficult or impossible because of disturbed economic conditions.

Investigations on beekeeping subjects were not systematically conducted in most European countries before the war, and since then some of the work which had been organized has been discontinued. Several countries have so far made no provision for such work. Under these circumstances it will probably be several years before the distribution of the Isle of Wight disease on the continent of Europe is fully known. It will be recalled that the discovery of the cause of this disease was the result of work by members of a university staff,

aided by private funds, and not a Government project. The interest in this subject promises to be the incentive for support for much-needed investigations, either from public or from private sources.

EMBARGOES TO PREVENT INTRODUCTION OF BEE DISEASES.

Several countries other than the United States have taken steps to prevent the introduction of the Isle of Wight disease and other diseases of bees. The embargo placed by the Dominion of Canada is well known to American beekeepers. Other British Dominions have taken similar precautions, notably the Union of South Africa, Jamaica, and Australia. There seems now to be an insistent demand from beekeepers of Italy for a strict prohibition of importations of bees into Italy. Because of the vast interest created by the work of Doctor Rennie and his associates, it is to be expected that other countries will follow the same course, and in all probability the free international shipment of bees will soon be a thing of the past.

Under regulation 5 (*b*) of the regulations formulated for the enforcement of the act of Congress of August 31, 1922 (see appendix), it is provided that importations of adult bees from any country other than the Dominion of Canada shall be conditioned on the determination of the Secretary of Agriculture, as a result of adequate scientific investigation, that no diseases dangerous to adult honeybees exist in the country in question, and that adequate precautions have been taken by such country to prevent the importation of adult honeybees from countries where such dangerous diseases exist. The purpose of this regulation is solely to safeguard American beekeeping interests, but it indicates clearly what steps are necessary for the protection of the beekeeping interest of any country against these dangerous diseases. This policy on the part of the United States Government will make it less probable that beekeepers of other countries wishing to ship queenbees into the United States will attempt to conceal the true situation; rather, they will urge that provisions for thorough investigations be made, if their trade with this country justifies the expense and labor of such investigations.

As a result of the freedom which Amercian beekeepers formerly enjoyed regarding the importation of bees, they now have three brood diseases, Nosema disease, the so-called paralysis, and perhaps other diseases of adult bees, all of which, so far as they are contagious or infectious, were assuredly brought to this country from abroad, since the honeybee is not native to America. The price which beekeepers of the United States are now paying for these accidental importations of diseases amounts to at least a million dollars annually, a high price to pay for the privilege of buying bees wherever one wishes, without knowledge as to the safety of the transaction. Under the law now in force, such importations may be safeguarded, so far as investigation has discovered the causes of diseases which are not yet present in this country. That there is still at least one disease of adult bees which is not present in the United States seems probable, because of the inability to find it during the past two seasons. Why the Isle of Wight disease is not widespread in the United States is a matter of mystery, but one of purely theoretical interest. If, as is believed and hoped, the United States is free from this disease, then the door has been closed in time, and it should be

the privilege of every American beekeeper warmly to support the enforcement of this law, and this is in fact the attitude of the vast majority at this time.

In the correspondence with the Bureau of Entomology regarding the regulations for importations, the necessity for further importations of queenbees is emphasized by those who wish to make such importations, and is minimized or denied by those who do not wish to make importations. This is a natural condition and occasions no surprise. It seems evident that there is no popular demand or great necessity for large importations at present, and, in fact, it is not proved that any actual damage would result to American beekeeping from a total prohibition of importations for a time. With the safeguards which have been provided under the law, it will be possible to make such importations as may be urgently needed for experimental and scientific purposes. As further investigations are made in the various foreign countries, it may be found safe to allow bees to enter from them without restrictions and for any purpose. Until accurate information is available from the various countries, it is the part of wisdom to limit the importations to those for experimental and scientific purposes and to keep a careful watch for information from all the countries from which any importations may be desired.

In addition to the countries named above as having prohibited or restricted the importation of adult bees, special attention should be drawn to the recent action of Switzerland. As has been pointed out, the Isle of Wight disease occurs in that country and in all Morgenthaler (*66*) now reports 19 cases of the disease in French Switzerland. Under these circumstances the prohibition of importations might seem unnecessary, yet the recent action of the Swiss Government puts such a prohibition into effect and outlines steps for the eradication of the disease already present. The situation with regard to the disease in that country is outlined in three recent articles by Leuenberger (*63*, *64*, *65*).

On April 18, 1923, the Swiss Bundesrat issued a proclamation (*68*) under which the Isle of Wight disease is included under the animal diseases covered by the Federal law of June 13, 1917, concerning the combating of animal diseases, and the ordinance for its execution dated August 30, 1920. Provision is also made for partial compensation by the Federal Government for colonies destroyed in the enforcement of this law and the several ordinances. Under date of April 25, 1923, the details for the control of the Isle of Wight disease are set forth by the veterinary office of the Swiss Government (*69*), in which the work of control is assigned to the foulbrood inspectors of the several cantons and the methods of compensation for colonies destroyed are outlined. The importation of bees and combs is forbidden from March 15, 1923, and as no exceptions to this prohibition have appeared, the prohibition seems to be absolute. It is the evident intention of the Swiss Government to eradicate the Isle of Wight disease which has already appeared in that country and to prevent any further introduction of the disease by this rigid prohibition.

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ADDITIONAL REFERENCES.

Since the preparation of the manuscript of this circular in the spring of 1923, several important papers on the Isle of Wight disease have appeared and some new records of the occurrence of the disease have been published, so that it seems desirable to make additional record of these findings as this circular goes to press. The additional literature cited is appended. The same arrangement of material is followed here as in the main list.

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APPENDIX.

UNITED STATES DEPARTMENT OF AGRICULTURE,
OFFICE OF THE SECRETARY.
SERVICE AND REGULATORY ANNOUNCEMENTS.

REGULATIONS GOVERNING THE IMPORTATION OF ADULT HONEYBEES INTO THE UNITED STATES.

The Act of August 31, 1922 (Public No. 293—67th Congress), entitled "An Act to regulate foreign commerce in the importation into the United States of the adult honeybee (*Apis mellifica*)," provides as follows:

That, in order to prevent the introduction and spread of diseases dangerous to the adult honeybee, the importation into the United States of the honeybee (Apis mellifica) in its adult stage is hereby prohibited, and all adult honeybees offered for import into the United States shall be destroyed if not immediately exported: *Provided,* That such adult honeybees may be imported into the United States for experimental or scientific purposes by the United States Department of Agriculture: *And provided further,* That such adult honeybees may be imported into the United States from countries in which the Secretary of Agriculture shall determine that no diseases dangerous to adult honeybees exist, under rules and regulations prescribed by the Secretary of the Treasury and the Secretary of Agriculture.

SEC. 2. That any person who shall violate any of the provisions of this act shall be deemed guilty of a misdemeanor and shall, upon conviction thereof, be punished by a fine not exceeding $500 or by imprisonment not exceeding one year, or both such fine and imprisonment, in the discretion of the court.

In accordance with the foregoing Act, notice is hereby given that the following rules and regulations have been prescribed by the Secretary of the Treasury and the Secretary of Agriculture, the same to become effective on and after the fifteenth day of May, 1923.

HENRY C. WALLACE,
Secretary of Agriculture.

MAY 12, 1923.

RULES AND REGULATIONS.

The following rules and regulations are promulgated under the authority conferred by the Act of Congress approved August 31, 1922, providing for the regulation of foreign commerce in the importation into the United States of the adult honeybee (*Apis mellifica*):

REGULATION 1.—*Definition.*—For the purpose of these regulations, it is understood that a disease dangerous to the adult honeybee is one which attacks adult honeybees, as distinguished from one which attacks the brood or developmental stages of the honeybee. Such diseases of adult honeybees are understood to include all diseases which attack adult honeybees, including queenbees, worker bees, and drones or male bees: *Provided,* That the disease caused by the protozoan parasite, *Nosema apis,* sometimes known as Nosema-disease, now widespread in the United States, shall not be considered as a disease dangerous to adult honeybees for the purposes of these regulations.

REGULATION 2.—Since, in the opinion of the Secretary of Agriculture, the importation of queenbees, with necessary accompanying worker bees, is the only kind which is necessary for the improvement of the stock of honeybees within the United States, it is understood that, for the purposes of these regulations, such expressions as the "importation of honeybees" or "importation of adult honeybees" shall mean the importation of queenbees and the necessary accompanying worker bees, except as hereinafter provided.

REGULATION 3.—The importation into the United States of the honeybee (*Apis mellifica*) in its adult stage, except as hereinafter provided, is prohibited, and all adult honeybees offered for entry into the United States, except as hereinafter provided in these regulations, shall be destroyed if not immediately exported.

REGULATION 4.—On representation by any person to the Department of Agriculture that there is adequate necessity for the importation of adult honeybees for experimental and scientific purposes, from any country other than those determined by the Secretary of Agriculture to be free of all diseases dangerous to adult honeybees, the Department of Agriculture will undertake to import such adult honeybees under the first proviso of the Act for the purpose intended, when the Department shall determine that such importations can be made without risk to the beekeeping industry of the country.

(2) All shipments of adult honeybees made for experimental and scientific purposes shall be addressed to the United States Department of Agriculture, Washington, D. C., and shall be subject to such examinations and holding in quarantine as may be necessary to determine the freedom of the shipment from diseases dangerous to adult honeybees. It is understood, as a further precautionary measure, that the Department of Agriculture will destroy all the worker bees accompanying such imported queenbees and will provide fresh worker bees and a fresh mailing cage for each such shipment. Any such importation made for experimental and scientific purposes which is found to be infected with any disease dangerous to adult honeybees may be destroyed or returned to the country of origin, at the option of the Department of Agriculture and no shipment will be distributed until the Department of Agriculture is convinced that the adult honeybees therein contained are free from all dangerous diseases. Any persons receiving adult honeybees distributed by the Department of Agriculture shall agree to the re-examination of the shipment from time to time, at the option of the Department, and shall relinquish the shipment and any increase therein to the Department of Agriculture for destruction or safeguarding, should any diseases dangerous to adult honeybees at any time develop in connection with it.

REGULATION 5.—In accordance with the second proviso of the Act, adult honeybees may be imported into the United States from countries in which the Secretary of Agriculture shall have determined that there exists no disease dangerous to adult honeybees.

(a) The Secretary of Agriculture, having determined that no disease dangerous to adult honeybees exists in the Dominion of Canada and being advised that, under order of the Deputy Minister of Agriculture of the Dominion of Canada, dated April 22, 1922, the importation of bees, used and second-hand hives, and raw hive goods and products, except honey and wax, from the continent of Europe into the Dominion of Canada, is prohibited, does hereby authorize that adult honeybees, unrestricted as to the definition thereof contained in Regulation 2 hereof, may be imported from the Dominion of Canada into the United States or any of its Territories or Districts free from any restriction whatsoever provided in these regulations, until otherwise ordered.

(b) Importations under the second proviso of the Act, from any country other than the Dominion of Canada, shall be conditioned on the determination by the Secretary of Agriculture that, as a result of adequate scientific investigations, no diseases dangerous to adult honeybees exist in the country in question and that adequate precautions have been taken by such country to prevent the importation of adult honeybees from countries where such dangerous diseases exist.

REGULATION 6.—Nothing in these regulations shall interfere with the regulations of any state pertaining to the control of the diseases of bees, either of the adult stage or of the brood, and a removal of the restrictions of this Act as applied to any country shall not be construed as granting permission for importations prohibited by the laws of the state into which shipment is contemplated.

HENRY C. WALLACE,
Secretary of Agriculture.
A. W. MELLON,
Secretary of the Treasury.

UNITED STATES DEPARTMENT OF AGRICULTURE,
OFFICE OF THE SECRETARY.
SERVICE AND REGULATORY ANNOUNCEMENTS.

SPECIAL RULES

For the Importation of Queenbees for Experimental and Scientific Purposes by the Department of Agriculture in Accordance With Regulation 4 of the Rules and Regulations Prescribed by the Secretary of the Treasury and the Secretary of Agriculture and Made Effective as of May 15, 1923.

Regulation 4 of the rules and regulations prescribed by the Secretary of the Treasury and the Secretary of Agriculture in accordance with the Act of August 31, 1922 (Public No. 293—67th Congress), is as follows:

REGULATION 4. On representation by any person to the Department of Agriculture that there is adequate necessity for the importation of adult honeybees for experimental and scientific purposes, from any country other than those determined by the Secretary of Agriculture to be free of all diseases dangerous to adult honeybees, the Department of Agriculture will undertake to import such adult honeybees under the first proviso of the Act for the purpose intended, when the Department shall determine that such importations can be made without risk to the beekeeping industry of the country.

(2) All shipments of adult honeybees made for experimental and scientific purposes shall be addressed to the United States Department of Agriculture, Washington, D. C., and shall be subject to such examinations and holding in quarantine as may be necessary to determine the freedom of the shipment from diseases dangerous to adult honeybees. It is understood, as a further precautionary measure, that the Department of Agriculture will destroy all the worker bees accompanying such imported queenbees and will provide fresh worker bees and a fresh mailing cage for each such shipment. Any such importation made for experimental and scientific purposes which is found to be infected with any disease dangerous to adult honeybees may be destroyed or returned to the country of origin, at the option of the Department of Agriculture and no shipment will be distributed until the Department of Agriculture is convinced that the adult honeybees therein contained are free from all dangerous diseases. Any persons receiving adult honeybees distributed by the Department of Agriculture shall agree to the re-examination of the shipment from time to time, at the option of the Department, and shall relinquish the shipment and any increase therein to the Department of Agriculture for destruction or safeguarding, should any diseases dangerous to adult honeybees at any time develop in connection with it.

Information is not at present available as to the number of queenbees which are urgently needed for experimental and scientific purposes, but the number of such importations will necessarily be limited by the Department's facilities for examining the imported material and for keeping the imported queenbees in quarantine for such time as may be deemed essential. In order, therefore, to limit the volume of such importations and to insure that the queenbees shall be imported solely for experimental and scientific purposes, as provided by the law, and in order adequately to safeguard the beekeeping interests of the United States, the following special rules are announced to govern such importations:

(1) Importations will be limited to the following classes of institutions and persons:

(a) Public institutions, such as Agricultural Colleges, Agricultural Experiment Stations, and similar institutions, which desire to conduct investigations on the various races of honeybees, may obtain queenbees through importation by the Department of Agriculture for such experiments.

(b) An individual, who can show that he is engaged in some special field of experimental and scientific work in beekeeping or with honeybees, may, on a satisfactory showing of scientific training and experience requisite for such work, obtain queenbees through importation by the Department of Agriculture for that purpose, provided there is reason to believe that the proposed experimental and scientific work will have value as a public service.

(c) Commercial queen-breeders, who urgently need queenbees for breeding experiments, may apply to the Department of Agriculture to have the necessary importations made. Such an application shall contain, or be accompanied by, evidence that the applicant is engaged in the rearing of queenbees on a commercial scale and shall indicate the purpose of, and the necessity for, the importations. If an applicant is not well known to the Department, he may be required to submit a list of persons qualified to substantiate statements made regarding his ability and standing as a breeder of queenbees.

(d) The Department of Agriculture does not consider that the experimental and scientific purpose for which importations may be made under said Regulation 4 includes the importation of queenbees for individual beekeepers merely for the purpose of requeening their own apiaries. In case, however, queens of

certain races cannot be obtained in the United States, and the testing in the United States of such races would be of value to the beekeeping industry, the necessary importations will be made by the Department, provided that those who make request therefor, and to whom the queens are to be distributed for experimental and scientific purposes, will agree to report to the Department twice annually on the merits of such races in comparison with races already known in the United States. Applications for such importations must show that it is impossible to secure such queens from commercial queen-breeders in the United States.

(2) Persons, institutions, and others, in urgent need of imported queenbees for experimental and scientific purposes, may submit a statement of their needs to the Department of Agriculture, giving the name and address of the foreign queen-breeder from whom the queenbees are desired, and, if approved, the Department will transmit an order to the breeder in the foreign country from which such queenbees are desired. No orders for imported queenbees will be placed by the Department of Agriculture with any but experienced and recognized breeders of queenbees in foreign countries and evidence must be presented by the applicant that the foreign queen-breeder is qualified to rear good queenbees and to mail them in a satisfactory condition.

(3) In the event that importations are made and the queenbees die enroute, the Department of Agriculture assumes no responsibility whatsoever, either in the shipment of the queenbees from the foreign country to the Department of Agriculture or in forwarding the queenbees to the person at whose request the importation was made. Every care will be exercised so that the queenbees may be safely mailed under the restrictions laid down in said Regulation 4. All shipments of queenbees will be made in accordance with the regulations of the Post Office Department governing such shipments in domestic mails.

(4) All persons receiving queenbees from foreign countries distributed by the Department of Agriculture shall agree further to cooperate with the Department in such additional examinations of the colonies containing the imported queenbees or their offspring, as shall be deemed necessary to protect the beekeeping interests of the United States from the introduction of diseases dangerous to adult honeybees. In the event that any later examination of the offspring of the imported queenbees is deemed necessary by the Department, the person receiving the imported queenbees shall agree to furnish the bees desired promptly or to permit such examinations by a representative of the Department as may be deemed necessary. Any person receiving such imported queenbees from the Department of Agriculture shall further agree to notify the Department immediately if any abnormal conditions are seen in the adult honeybees in the colony headed by the imported queenbee, or in any other colony in the same apiary, so as to permit immediate examinations of any apparently abnormal adult honeybees.

The foregoing special rules are hereby adopted and shall be in force until further notice.

C. W. Pugsley,
Acting Secretary of Agriculture.

June 19, 1923.

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FUNGOUS DISEASES OF THE HONEYBEE

BY

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Assistant Apiculturist, Division of Bee Culture
Bureau of Entomology

UNITED STATES DEPARTMENT OF AGRICULTURE, WASHINGTON, D. C.

TECHNICAL BULL. No. 149 JANUARY, 1930

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

FUNGOUS DISEASES OF THE HONEYBEE[1]

By C. E. BURNSIDE

Assistant Apiculturist, Division of Bee Culture, Bureau of Entomology

CONTENTS

INTRODUCTION

The recognition and control of bee diseases is of prime importance in the commercial production of honey. The serious bacterial diseases of the brood have been extensively studied, and the life histories of their causative organisms and the methods for control are fairly well understood. There exist, however, other diseases of the brood and of adult bees, seemingly of lesser consequence, the causes of which are not yet known and for which satisfactory methods of control have not been determined.

The writer has shown in an earlier paper (*5*)[2] that a considerable number of species of fungi occur regularly on adult bees, and less frequently on their brood, which completely mummify all of the softer tissues. Most of these species of fungi are capable of establishing themselves on the brood combs under conditions that often pre-

[1] This bulletin, prepared in part fulfillment of the requirements for the degree of doctor of philosophy at the University of Michigan, is a joint contribution from the Bureau of Entomology, U. S. Department of Agriculture, and the University of Michigan. The experimental work was done in the laboratories of the department of botany of the University of Michigan and the bee culture laboratory of the Bureau of Entomology at Somerset, Md. The author acknowledges the valuable advice and assistance of C. H. Kauffman, of the University of Michigan, under whose direct supervision this work was done; of H. H. Bartlett, of the University of Michigan; of James I. Hambleton, senior apiculturist, in charge of division of bee culture, Bureau of Entomology; and of E. F. Phillips, formerly apiculturist in charge of bee culture investigations, Bureau of Entomology, and now professor of apiculture in the New York State College of Agriculture. Special acknowledgments are due to Charles Thom and M. B. Church, of the Bureau of Chemistry and Soils, for assistance in the identification of the forms used.

[2] Reference is made by italic numbers in parentheses to "Literature cited," p. 40.

vail within the hive during late winter and spring. The ecology of these fungi on bees in all stages and their relation to bee diseases have never been fully worked out.

During the past three years a mycological study has been made by the writer, in culture and on bees, of a considerable number of species of fungi that were isolated from adults, larvae, and combs. Experiments have been performed to determine whether the fungi that are commonly found on bees are purely saprophytic or whether, under conditions favorable for infection, they can attack and kill healthy bees or brood.

HISTORICAL

The existence of diseases of bees was first recorded by writers before the beginning of the Christian era, but the descriptions are too meager to identify them. The more careful study in Europe of diseases of brood dates from the work of Schirach (*21*) in 1771, but for more than a century following it was quite generally believed that there was only one such disorder. Succeeding reports in Europe supported this view. During the decade just preceding the twentieth century American beekeepers came to believe that more than one disease of the brood existed, and that these diseases were of decidedly different characters. This has been conclusively proven in the United States, chiefly by the investigations of White, who describes and figures two bacterial brood diseases, a filterable virus disease, and one protozoan disease of adult bees, in a series of papers dating from 1917 to 1920. The diseases described by him are sacbrood (*28*), Nosema disease (*29*), American foulbrood (*30*), and European foulbrood (*31*). More recent valuable additions to the knowledge of brood diseases and their control have appeared in the publications of Sturtevant (*22*, *23*), and Zander (*32*, *33*).

The study of diseases of bees due to fungi has been much later in its development than the study of those caused by bacteria. Much the greater share of the attention of investigators of bee diseases has been given to the two serious bacterial brood diseases, American foulbrood and European foulbrood, caused respectively by *Bacillus larvae* and *B. pluton*. In America only two reports are on record of diseases of bees and their brood caused by hyphomycetous fungi. In 1896 Howard (*14*), of Texas, described a new brood disease which he called "pickled brood or white fungus," caused by a species of Aspergillus to which he gave the name "*Aspergillus pollini*." Two years later he described (*15*) the same disease as occurring in both pupae and adult bees and stated his belief that this disease had been mistakenly diagnosed by beekeepers as paralysis.

In both reports Howard gives descriptions and illustrations of the disease, which he called "pickled brood or white fungus," which are more readily applicable to the disease known as sacbrood than to those caused by fungi. He ascribed this disease to the pathogenic Aspergillus, *A. pollini*. The dead larvae are described as at first white and watery, later becoming black and swollen, and finally drying down to black scales. In no case was the fungus, which Howard assumes to be the cause of the disease, observed on the larvae, and isolations of the organism were not made. In adult bees the disease is described as causing black, shiny, apparently frozen abdomens.

The affected bees become much weakened, are capable of only feeble, trembling movements, and finally die. As in the case of the brood killed by the "pickled brood" disease, the fungus was not observed on or within the tissues of the infected bees, but when cultures were prepared from the alimentary canals of these bees, *A. pollini* developed constantly to the exclusion of all other fungi.

It appears probable, therefore, that Howard may have observed one or more species of Aspergillus on brood combs and succeeded in culturing these, or other species, from the alimentary canals of adult bees. His technical descriptions of *Aspergillus pollini* are entirely too meager to make it possible to determine which of the numerous species of Aspergillus were observed. If they are pathogenic for bees, it is evident that he either did not observe a true mycosis of bees or confused the condition with other disturbances.

Diseases of bees caused by fungi have not been reported from North America since then. This may probably be attributed to the fact that fungous diseases of bees appear less destructive than the common bacterial diseases of bees and seldom become epidemic.

In Europe, on the other hand, two species of fungi, *Aspergillus flavus* Link and *Pericystis apis* Maassen, are widely recognized as the causative organisms of diseases of brood and adult bees. Of these, *A. flavus* is considered of the greater economic importance, since it attacks worker brood and adult bees, whereas *P. apis* usually attacks only drone brood. The two brood diseases stone brood (Steinbrut), caused by *A. flavus*, and chalk brood (Kalkbrut), caused by *P. apis*, have received more attention from European beekeepers and investigators than has the disease of adult bees caused by *A. flavus*. This might readily be expected as a direct result of the nature of the diseases and the reaction of infected adult bees. Pericystis mycosis is mentioned by Claussen (*8*), Bahr (*2*), and Morgenthaler, Kessling, and Hunselmann (in communications with Claussen). Claussen describes it as benign and transient rather than malignant, affecting capped as well as uncapped drone brood and passing over to worker brood in severe or exceptional cases. He states that dead and diseased larvae may be thrown out of the hive by the bees or allowed to remain in the brood combs, where they become mummified after they are overgrown with white mycelium. The bees, however, usually allow any brood killed by *A. flavus* to remain in the combs for a considerable length of time, or at most only partially remove it, since destruction of the cell walls is often necessary for complete removal.

Slight infection among the brood quickly attracts the attention of an observing beekeeper, whereas he may completely overlook a considerable number of adult bees dead of this disease, owing to the fact that during the active season worn-out field bees die normally in considerable numbers about the hive. The writer, therefore, believes that the importance of Aspergillus mycosis as a brood disease may have been overestimated in comparison with its importance as a disease of adult bees.

Recent research in Europe seems to indicate that other fungi than the foregoing may under favorable conditions infect and kill bees and their brood. Fielitz (*10*), working with three fungi, *Trichoderma lignorum* Tode, *Mucor mucedo* Linné, and *Penicillium glaucum*

Link, found on mummified bees in Germany, was able to infect capped and uncapped brood and adult bees by artificial inoculation with the first two. *T. lignorum* was shown to be capable of becoming actively pathogenic when introduced into healthy colonies on brood combs. When similarly introduced, *M. mucedo* attacked and killed brood in capped cells and an occasional adult bee. In his experiments with *P. glaucum* neither the bees nor their brood were attacked, although it is one of the commonest organisms in the hive.

While making anatomical studies of honeybees affected with constipation, Lardinois (*17*) claims to have observed *Saccharomyces apiculatus* (Reess) Hansen constantly associated with lesions in the tissues. The same organism occurred in the intestines of dead pupae that were thrown out of the hive, in the food of larvae, and in honey. Lardinois asserts his belief that *S. apiculatus* is the sole cause of May disease and that disturbances commonly recognized as constipation, paralysis, convulsions, staggering, malformation, and death of brood are, in reality, all forms of the disease which he called saccharomycosis. He does not support these conclusions by inoculation experiments but draws them solely from the fact that this yeast occurs in "lesions" and in the intestine of bees affected with "May disease." Doctor Lardinois believes, however, that these conclusions may be easily verified.

One is quickly led to believe that bee diseases caused by pathogenic fungi may be more widespread than is commonly supposed. A comparison of conditions in Europe, where a number of recognized fungi are known to cause diseases of bees, with conditions in this country would make it seem likely that the same fungi are capable of attacking bees here. The publications of Thom and Church (*24; 25, p. 200*) show that a great number of strains of *Aspergillus flavus* are found in America. *Mucor mucedo* and *Trichoderma lignorum* also occur elsewhere than in Europe. *Pericystis apis* and *P. alvei* Betts, which, according to Claussen (*8*), differ in certain morphological characters and in their ability to attack brood of bees, have never been reported from North America. Forms of *Saccharomyces apiculatus*, which Lardinois has stated cause disease of bees in France, are widely distributed in North America. The writer has frequently found these forms in North America within the alimentary canal of bees and in honey.

INVESTIGATIONS WITH PATHOGENS

DESCRIPTIONS AND LOCATIONS OF APIARIES USED

Apiaries located in the vicinity of Bronson, Mich., were used in these investigations for securing specimens of fungi and for making tests for pathogenicity and control. These apiaries are situated on boulder clay and sandy drift formations in a general farming community. Three honey flows occur annually in the region, providing a plentiful supply of stores at all times when weather conditions are favorable.

The experimental apiary of the botanical laboratory of the University of Michigan at Ann Arbor was also used in these investigations. This apiary was located on the roof of the Natural Science Building, thus affording excellent conditions for recovering diseased

bees. With the beginning of cold weather the colonies were placed in an attic where a temperature of about 55° F. was maintained, affording facilities for manipulating the bees during cold weather without danger of chilling them. The general conditions here with respect to honey flows and weather factors are essentially the same as at Bronson, excepting that here the early spring honey flow is somewhat heavier on account of the surrounding fruit farms. On the other hand, the fall honey flow from wild flowers is somewhat less.

Two apiaries of the bee-culture laboratory of the Bureau of Entomology at Somerset, Md., were used during the summers of 1924, 1925, and 1926. One of these was located at the laboratory, while the other was about a quarter of a mile away. Forest trees, particularly species of Acer and Salix, provide a source of nectar during early spring, but the main honey flow is from the tulip tree (*Liriodendron, tulipifera*). Occasionally basswood (Tilia), spruce (Picea), and locust (*Robinia pseudoacacia*) yield appreciable quantities of nectar. The fall flow here is light and of short duration.

RACES OF BEES

The bees used for the tests for susceptibility were for the most part Italians and Italian hybrids, including workers, drones, and queens. No other races were used for inoculation experiments. Pathogenic organisms were isolated, however, from Carniolan bees.

The colonies used for inoculation experiments were, most of them, in 5-frame hives (nuclei) containing three or more frames of brood. Normal colonies in standard 10-frame hives were also occasionally used.

THE FUNGI STUDIED

The fungi used were isolated from bees collected from widely different sources.

The greater number of forms were from bees from the experimental apiaries. The first isolations of fungi were made from dead bees and their brood collected at Bronson, Mich., during the early brood-rearing season of 1924, and others were made from bees collected at this same apiary during the spring of 1925 and that of 1926. During the summers of the three years that this work was in progress fungi were collected from the apiaries of the bee-culture laboratory of the Bureau of Entomology. Among the forms obtained here were duplicates of most of those collected at Bronson and elsewhere. Important collections were also made from the experimental apiary at the University of Michigan during the academic years of 1924–25 and 1925–26.

In addition to the collections from the experimental apiaries, fungi have been isolated from samples of bees or brood sent to the bee-culture laboratory from all of the important beekeeping regions of the United States. Although the forms isolated from such samples duplicated those obtained from the experimental apiaries, their presence gave an indication of the extent of the range of these forms. All of the Aspergilli in the following list appeared with sufficient regularity to indicate their distribution among bees in all parts of the United States, and two species, *A. flavus* and *A. fumigatus*, were isolated on several occasions from bees imported from Europe. The

yeasts were also isolated from bees from numerous sources within the United States. *Trichoderma koningi* was isolated from adult bees in Maryland and from mummified larvae from Oregon. *Pericystis apis* was also isolated from mummified brood sent from England and from Germany.

The fungi included in the following list were found to attack and kill bees when the latter were inoculated experimentally. Among the Aspergilli, with the exception of *Aspergillus oryzae* and *A. parasiticus*, many cultures other than those indicated were isolated from bees and tested for parasitic properties. This is true especially of the *A. flavus-oryzae* and the *A. fumigatus* groups.

Aspergillus flavus (Link), sensu Thom and Church. Author's collection: 1, 3, 4, 5, 7, 9, 12, 28, 12340. Thom and Church collection: Ao5c, 183, 108.

Aspergillus oryzae, (Ahlburg, Cohn), sensu Thom and Church. Thom and Church collection: 113 L, Ao5b.

Aspergillus effusus, VIII; D. C.

Aspergillus parasiticus Speare.

Aspergillus flavus-oryzae, sensu Thom and Church. Thom and Church collection: Aop, Apb, Aob, Ao5a.

Aspergillus fumigatus (Fresenius), sensu Thom and Church. Author's collection: 1, 33, 12287, 12288. Thom and Church collection: Yates IV, 118, 4063–c–18.

Aspergillus nidulans (Eidam), sensu Thom and Church. Author's collection: 1. Thom and Church collection: 110, 4010.4, 4415.

Aspergillus ochraceus (Wilhelm), sensu Thom and Church. Author's collection: Conn.; D. C. Thom and Church collection: 112, 2399, 4020.4, 4065.1, 4640.476, 4640.483.

Aspergillus glaucus (Link), sensu Thom and Church. Author's collection: 1, Ann Arbor, Mich. Thom and Church collection: 3528.7.

Saccharomyces ellipsoideus Hansen.

Saccharomyces cerevisiae Hansen.

Saccharomyces apiculatus (Reess), Hansen.

Mycoderma cerevisiae (Desm), Hansen. Author's collection: I, II.

Torula sp.

Mucor hiemalis Wehmer.

COLLECTION OF FUNGI FROM COLONIES AND FROM INDIVIDUAL BEES

Fungous pathogens of honeybees were collected from colonies and from individual bees in the vicinity of experimental apiaries. Early in the spring the interior of the hives and the ground about the apiary were examined for infected bees. Similar examinations were continued during the summer and fall, and less frequently during the winter in colonies wintered indoors. Adult bees, including queens and drones, with fungus-infected tissues or with conidiophores and conidia upon their outer surface yielded pathogenic forms. Crawling bees, unable to fly and apparently diseased, were collected. These bees were either used immediately for isolation experiments or were caged, under conditions favorable for the continued development of fungi, for examination later. Usually the greater number of such bees died within a short time after having been caged. At other times their condition appeared not to shorten their lives. The number of infected bees appeared to increase noticeably after warm summer rains and those collected yielded a higher percentage of pathogenic organisms. Fungus-infected larvae were found in weak colonies in early spring. Infected larvae were also found in colonies in which facultative parasites were growing on the brood combs. It

was not uncommon to find brood attacked during the spring in colonies which, owing to weather conditions or other factors, had a high mortality when normally the population should have been increasing. The quantity of diseased brood was found to be less during dry weather. At all times of the year strong colonies were found to be relatively free from pathogenic fungi.

EXAMINATION OF DISEASED BEES AND ISOLATION OF PATHOGENIC FUNGI

The methods employed in the isolation of pathogenic fungi from bees must depend to a considerable degree upon the species of infecting organisms and the extent and state of their development. Samples of dead bees or bees in various stages of development of the disease were therefore examined microscopically to determine the stage of growth of the fungus. Isolation of filamentous pathogens was least difficult when spores practically free from contaminating organisms were present upon the body of the bees. The tip of an inoculating needle drawn to a sharp point was moistened by dipping it into sterile agar. The tip of a spore-bearing branch with mature spores was lightly touched and a few spores transferred to an agar plate. Pure cultures of the organism desired were often obtained by this method without additional effort. When more than one fungus was fruiting upon the body of the bee or even when scattered spores of other fungi were present, this method usually resulted in mixed cultures. Pure cultures of the Aspergilli, which are mostly rapid growers, were often obtained from mixed cultures by cutting off the tip of a young mycelium and transferring it with a small quantity of agar to a fresh plate. When contamination with other fungi was heavy, or when the contaminating organisms grew more rapidly than the fungus to be isolated, the dilution-spray method devised by Kauffman (*16*, *p. 364*) was employed with good results. A suspension of conidia was first made in a tube of sterile tap water. This was diluted until droplets blown from a glass tube drawn to an extremely fine bore at the tip contained an average of about one spore. One or two plates of nutrient agar were sprayed with this suspension, the spray being so regulated that droplets on the agar remained separated by a distance of about 3 millimeters. When germinating spores first appeared single spores with germ tubes were cut and transferred with small pieces of agar to separate poured plates. Two or more such cultures were prepared in each instance. When contamination occurred transfers were made of the few hyphae from the single spore mycelium as soon as the contaminating organism became visible.

When spores were present only within the exoskeleton of the bees, and especially when within the alimentary canal, isolation of pure cultures was difficult on account of the presence of great numbers of bacteria. When these conditions were met with, the acidity of the nutrient agar upon which the suspension of spores was sprayed was made slightly greater with tartaric acid, and the cultures were kept at a temperature of about 10° C. until the spores had germinated. Though the growth of the fungus was retarded when kept at this temperature, the growth of bacteria was practically inhibited. Single germinating spores were then cut out along with any adhering

bacteria and transferred to sterile plates. These cultures were kept in the cold room until fungus mycelium had grown to a distance of several millimeters beyond the zone of bacterial growth. The tips of hyphae, apparently free of bacteria, were then cut off and transferred to sterile plates. This process usually resulted in pure cultures. Whenever bacteria appeared, the process was repeated as often as was necessary.

A method modified somewhat from the foregoing was used when only mycelium was present within the tissues of the bees. Small pieces of tissue containing mycelium were placed on plates of nutrient agar and kept at 10° C. It was not always necessary to place these cultures in the cold room when they could be closely watched and isolations made from the first hyphae that grew into the agar.

Hyphae grew out rapidly from the infected tissue, and the tips of the first appearing hyphae were cut off and transferred before bacteria or mycelium from spores could spread beyond the point of inoculation. Pure cultures were made from these after spores had matured.

The isolation of yeasts from the alimentary canal of the bee required a more delicate technic than the isolation of filamentous fungi because of the presence of other contaminating forms. When yeasts occurred within the tissues or blood of bees they were readily isolated since they were usually found here in pure or nearly pure culture. In the isolation of yeasts a sufficient quantity of acid was added to the medium to retard the growth of bacteria, thus facilitating the isolation of pure yeast cultures.

To obtain pure yeast cultures the spray method described above was used. A suspension of yeast cells was sprayed on beer-wort agar or on Leonian's agar in Petri dishes. The location of the droplets after evaporation was marked by a perceptible deposit of lime from the tap water. After from 12 to 24 hours these spots were examined under the microscope, and those that contained yeast colonies developed from single yeast cells were marked by scratching the agar about them with a sterile needle. When these colonies had developed to contain several hundred cells second dilutions and sprays were made from them. From these cultures, or from similar succeeding ones when bacterial contamination was heavy, yeast colonies free from bacteria were obtained. Colonies, the origin of which could be traced to a single cell, were chosen for isolation a second time to avoid the possibility of mixed yeast cultures. When the original culture was obtained from the rectum or ventriculus of a bee contamination with bacteria was usually heavy, and several repetitions of the process were often necessary to obtain bacteria-free cultures.

CULTURE METHODS

For the culture of the organisms studied the usual mycological equipment was used.

Erlenmeyer flasks of 500 and 1,000 cubic centimeter capacity, filled to a depth of about 3 centimeters with liquid nutrient media, were used for the culture of pathogenic organisms in experiments to determine the production of metabolic toxins. Flask cultures iden-

tical with these were also prepared to inoculate large quantities of nutrient solution to be used in the preparation of brood-comb cultures.

PREPARATION OF THE CULTURES

All of the fungi tested and found to be pathogenic for bees were cultured on one or more of the solid or liquid nutrient media used. Cultures of these fungi were prepared by inoculating the nutrient medium in the culture dish with a single spore or with spores or mycelium from pure cultures. Normal room temperatures were generally used although cold rooms and electric ovens were employed at times. Combs were sterilized in a 20 per cent aqueous solution of formaldehyde. Two liters of nutrient solution containing spores of the desired organism taken from a pure culture were poured into each comb. These combs were kept in a sterile moist chamber until spores had matured over the greater part of their surface. Practically pure cultures were obtained by this method. The few contaminating spores which settled on the combs while the spore suspension was being poured were soon overgrown and rarely matured.

It was found better to keep the moist chambers out of doors while the fungi on the combs were developing, or at least in rooms where beekeeping equipment, particularly extracting and brood combs, were not stored, since under proper atmospheric conditions the combs are overgrown with fungi, principally Penicillia and Aspergilli. An abundance of spores of such pathogenic organisms within the room will add to the danger of spreading the molds to other combs.

One strain of *Aspergillus ochraceus* was found to produce but slight growth on liquid media. Cultures of this organism on combs produced few or no spores and were usually soon overrun by species of Penicillium. Inoculation of colonies with this organism were generally unsuccessful. This difficulty was overcome by culturing the organism on heavy sheets of reinforced blotting paper. The blotting paper was first sterilized and then saturated with nutrient agar before inoculation by spraying with a suspension of spores. After a crop of spores had matured the sheets of blotting paper were suspended in the hive containing the colony to be inoculated.

THE MEDIA EMPLOYED

Several nutrient media, both synthetic and natural, were used during this work for the artificial culture of the organisms. Solid media which were used extensively for isolation, study, storage, and shipping purposes, were prepared by the addition of agar, and less frequently gelatin, to the nutrient solutions. The agar and gelatin were omitted in preparing cultures for inoculation purposes when it was desired to have a large quantity of spores that could be readily freed from the medium. Liquid nutrient media were also used when the object was to extract toxic products of pathogenic organisms, and for the culture and study of yeasts. Natural media, such as potato, carrot, and milk were rarely used. Occasionally liquid media to which a small quantity of tartaric acid was added were used when it was desired to eliminate bacteria from the yeast cultures. The media used in the course of these investigations are described on the pages that follow.

MALTOSE AGAR

Maltose	grams	5.00
Magnesium sulphate	do	.10
Calcium nitrate	do	.50
Potassium phosphate, dihydrogen	do	.25
Agar	do	15.00
Water	liter	1

Maltose agar, prepared according to the foregoing formula, which is the standard culture medium in use in the cryptogamic laboratory at Ann Arbor, Mich., was used for isolation and culture of filamentous fungi and for isolation of yeasts. This agar remains clear and light colored and may be autoclaved at 15 pounds for 20 minutes, or even at higher temperatures and for longer periods, without hydrolysis sufficient to prevent solidification.

Single germinating spores are easily located when sprays are made on maltose agar, and transfers are facilitated on account of its firmness. Most of the pathogenic filamentous fungi used in these investigations grow well on it and produce spores abundantly. The yeasts grow slowly, and for this reason it serves well for their isolation, but it is unsatisfactory for culturing, and for the study of these organisms.

LEONIAN'S NUTRIENT AGAR

Potassium phosphate, dihydrogen	grams	1.25
Magnesium sulphate	do	.625
Peptone	do	.625
Maltose	do	6.250
Malt extract	do	6.250
Agar	do	13.00
Water	liter	1

Leonian's agar is darker and softer than maltose agar and is therefore less desirable for the isolation of single germinated spores. On account of its softness, cultures do not ship well on it unless partially dried. It was found to give the best results for the isolation of filamentous pathogenic fungi from the infected tissues of bees. Hyphae from small pieces of infected tissue spread more rapidly into this agar than into peptone agar; consequently the danger of contamination from germinating spores of fungi other than the one desired is lessened, since spores appear to germinate with equal rapidity on either of these agars. Conidia of the filamentous fungi form early and abundantly on Leonian's agar, and all of the yeasts encountered in these investigations grew well on it.

CZAPEK'S SOLUTION AGAR

Sodium nitrate	grams	3.00
Potassium phosphate	do	1.00
Magnesium sulphate	do	.50
Potassium chloride	do	.50
Ferrous sulphate	do	.01
Sucrose	do	30.00
Agar	do	15.00
Water	liter	1

All of the Aspergilli considered here and described by Thom and Church (*25*) were grown by them upon this medium. It was therefore used for the culture and identification of all of the forms of Aspergilli that have been found to be pathogenic to honeybees.

Isolations of single spores can be made as readily from Czapek's solution agar as from maltose agar, consequently it was frequently used for this purpose.

Beer-wort Gelatin

For the study of yeasts, beer wort was prepared from malted barley and hops as follows: Two hundred grams of the finely ground barley was soaked for an hour, with occasional stirring, in a liter of distilled water at 60° C. Four grams of hops was then added and the mixture boiled for an hour during which time it was stirred at intervals of a few minutes. Water was added from time to time to maintain the original volume. The barley meal was then separated from the liquid by straining it through closely woven cloth, and the liquid was cleared with the white of eggs. The quantity of maltose present was determined, and water was added until the liquid beer wort contained approximately 3 per cent of this sugar. Gelatin was added to the clear beer wort when a solid medium was desired.

Potato Media

Culture media for yeasts were prepared by cutting pieces of potatoes the proper size for the culture dish and sterilizing them after stoppering or covering. Potato-broth agar was prepared at first for the purpose of storing yeasts, but since there was no particular advantage in its use, it was replaced with beer-wort gelatin.

Liquid Nutrient Media

Synthetic and natural liquid nutrient media were prepared according to the formulas just given under solid media, except that agar and gelatin were omitted. Honey, dextrose, and levulose were sometimes substituted for the sugars given in these formulas. Beer wort was used extensively in the study and identification of yeasts, since most of the morphological and cultural characteristics of yeasts reported in the past were made from growth in this nutrient medium.

Sucrose, dextrose, levulose, maltose, galactose, dextrin, inulin, raffinose, d-mannose, and l-arabinose were tested in liquid media to determine the ability of the yeasts listed on page 6 to ferment these sugars.

EXPERIMENTS WITH BEES

PRELIMINARY INOCULATION WITH MOLDED COMBS

In a previous study of the fungi associated with honeybees (*5*, *p. 63*, *68*), it was observed that species of two groups, the Penicillia and the Aspergilli, occur with greater frequency than do other forms. These fungi, Penicillia and Aspergilli, were found within the bodies of bees where the mycelium had permeated all of the softer tissues, resulting in a characteristic mumification. Formation of spores of these fungi was observed to occur both within the exoskeleton and upon the exterior. After these observations a question arose as to the relation of these organisms to the honeybee. Was the bee attacked before death, or afterwards? If before, death might have resulted from infection by the fungus.

To determine this point preliminary experiments were conducted in the writer's apiary at Bronson, Mich., during the spring of 1924. A search was made of the entire apiary, and the combs showing the best growth of mycelium and spores of Penicillia and Aspergilli were selected. Both brood combs and extracting combs were found to contain these forms. Brood combs overgrown with Penicillia were readily found in colonies that had wintered badly or had died during the winter. Combs even partially covered with Aspergilli were less abundant. In order to find a sufficient number of combs containing Aspergilli, it was necessary to examine not only the brood combs but the extracting combs as well.

The combs containing the fungi to be tested for pathogenicity were placed in weak, but apparently healthy, colonies. As a result of these inoculations a varying degree of loss of bees was noticeable, and in one case the death of the entire colony resulted. The infected bees appeared to die at a considerable distance from their hives, and dead bees were not found in unusual numbers on the bottom board or near the hives. Numerous bees unable to fly were found crawling about the apiary during the time the inoculation experiments were in progress. It was found that about 50 per cent of the dead bees collected from the fronts of the colonies that had been given infected combs produced conidiophores of apparently the same species as those used in the inoculations. In order to promote the production of conidia the dead bees were kept in moist chambers at room temperature for from 5 to 7 days.

The results obtained in these preliminary experiments were considered sufficient to warrant further study. The experiments which follow were therefore made with pure cultures of a considerable number of fungi suspected of possessing qualities pathogenic to bees.

INOCULATION EXPERIMENTS WITH PURE CULTURES

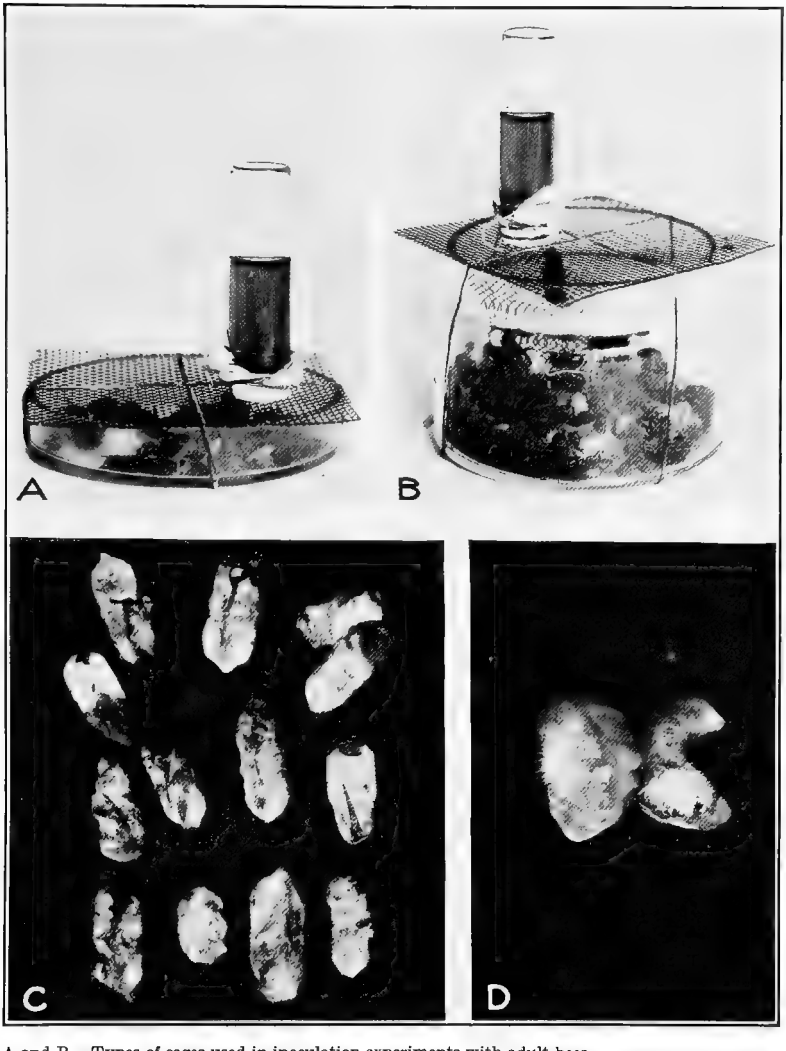
Experiments with pure cultures of the organisms were of two general types. In the one, adult bees were inoculated and kept in cages during the experiment. In the other, large quantities of the inoculum were used on bees in normal colonies.

EXPERIMENTS WITH CAGED BEES

The types of cages used for confining the bees during the experiments are shown in Plate 1, A and B. The cage shown in A was found to be the most convenient, as the removal of individual bees was facilitated. In this cage, also, the food supply was easily reached by the bees.

Some trouble was experienced at first in devising a method of feeding that would furnish the bees an abundant supply of food at all times without danger of daubing them with it. This smearing invariably happened in the use of candy in culture dishes when the nutrient agar was not thoroughly dried, since water was rapidly taken up from the agar by the candy.

A satisfactory method was finally found and used in the succeeding experiments. Small vials were filled with sugar solution or honey and covered with one or more thicknesses of cheesecloth fastened with rubber bands. These were inverted on the screen covers



A and B.—Types of cages used in inoculation experiments with adult bees.
C.—Brood dead of Aspergillus mycosis produced experimentally by inoculation with a culture of *Aspergillus effusus* on a brood comb.
D.—Larvae recently dead of Aspergillus mycosis caused by the penetration of the larval skin by germ tubes of *A. flavus*.

of the cages so that the food could readily be reached by the bees. (Pl. 1, A and B.) The number of thicknesses of cheesecloth required depended upon the density of the sirup used. Heavy honeys required but one thickness, but for dilute sugar sirups two or more thicknesses of cloth were necessary to prevent leakage.

Bees in cages were inoculated by being placed on cultures of the fungi or by mixing the spores with their food. Within the laboratory, where cultures of fungi were kept, it was unsafe to inoculate bees, by the first of the two methods, with fungi that form dry and dustlike spores; for by fanning their wings the bees sent a dust of spores into the air. Consequently, inoculations were made outside of the laboratory. When spores of fungi were mixed with the food given to the bees the danger of contamination of cultures within the laboratory was largely avoided. Abundant space outside of the laboratory being available, however, the first method of inoculation was generally used, since it probably approached nearest to the natural methods by which bees become infected. Complete inoculations resulted from placing the bees for one or more minutes on plate cultures that had matured a good crop of dry spores. After inoculation the bees were either removed to sterile dishes or caged, until the experiment was complete, on the culture upon which the inoculation was made. A cone made by cutting off the smaller end of a funnel, so that a hole with a diameter of about 1½ inches was left at that end, was used for transferring the bees from the cultures to the cages. The small end was placed over the cage, and the culture dish containing the bees was opened quickly and inverted in the mouth of the funnel, completely closing it. When the bees had entered the cage the cone was removed and the screen cover quickly placed in position and fastened with rubber bands. After a little practice this operation of transferring could be successfully performed without injuring the bees, success depending upon speed and precision of manipulation.

As a measure of precaution, when several fungi with dustlike spores were being tested at the same time, inoculations on dried cultures were made out of doors and the cages placed in different rooms, or in different parts of the same room. This prevented, to a large extent, infection with pathogenic fungi from other cages. When this precaution was not observed, a high percentage of infection, at times reaching more than 70 per cent, could be traced directly to the crossing over of spores from adjacent cages. When spores of fungi were mixed with the food given to the bees these precautions were unnecessary.

Selection of Bees for the Experiments

Old bees from the field force of a colony die rapidly when caged, even under conditions of temperature, moisture, etc., that duplicate those of the hive. Their early death when caged appears to be due in part to continued exertion in an effort to escape. Records of the death rate among infected bees are therefore unsatisfactory when field bees are used. Young bees for caging were obtained by setting aside frames of emerging brood a few days before bees were needed, or by lightly shaking frames of bees to rid them of most of the old bees, as young bees cling more tightly to the combs than do the older

ones. Young bees are also more easily handled, since they are less excitable when caged. Young drones and queens, which may or may not have recently emerged, are quite as satisfactory for these experiments as are worker bees, and were frequently included.

EXPERIMENTS WITH COLONIES

Colonies were inoculated by placing matured dry cultures of fungi on brood combs or on strips of blotting paper between the brood combs. One or two of these cultures were put in each of the experimental colonies. Complete infection was obtained somewhat sooner with two comb cultures than with one, though in order to secure complete infection of a colony it was necessary to use cultures with an abundance of dry spores on the greater portion of the surface. Spores were soon spread to all parts of the hive by the worker bees and after three or four days were found in the food of the brood and in the alimentary canal of all the adult bees examined. Colonies were less frequently infected by mixing spores with dilute sugar sirup and confining the bees to the mixture for food.

BLOOD INOCULATION EXPERIMENTS

The blood of the honeybee is well protected from the entrance of microorganisms by the tough exoskeleton. As far as is known, infection by pathogenic organisms occurs only by way of the alimentary canal. Practically nothing has been done to determine the resistance, or lack of resistance, of the blood of the honeybee to infection by microorganisms. A series of blood inoculations of adult bees was therefore made with the fungi used in these investigations.

While they were being inoculated the bees were held between the thumb and the second finger, with the wings turned upward and held with the fore finger. The propodeum was moistened by lightly rubbing it with a wad of cotton or filter paper saturated with a water suspension of the organism under observation until a film was spread over the surface. This portion of the exoskeleton was carefully punctured with a fine-pointed sterilized needle. When the needle was withdrawn the water containing the spores was spread into the wound. As an alternative for the first method, the needle was dipped into the suspension of spores and the tip inserted into the wound a second time.

Checks, to determine the effect of the punctures upon the bees, were prepared by a similar treatment, except that spores were omitted from the water with which the propodeum was moistened or in which the needle was dipped. Inoculations were also made at other points on the body, but fatal injuries resulted more often when inoculations were made in places other than the propodeum. Inoculated and check bees were kept in cages until the experiment was completed.

EXPERIMENTS WITH BROOD

In addition to the inoculation of colonies in which spores reached the food of all uncapped brood, uncapped larvae were inoculated directly from plate or tube cultures. Dried cultures in Petri dishes or tubes were scraped with a scalpel or inoculating needle to loosen

and separate the spores. The dry spores were then shaken only lightly over the brood. If too many spores were shaken into the cells, the worker bees set to work at once to clean them out, and sometimes removed the brood before any infection could be determined. If the inoculated brood is not removed by the workers within 24 hours, it is usually allowed to remain in the comb unless death from infection occurs.

A somewhat surer method was used with fungi that do not produce abundant spores, and when the evidence obtained with dry spores was not considered conclusive. Water suspensions of spores were prepared and small drops added to the larval food with a pipette drawn to a slender tapering point. Larvae were rarely removed from the cells following this treatment except after infection occurred and death had resulted. Close observation, however, was always necessary to determine the exact fate of the inoculated brood. In strong colonies the workers often remove the brood before infection can result if the inoculation is heavy, or they may remove them from the cells, often piecemeal, before symptoms of the disease appear. This method offers quick and easy means, however, of testing the pathogenicity of a large number of organisms for brood of bees.

While examining brood inoculated by the methods just described it was occasionally noticed that tufts of mycelium appeared on the brood before other symptoms of diseases had developed. (Pl. 1, D.) Microscopical examination of such larvae showed that these tufts had developed from mycelium beneath the skin of the larvae. This mycelium was much more abundant in the tissues beneath the tufts than elsewhere. If infection occurred within the alimentary canal one would expect to find mycelium more extensively developed there, at least immediately following infection, than in the tissues just beneath the larval skin. It appeared that the mycelium observed on a few larvae soon after inoculation originated from infection through the skin rather than from within the alimentary canal.

To find whether germ tubes from spores germinating upon the moist skin of larvae can penetrate the skin to the tissues beneath, masses of spores, each about the size of a pinhead, were smeared on a number of larvae in brood combs. The position of these within the comb was marked by removing the brood from the two surrounding rows of cells. The infected larvae were examined twice daily during the three days following. In another method that was used larvae were removed from the comb and kept in watch crystals on a 70 per cent solution of sugar sirup or honey during the experiment. Masses of spores were placed on the larvae in the same manner as if they had been left in the comb. The watch crystals with the larvae were kept between observations in an incubator at 36° C. This method was finally used to the exclusion of the former, for when the larvae were left in the hive, spores placed upon the skins were often removed by the workers or mixed with the food in the cells as the result of movements of the inoculated larvae. This frequently resulted in infection through the alimentary canal. If care was used in placing the moist mass of spores on the skin of the larvae in watch crystals, spores rarely reached the alimentary canal. Larvae move very little when on sirup; consequently the spores remained in the position in which they were placed.

SECONDARILY INFECTED BEES

The accurate determination of the effects of an inoculation upon adult bees demands frequent observations and records. If examination is delayed beyond two days after death, secondary organisms may materially affect the symptoms and obscure the pathogenic organism. Examination during the last stages of disease before death, or immediately after death, furnishes the most conclusive evidence of the pathogenic relations of an organism. The presence of vegetating microorganisms in bees at this time is proof of their growth and multiplication within living bees. As apparently normal adult bees harbor large numbers of certain bacteria and yeasts within the alimentary canal, particularly within the ventriculus and the small and large intestines, their presence is of little pathogenic significance.

On the other hand, much weight may be given to the presence of vegetating organisms of filamentous fungi within the alimentary canal of living bees. The presence of vegetating organisms of any sort within the blood or tissues is evidence of their pathogenic growth, since the tissues and blood of healthy bees are always sterile. Under favorable conditions, however, they may be invaded after death by organisms present within the alimentary canal.

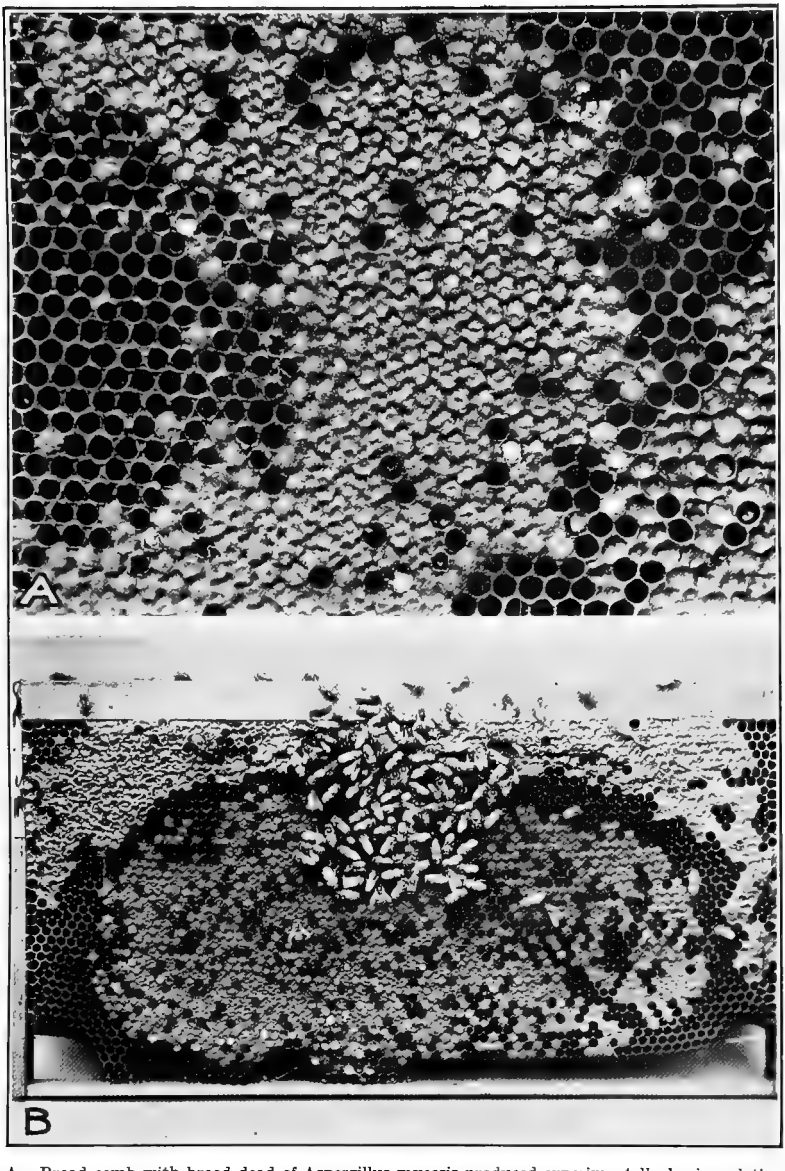
What has been said concerning diseases of adult bees is true to a lesser extent for diseases of their brood. Normally brood harbors fewer bacteria than do the adult bees. During the first two or three days of the life of the larvae, when their food consists only of pap, which is believed to be a glandular secretion, it is often difficult to demonstrate the presence of microorganisms within the digestive tract either microscopically or by culture. When such young larvae die of other causes than bacterial disease the remains frequently dry down without being attacked by putrefactive bacteria or fungi. Microorganisms are found within the alimentary canal of older larvae and pupae, after pollen and honey are mixed with the food, but they are fewer in number than within the worker bees of the field force. Secondary invaders rarely appear after the death of infected brood with the possible exception of those dying from European foulbrood. The causative organism of American foulbrood can be recognized in old brood remains, whereas the absence of putrefactive organisms is the rule with sacbrood.

During these experiments examinations were made at frequent intervals after the inoculations were made. Adult bees were watched more closely than brood in order to observe the symptoms of disease before they were modified by secondary invaders. Records were kept of microscopical and macroscopical examinations and were compared with similar records of bees used as checks upon the experiments.

RESULTS OF INOCULATIONS AND SYMPTOMS OF THE DISEASES PRODUCED

SYMPTOMS PRODUCED BY THE ASPERGILLI

The inoculation of bees with a number of species of Aspergillus resulted in the rapid appearance of mycosis and the death of the bees. With some of the more virulent species all of the bees inoculated and



A.—Brood comb with brood dead of Aspergillus mycosis produced experimentally by inoculation with a culture of *Aspergillus flavus* on a brood comb.

B.—Brood comb with brood and young bees from a colony inoculated experimentally with *A. flavus*, taken after the older bees had deserted the hive before succumbing to Aspergillus mycosis.

kept in cages were attacked and killed within from two to four days. With less virulent species the bees lived somewhat longer and a varying percentage of the inoculated bees escaped infection. At times, with species not ordinarily found on bees, only about 5 or 10 per cent of the inoculated bees died of infection.

The results obtained in colonies were quite similar to those obtained with bees in cages. When colonies were infected with one or two brood-comb cultures of virulent species of Aspergillus, all, or nearly all, of the brood and bees of the colony were killed by the fungi. Illustrations A and B of Plate 2 show brood combs from infected colonies after most of the field bees had died from infection with *Aspergillus flavus*.

With less virulent organisms the death rate for the first day was considerably lower, and at times only part of the population of the colony was killed. In the latter case the activity of the colony returned to normal after the infected combs had been cleaned and the dead brood removed from the brood combs.

When the inoculations were made by placing spores of pathogenic Aspergilli in the blood of bees through needle punctures, disease and death usually resulted. A few larvae were attacked as a result of direct penetration of the skin by germ tubes. (Pl. 1, D.)

Symptoms of Mycosis in Adult Bees

The first noticeable symptoms of infection by Aspergillus in adult bees is their weakening and restlessness, and the continued effort on the part of the sick bees to escape from the cluster of healthy ones. In cages the earliest affected bees can be picked out from among those not yet infected by selecting bees that continue to crawl at a time when the greater number of bees are quiet. Crawling continues for several hours, accompanied by a gradual weakening and loss of definiteness in the movements. In from one to four hours the crawlers become too weak to stand or crawl normally and move with a staggering motion. They frequently fall and eventually become unable to right themselves. During the crawling period bees sometimes lose the use of one or more legs. The affected legs are dragged or used ineffectively in crawling. Weakness increases until the infected bees are capable of only feeble movements of the legs, mouth parts, and abdominal segments.[3]

Bees that are naturally infected or are artificially infected within the colony show the same symptoms of disease as are shown by bees kept in cages. As soon as attacked by the fungus, field bees fly or crawl from the hive and usually die at a considerable distance from the entrance. During weather favorable for flight probably not more than 5 or 10 per cent of the infected bees die within or directly in front of the hive. Most of these die at night and are young bees

[3] These observations are comparable to those made by Turesson (26) on the toxicity of molds to the honeybee and the cause of bee paralysis. As a result of his experiments Turesson concluded that paralysis among bees is caused by the eating of fungous spores and mycelium. He claims to have found very little difference in the toxicity of the different species that he tried and believes that toxic substances are present in most fungi. He speaks of observing the germination of spores and development of mycelium within the alimentary canal, but apparently did not suspect any of the organisms capable of parasitizing bees. The writer's conclusions differ from the conclusions of Turesson on this point.

that were infected before their first flight. These young bees avoid the light and consequently remain in the hive until the disease is well advanced. In artificially infected colonies the first crawlers appear in from one to three or four days, depending largely upon the virulence and rate of growth of the fungus, and the quantity of inoculum used. Bees were seen to leave the hive and fly heavily. Some fly for only a short distance, then fall to the ground as if too weak to maintain flight. Several such short flights may be made by diseased bees while sufficient strength remains. When too weak to fly, they crawl and frequently attempt to take wing from the tops of grasses or stems, but fall to the ground only to try again. Death usually occurs within a few hours after they have come to the stage where they are no longer able to fly. When not too weak, the crawlers taken from the shade into bright sunlight or a warm room may recover sufficient strength to take wing again. This apparent recovery lasts for only a short time, and death occurs as soon as if the bees had remained exposed to the lower temperatures. Cowan (*9, p. 189*) attributes the loss of flight in bees infected with *Mucor mucedo* to pressure exerted by developing mycelium and the consequent inability to distend the air sacs. In the case of Aspergillus infection, however, a general weakening as a result of disease seems to account for their inability to fly.

No important changes in appearance occur at death. The body of the bee retains its normal color and shape. The abdomen may or may not be distended, since this depends upon the quantity of food consumed by the diseased bee just previous to death. The body appendages retain their normal position in relation to the body, which may lie in any position. A slight increase in the hardness of the abdomen may have occurred at death, though this hardening has been immediately preceded by a softening of the infected tissues. Soon after death, if the original infection was heavy and the saprophytic development of putrefactive bacteria is not too rapid, the abdomen becomes noticeably harder than in normal bees or in bees dead of disturbances not of a fungous nature. The muscles of the thorax, on the other hand, usually become considerably softer soon after death, as can be noted by crushing them between the fingers or under a cover glass. This character is of little diagnostic value, however, as the same symptom is present in bees dying of other causes. The increase in firmness of the abdomen at this time is, therefore, the only distinctive external symptom of fungous infection. After death the firmness remains or increases, and the tissues of the abdomen may become quite hard. The thoracic muscles, after first softening, also increase in rigidity, and then upon drying become of chalklike consistency.

When the abdomens of these dead bees are dissected for microscopic examination the digestive tract and surrounding tissues are found to be tough in texture and are less easily crushed than in the case of healthy bees or of bees dead of other diseases. While the ventriculus of the healthy bee spreads evenly when pressed under a cover glass, the ventriculus of a bee dead of mycosis offers considerable resistance to pressure. Teasing it apart with needles is often necessary to prepare mounts that will transmit sufficient light for satisfactory examination.

When dead bees infected by aspergillus are kept in a moisture-saturated atmosphere at ordinary temperatures the fungous growth continues after death and attacks all of the softer tissues of the abdomen, thorax, and even the head. Tufts of mycelium and conidiophores develop at the body openings. Conidiophores also grow through the thinner parts of the exoskeleton, at the articulation of the body appendages, and at the junction of the abdominal segments. (Pls. 3, A and B; 4, A; and 5, A.) When conidiophores do not develop upon the surface of the dead bees they may do so inside the exoskeleton of the abdomen or thorax. (Pls. 4, B, and 5, B.) Conidiophores do not develop in a very dry atmosphere, whereas an excess of water aids the growth of bacteria and thus prevents the development of conidiophores.

After inoculation and before symptoms of mycosis have appeared the spores present within the alimentary canal increase in size as a result of imbibition of water, except within the honey stomach, where the contents are usually highly concentrated. When several times their original size the spores germinate, and an extensive mycelium develops. (Pl. 6.) Spores that germinate within the ventriculus may limit their growth for a time to the food contents, but the wall is soon penetrated by the developing mycelium. A similar process of development may occur within the pharnyx, proventriculus, small intestine, or rectum, and less frequently within the honey stomach. The Malpighian tubules usually remain free from attack until after the death of the bee. The trachea likewise remain free from attack, even in advanced stages of decay after death. Spores appear to be unable to germinate within the trachea, owing to dryness resulting from aeration. In addition, these organs are protected from perforation by the tough layers of the interior surface. The small quantity of available food-supplying material within the tracheal walls likewise limits fungous growth. All of the softer tissues of the three divisions of the body may be attacked by the fungous mycelium. The thoracic muscles are a favorable medium for the development of pathogenic species of Aspergillus.

The infection of adult bees with pathogenic fungi results normally only from the germination of spores within the alimentary canal. Spores failed to cause infection when placed on various parts of the exterior body surface of adult bees, and germ tubes from spores cultured in small quantities of nutrient media did not penetrate the tissues when placed on the soft covering of the neck or in the folds of the abdominal segments. Spores spread over the openings of the spiracles likewise failed to cause infection.

Symptoms of Mycosis in the Brood

In brood, as with adult bees, Aspergillus infection shows a number of more or less arbitrary stages, depending upon the degree of development of the infecting fungus. The changes in symptoms may occur with considerable rapidity within a period of a few days owing to the rapid growth of mycelium. Both larvae and pupae are attacked by any of several common species, but after the feeding state, and especially after the cells are capped, the chances of infection are considerably reduced. In these experiments the larvae were attacked

in greater number than were the pupae. The symptoms of disease in the larvae and pupae are quite similar; consequently no attempt is made to differentiate between them.

The symptoms manifested by infected brood before death are not readily noticeable. There may be a slight increase or alteration in movement in larvae that become infected before the quiescent period or in pupae that are infected just previous to the time for emerging. Ordinarily this passes unobserved, since the extent of the movement of which brood is capable, within the cells of the brood comb, is very limited. During their active period diseased larvae may turn to one side or the other or turn completely over, although this is not a usual occurrence. Both larvae and pupae usually die while in their normal position in the cells.

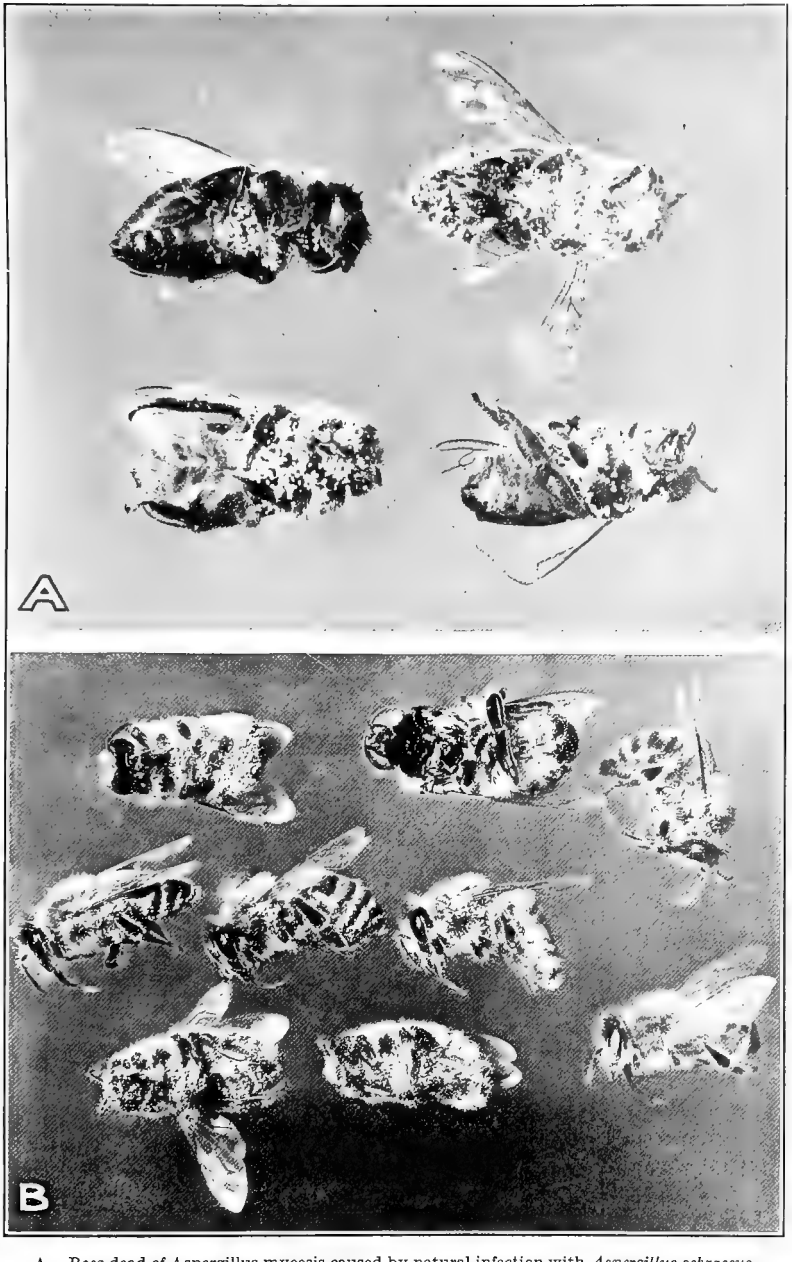
It is difficult to determine when death occurs, especially during the quiescent period. There are no changes in the outward appearance of infected brood for a short time before and after death. Larvae or pupae, at an age when they are normally capable of movement, may be assumed to be dead when all movement has ceased. The fact can be determined by examination under a reading glass which magnifies four diameters or more, or under a binocular microscope. The skin of living healthy larvae normally presents a moist and glistening appearance. Movements of the mouth parts and breathing movements are readily noticeable. With death all movements stop, and the skin becomes dry and dull after a few hours.

After death, before mycelium appears outside the skin, changes in appearance and texture develop. Firmness increases as a result of the interweaving of mycelium in and about the digestive organs and tissues. The color in this stage changes from the glistening white of the healthy larva to a dull, dry, creamy white. Drying, as indicated by wrinkling and shrinking, has already begun. The anterior end of the larva, which usually shows the first indications of drying, may be sharply curved ventrally, bringing the anterior and posterior ends closer together.

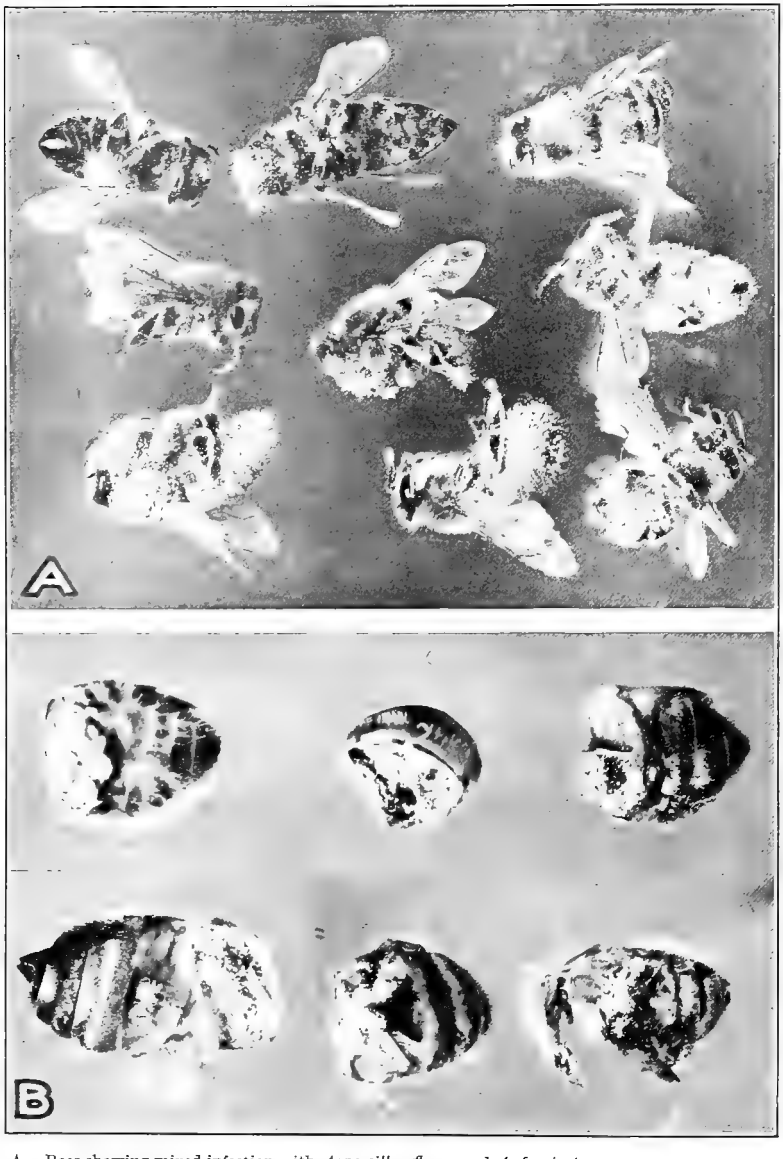
Soon after color changes appear the mycelium breaks through the skin. It appears first in a circle of radiating hyphae near the anterior extremity and gives to the dead larva the appearance of possessing a collar. Development of mycelium over the surface forms a false skin composed of closely woven hyphae. (Pl. 1, C.) The segmental markings are retained in this false skin; consequently close observation is necessary to distinguish between this and the true skin. The false skin of mycelium may be removed without injuring the true skin if care is used.

The development of conidiophores upon the surface of the larva begins at about the time of the maturation of the false skin. They form most abundantly at the anterior and less abundantly at the posterior extremities where the mycelium is in direct contact with the air. (Pl. 1, C.) When the conidiospores mature, the white color of the false skin is replaced, wherever the dead larvae are not in direct contact with the cell walls, by the color of the spores of the infecting organism. (Pl. 1, C.)

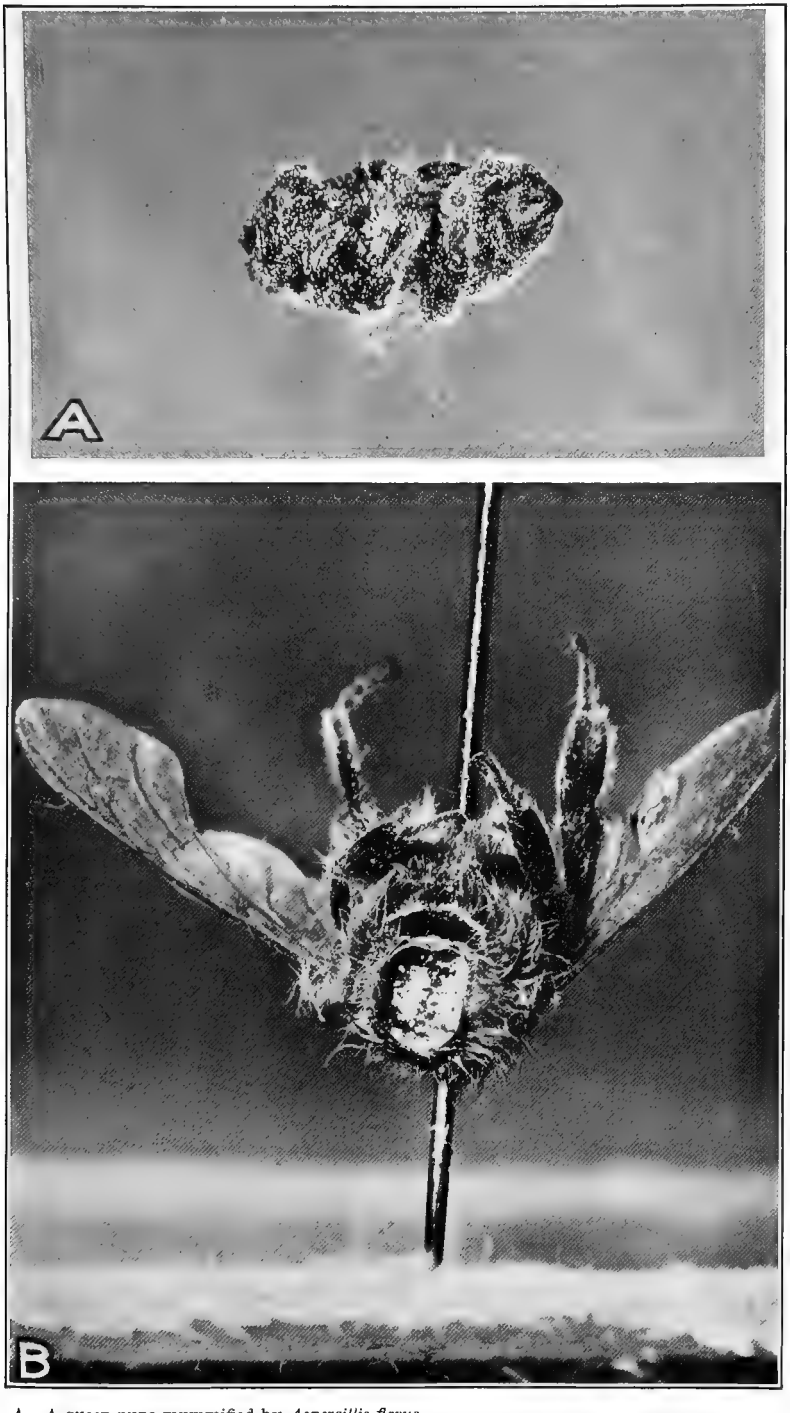
After the spores are mature changes take place much more slowly. Aging of the spores is accompanied by darkening and a loss of bril-



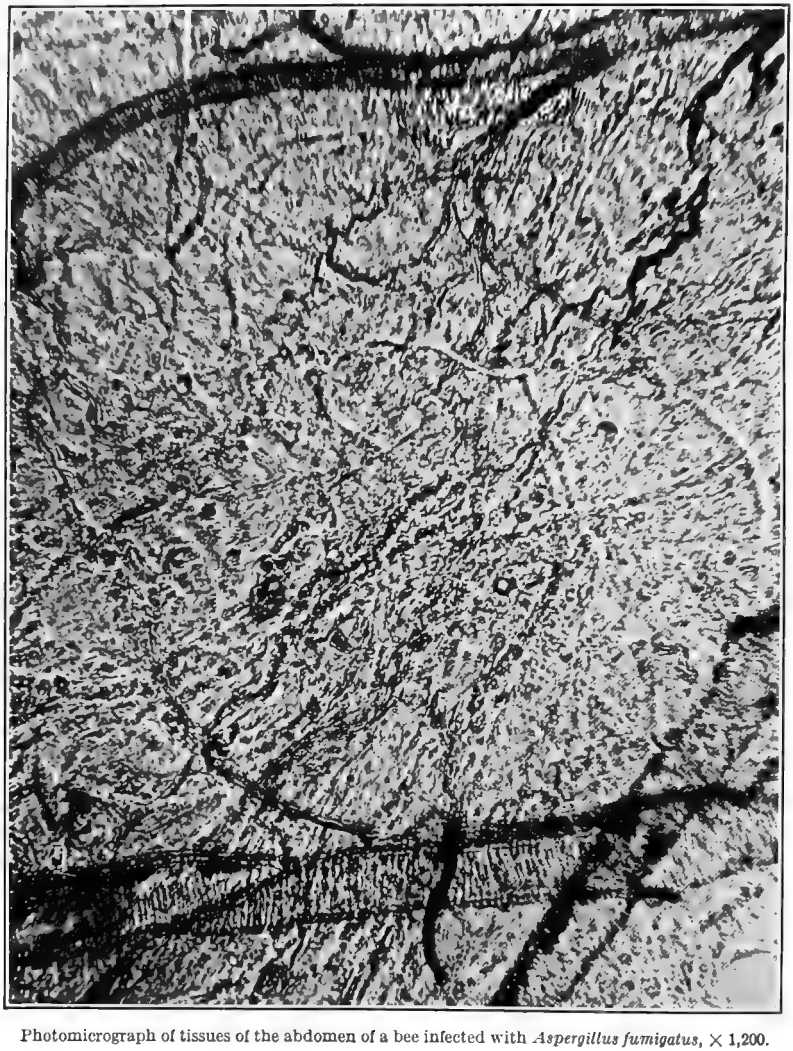
A.—Bees dead of Aspergillus mycosis caused by natural infection with *Aspergillus ochraceus*.
B.—Worker bees dead of Aspergillus mycosis caused by natural infection with *A. flavus*.



A.—Bees showing mixed infection with *Aspergillus flavus* and *A. fumigatus*.
B.—Dissected abdomens of bees dead of Aspergillus mycosis produced experimentally, the penetration of the exoskeleton by the conidiophores having been prevented by keeping the bodies in a dry atmosphere.



A.—A queen pupa mummified by *Aspergillis flavus*.
B.—Decapitated bee (naturally infected) showing conidiophores of *A. flavus* that matured within the thorax.



Photomicrograph of tissues of the abdomen of a bee infected with *Aspergillus fumigatus*, × 1,200.

liancy, and continued drying of the body results in the formation of a mummy of considerable hardness.

Infection of brood from fungous spores within the digestive organs occurs in a manner quite similar to the infection of adult bees (p. 19). Infection of larvae is not limited in its origin strictly to the digestive organs, although this is the usual channel of infection. Spores of two species of Aspergillus, *A. flavus* and *A. fumigatus*, placed on the skin of larvae produced local infection at the point of contact. Germ tubes penetrated the skin and attacked the subcutaneous tissues in about 15 per cent of the cases in some experiments. This mode of infection occurred in larvae in combs, and in larvae kept on honey or sugar solution in watch crystals. Soon after the skin is penetrated and the mycelium has become well established in the tissues tufts of aerial hyphae and conidiphores appear at the point of infection. (Pl. 1, D.)

Although these experiments would seem to indicate that direct penetration by germ tubes from spores germinating on the surface of larvae is possible, it probably rarely occurs. Even in the experiments where masses of spores were placed on the surface of the larvae, germ tubes generally failed to penetrate the skin.

Results of Inoculation of the Blood of Bees with Spores of Aspergillus

In the experiments with caged bees a few wounded ones were observed that appeared to be attacked in the wound by the fungus. Bees that were inoculated in the tissues or blood were killed more quickly than when spores of pathogenic fungi were taken into the alimentary canal. All of the species of Aspergillus that were found capable of attacking bees through the alimentary canal proved to be pathogenic when introduced into the tissues or blood. Other species that did not attack bees when taken into the digestive organs were found to be capable of attacking bees when inoculations were made by wounding.

This method of infection could seldom occur in nature on account of the constant protection afforded by the body covering.

Study of the Physical and Chemical Action of the Fungus on the Infected Bees

The germination of spores of filamentous fungi within the alimentary canal of bees and the growth of mycelium here and in the tissues result in disease and death. It has been stated by Vincens (*27*) that "the parasite seems to act by mechanical obstruction of the digestive passages or by paralysis of the muscles of the intestine."

As a result of the study of freshly prepared mounts, the writer believes that the blocking of the digestive tract is of minor consequence. When the first symptoms appear the quantity of mycelium present within the alimentary canal of infected bees is in most cases so small that it is difficult to understand how it can affect the movements of food. Death usually occurs before a sufficient quantity of mycelium has developed to cause congestion within the digestive tract. Frequently, when feeble movements of the appendages or abdominal segments are the only signs of life, only a few scattered hyphae can be found within the contents of the alimentary canal.

It appears that one must look for other causes responsible for disease and death in the bees infected by fungi.

To determine these causes of death, a histological and chemical study was made of infected tissues from diseased and dead bees. The chemical action of the fungus was determined by observing the infected tissues, and by testing the action of certain metabolic products produced by the fungus, when applied to the tissues in the absence of the fungus.

It was determined by examination of sections of the ventriculus that hyphae penetrate the tissues soon after the spores germinate. The wall is usually attacked first and is soon permeated with mycelium. Other tissues of the abdomen and thorax are also attacked by the fungus.

That a mechanical effect is produced in the tissues by the advancing mycelium is shown by the forcing apart of the muscle fibers when the hyphae grow among them. Later the mycelium becomes densely interwoven among all of the softer tissues, and the mechanical effect of this is probably sufficient to cause death.

In view of the rapid softening of the tissues and the appearance of weakness, with the consequent crawling condition, among infected bees, it appears that enzymatic and toxic substances contribute materially to the symptoms. The tissues of the bee are attacked by the fungous enzymes in advance of the growing mycelium, but this digestion is not completed until the mycelium is well developed. The resultant softening of the muscle tissues of the thorax is followed by a loss of the brownish tinge characteristic of healthy tissues and the appearance of a dull white color. A sarcolemma is absent about the wing-muscle fibers of the honeybee, and no support other than the sarcoplasm is known to exist. The semifluid sarcoplasm surrounding the sarcostyles is softened, permitting the muscle fibers to separate readily. This probably accounts for the softening and collapse of muscle tissue and the ready separation of the threads of muscle fibers under pressure.

Softening of the sarcoplasm surrounding the muscle fibers is followed by the death of the sarcoplasm of the sarcotyles and the disappearance of their membranous walls. In advanced stages of digestion, i. e., after death, the sarcostyles break up into segments, frequently separating at the telophragmata. After complete digestion only formless granules remain.

The muscles of the thorax are most easily studied, but the wall of the ventriculus may be used successfully since it is attacked soon after infection occurs. The digestion of sarcoplasm of the tissues of the bee is probably largely proteolytic in nature, for protein is the chief constituent of muscle tissue. It appeared, however, from some comparative measurements made by the writer that pathogenicity of the organisms studied is in no way related to the quantitative production of protease. It is possible that the sarcoplasm is killed by fungous toxins before enzymatic action begins.

The observations described above on infected tissues indicate that the changes which occur after the death of the tissues are apparently due to enzymatic action. If this is true, muscle tissue should be similarly affected in fungus-free culture media or in solutions of extracted enzymes.

In contrast to the findings of Eduard Buchner (*4*), in his work on the extraction of zymase from yeast, the enzymes of several species of Aspergillus and Mucor were found to exist in much greater quantities in the medium in which cultures have grown than within the mycelium. Evidence in support of the view that the effect of pathogenic fungi on bees is chiefly enzymatic and toxic was obtained from the following experiments:

Enzymes from the liquid medium in which pure cultures of *Aspergillus flavus*, *A. effusus*, and *A. fumigatus* had matured were extracted by precipitation with alcohol. After thorough drying the precipitate was dissolved in distilled water. The digestive tract and muscle tissues of bees that had been recently killed, entire bees, and each of the three divisions of the body were submerged in this solution and kept at 37° C. while the action was observed. Tissues directly exposed to the action of enzymes were soon discolored and in from 5 to 10 hours offered no noticeable resistance to pressure. Tissues of thoraces, abdomens, and heads of bees with the exoskeleton of each of these body divisions attached were affected similarly but somewhat more slowly. The chitinous exoskeleton appeared to be unaffected after 24 hours, whereas no evidence of enzymatic action on the internal tissues of entire bees was noticeable after 30 hours. It appears that the exoskeleton is not extensively attacked by any of the enzymes produced by these fungi, although conidiophores and mycelium penetrate the body wall of the bee when excessive moisture is present. Ordinarily the only channel of infection is through the alimentary canal.

The experiments described above were repeated with the filtered medium from cultures, with sterile medium that had not been used for culturing, and with distilled water. With the medium from cultures the action was similar but somewhat slower than with extracted enzymes. With sterile, freshly prepared medium and with water as checks, no enzymatic action upon tissues was noticeable after 48 hours. The medium, which was of a high sugar content (25 per cent), appeared to act as a preservative, since bacterial decay of the tissue occurred later than in distilled water.

Demonstration of a Toxic Substance Produced by Aspergillus flavus A05c

It is a familiar fact that many of the diseases of animals and plants are due to microorganisms which elaborate toxic substances of some form or other. The exact nature of these substances, which are at times partially responsible for the symptoms of the various diseases, is not completely known. The inability of chemists to determine their constitution is probably due to the impurity, complexity, and unstable nature of the molecule. It is possible by various procedures to extract from cultures of pathogenic organisms substances that are more or less toxic. When toxins are properly administered in the absence of the pathogenic organisms they may produce the symptoms of the disease. Severe poisoning may also be produced by toxic products of nonpathogenic organisms. The potency of these toxins, which may be many times as effective as strychnine, is well known. Toxic substances are not uncommon among the

higher plants, and among the mushrooms there are numerous species whose toxins have caused the deaths of great numbers of mushroom eaters. Although numerous species of the Hyphomycetes are active parasites of other fungi, higher plants, and animals, very little has been done to demonstrate the nature of the toxic substances elaborated by these organisms.

In the progress of some plant diseases caused by fungi the cells of the host are killed or altered far in advance of the developing mycelium of the parasite. This is particularly true among the rusts where zones of dead cells may surround the infected spot. The death of the cells of the host not in direct contact with the fungus can probably be attributed to toxic products of the parasite.

A few reports of toxic substances produced by filamentous fungi are on record. In 1906 Paladino-Blandini (*20, p. 608*) prepared an alcoholic precipitate from the mycelium of *Rhizopus nigricans* which was toxic to rabbits when injected intravenously. In 1915 Blakeslee and Gortner (*3*) published a more complete study of this toxin and its action upon rabbits. In earlier experiments by these two investigators (*11*), results with rabbits were negative when the fungus was administered by feeding in large doses. (Such an alcoholic extract would be a protein, and its nontoxic nature when fed can be explained by its digestion to nonpoisonous compounds before absorption.) In 1896 Gosio (*12*), working with a species of Penicillium, found that the culture medium gave phenol reactions. When injected into rabbits and rats, phenol poisoning resulted. In the study of maize deterioration, Alsberg and Black (*1, p. 13, 43*) isolated a characteristic phenolic substance from *Penicillium puberulum* and *P. stoloniferum*. This substance could only be isolated from the medium. Turesson (*26*) as a result of his work on the toxicity of fungi concludes that it is of wide occurrence in fungi.

To determine whether or not such a toxic substance injurious to living bees is produced by these fungi experiments were begun with two pathogenic organisms, *Aspergillus flavus* Ao5c (Thom collection) and *A. fumigatus*, isolated from an infected worker bee.

Direct injection into the blood of bees was considered impracticable; consequently feeding experiments were adapted as a test for the toxicant. Food prepared by the addition of honey to the medium in which these Aspergilli had been grown and to juices pressed from mycelium was fed to caged bees. When the unheated medium or fungous juice from cultures was used it was necessary to filter it, using the best grade of filter paper, to eliminated spores of the pathogenic organisms. This was partially accomplished only with *Aspergillus flavus*. *A. fumigatus* was not used after the first attempt on account of the small size of the spores. Although the death rate among the bees fed with medium in which cultures had matured averaged higher than among the checks which were fed freshly prepared medium, the method was discarded because the death rate among the checks, due to salts, was higher than normal. When bees were fed with the fluids pressed from mycelium, or with the wash water from the mycelium, the death rate remained about normal. It appeared, therefore, that if a toxic substance is produced, it must be sought in the medium rather than in the mycelium of the fungus.

EXTRACTION OF THE TOXIN FROM THE MEDIUM

It appeared, as a result of the experiments discussed above, that the toxic substance must be extracted in order to avoid error from other factors that affected the death rate. Since nothing was known of the nature or constitution of the toxic substance, it was necessary to determine the best method for extracting it.

Alcohol extracts

Extraction of proteins with alcohol was tried first. The precipitates obtained from the medium in which fungi had been cultured and from the juices pressed from the mycelium after grinding it with sand were dissolved in water and fed with dilute honey to bees in cages. The bees took this food readily, but the death rate remained normal throughout two trials. It is not proved by these experiments that a poisonous protein is not produced, since such a poison may be digested to nonpoisonous compounds before absorption, as with higher animals (*11*, *p. 357*). On the other hand, absorption by the honeybee of some of the simpler sugars appears to be a question of only from one to a few minutes.

Ether extract

The medium from 10-day-old flask cultures of *Aspergillus flavus* Ao5c and the fungous juices pressed from the mycelium after it had been washed and ground with sand were shaken five times with small quantities of ether. After each shaking the ether was separated from the medium with a separatory funnel. The ether was then washed with a large volume of distilled water, separated, and evaporated to dryness in evaporating dishes. Brown amorphous residues were obtained in larger quantities from the medium than from the fungous juices. Each of the residues was dissolved in 5 cubic centimeters of distilled water and added to about 5 cubic centimeters of clover honey. This was given as food to caged worker bees. The bees that received the extract from the fungous juices lived normally; but some of the bees receiving the extract from the culture medium were noticeably affected after 4 hours, and after 10 hours all that had been fed with this extract were dead. The experiment with the culture medium extract was repeated with four lots of bees, the same food being used for each succeeding lot after all of the bees in the previous lot had died. A constant decrease in potency was noticed with each successive trial. With the second trial all of the bees were dead at the end of 18 hours; with the third, after 30 hours; with the fourth, after 2 days; and with the fifth, after 7 days. (Table 1 and fig. 1.)

This experiment was repeated several times with similar results except that the earlier deaths were not obtained again in succeeding experiments. Extracts from old cultures that had been kept at room temperature and from young cultures prepared at about the time spores were forming appeared to contain only small quantities of the toxic substance. The greatest accumulation of toxin within the medium seems to be present at about the time the cultures are

mature, as indicated by the appearance of a deep yellow-green color in most of the conidia. The disappearance of the toxin from cultures and the rapid loss of potency after extraction indicate that it is of a transient nature. This is shown in Table 2 and Figure 2. When freshly prepared ether extract was fed to bees the entire lot died in

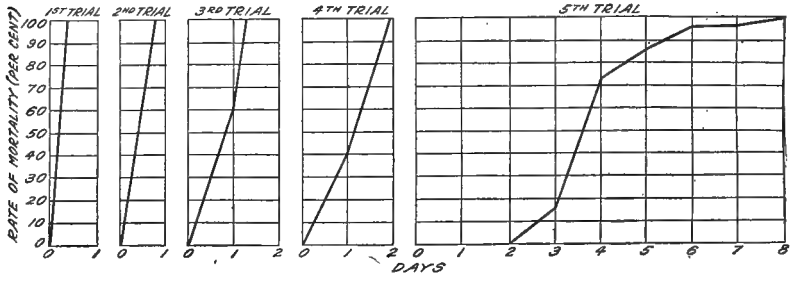


FIGURE 1.—Rate of mortality of honeybees at daily intervals after the commencement of feeding with honey containing ether extract of the nutrient medium in which *Aspergillus flavus* had been cultured. The food was transferred to a new cage of bees as soon as the bees of the previous trial were dead

five days. Fifteen days later the same food was given to a second lot of bees. This trial showed a death rate that was nearly normal for caged bees.

TABLE 1.—*Length of life of bees in cages when ether extract of the culture medium of Aspergillus flavus Ao5c was mixed with their food*

Age of extract when fed to new lot of bees	Number of deaths on days stated								Total deaths
	1	2	3	4	5	6	7	8	
Freshly prepared	[1] 24								24
24 hours	[1] 26								26
2 days	15	10							25
4 days	10	16							26
8 days	0	0	5	16	4	3	0	1	29

[1] Although this table indicates only that the bees of the first and second tests were dead after 24 hours, the actual time was 10 hours in the case of the first trial and 18 hours in the second.

TABLE 2.—*Length of life of bees in cages when ether extract of the medium in which Aspergillus flavus Ao5c had been cultured was mixed with their food*

Age of extract when supplied to a new cage of bees	Number of deaths on days stated																				Total deaths
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
Freshly prepared	7	4	4	9	1																	25
15 days	0	0	0	0	0	1	1	2	0	1	1	2	2	1	3	3	3	2	2	1	1	26

Chloroform extract

To find whether poisonous organic bases or other poisonous substances soluble in chloroform were present, the liquid nutrient medium (Leonian's formula) was thoroughly drained from half a dozen flask cultures of the mature organism, *Aspergillus flavus* Ao5c.

The fungous fluids were pressed from the mycelium after it had been washed and ground with sand. Both the medium and the fungous fluids were each shaken five times with small volumes of chloroform and separated with a separatory funnel after each operation. After washing in an evaporating dish the chloroform was evaporated to dryness, and the residue from each solution was taken up with about 5 cubic centimeters of distilled water and given to worker bees with an equal volume of honey. In each case the entire quantity of food was consumed without evidence of poisoning. Repetitions of this experiment at different stages in the development of the fungus gave entirely negative results. It would seem therefore that bases capable of poisoning bees when administered with food are not produced by *A. flavus* Ao5c.

Chemical Nature of the Toxic Substance

In most cases chemists have been unable to determine the constitution of toxic substances elaborated by microorganisms; therefore only

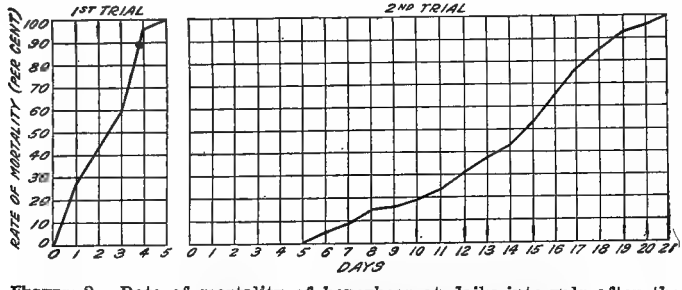


Figure 2.—Rate of mortality of honeybees at daily intervals after the commencement of feeding with honey containing ether extract of the nutrient medium in which *Aspergillus flavus* had been cultured. First trial made immediately after the extract was prepared, second trial 15 days after the extract was prepared.

a few tests were made to determine the general nature of this substance. Tests for phenolic compounds were made by floating small quantities of freshly prepared ether extract on very dilute ferric chloride solution. The absence of color reaction in all cases indicated the absence of such compounds in the extract. When the extract from flask cultures in 5 cubic centimeters of water was mixed with 5 cubic centimeters of honey, the resulting solution showed a hydrogen-ion concentration of about pH 4.6 by the colorimetric method of Clark and Lubs (*7*) and Clark (*6*), whereas equal volumes of water and the same honey showed a concentration of about pH 5.

Something of the nature of the toxic substance is shown by the following experiments: Freshly prepared extract was divided into three equal parts. One part was fed directly to bees in cages. The other two parts were made neutral, or slightly basic, by the addition of potassium hydroxide. One of these parts was immediately adjusted by hydrochloric acid to a hydrogen-ion concentration of about pH 4. 6. Honey was added to each of these two lots and they were fed to bees. The bees given the original unneutralized extract showed definite evidence of poisoning after 24 hours. The death rate was considerably higher than normal, and all of the bees had died

before the end of the fifth day. Bees fed upon honey with the ether extract, which had been made neutral with potassium hydroxide, showed a normal death rate for 15 days, when all of the food was consumed. Bees given the solution that had been readjusted to about pH 4.6 after neutralization died at about the same rate as bees fed on the ether extract that had not been neutralized. (Table 3 and Fig. 3.)

TABLE 3.—*Length of life of bees in cages when ether extract of the culture medium of Aspergillus flavus Ao5c was mixed with their food*

pH value of the food containing fungous extract	Number of deaths on days stated															Total deaths
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Freshly prepared; about pH 4.6	0	4	6	6	3	6	----	----	----	----	----	----	----	----	----	25
Made basic with potassium hydroxide	0	3	1	0	0	0	0	0	1	0	0	2	1	3	(1)	11
Changed from basic to pH 4.6 with hydrochloric acid	0	5	6	6	2	3	0	3	----	----	----	----	----	----	----	25

[1] The total number of bees used in this experiment was also 25. The food containing the fungous extract was consumed by the bees after 14 days, when records of the daily death rate were discontinued.

If, as is indicated by the foregoing experiment, the toxic substance is of basic nature, it should be dissolved out with chloroform if the medium is first made basic. Potassium hydroxide was added to the medium from 12-day-old cultures until it gave a distinct basic reaction. Chloroform was then shaken with the medium, separated, and evaporated to dryness, as was done with ether. A flocculent precipitate, which formed upon the first addition of chloroform, was separated, drained, and dissolved in a small quantity of water. By the addition of honey to this precipitate a food for bees was prepared. A food was prepared also with the residue left upon evaporation of the chloroform. No poisoning resulted from either food. After six days each preparation was adjusted to a pH value of about 4.6 by the addition of sulphuric acid and fed to recently caged bees. The death rate in both cages remained normal. The toxic substance, if present at first, appeared to have been entirely destroyed in the basic condition.

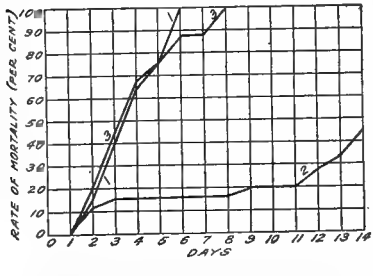


FIGURE 3.—Rate of mortality of honeybees at daily intervals after the commencement of feeding with honey containing ether extract of the nutrient medium in which *Aspergillus flavus* had been cultured: 1, With freshly prepared extract; 2, with extract made slightly basic with potassium hydroxide; 3, with extract adjusted to a concentration of about pH 4.6 after it had been made slightly basic

That it was nearly all removed from the medium was shown by adjusting the medium to its original pH value and extracting with ether immediately after the extraction with chloroform was complete. When this ether extract was fed to bees the death rate was about normal.

In order to determine whether the toxic substance could be recovered by the adjustment of the pH value immediately after

extraction with chloroform, an extraction was made from a new set of cultures. As soon as the chloroform had evaporated the residue was taken up with water, mixed with honey, and adjusted to about pH 4.6. The precipitate that formed with the first addition of chloroform was treated in the same way. The death rate was increased when either the residue left after evaporation of the chloroform or the precipitate was fed to caged bees. The death rate from the residue was much higher than that from the precipitate, which shows that there is a greater amount of toxin in the chloroform. (Table 4 and fig. 4.) It may be that the presence of the toxin with the precipitate can be accounted for by adsorption.

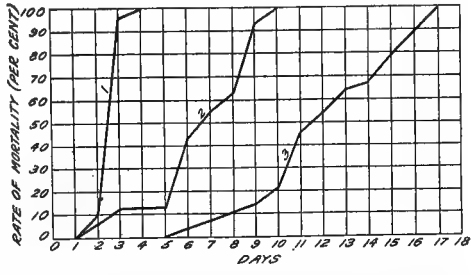


FIGURE 4.—Rate of mortality of honeybees at daily intervals after the commencement of feeding with extract of the medium in which *Aspergillus flavus* had been cultured: 1, With chloroform extract from medium made slightly basic with potassium hydroxide before extracting. The food containing the extract was then adjusted to a concentration of about pH 4.6 with sulphuric acid just before it was given to the bees. 2, With the precipitate obtained upon the addition of chloroform to the basic medium. The food containing the extract was adjusted to a concentration of about pH 4.6 before it was given to the bees. 3, With ether extract of the medium adjusted to an acid reaction after extractions had been made with chloroform

TABLE 4.—*Length of life of bees in cages when the extract from the culture medium of Aspergillus flavus Ao5c was mixed with their food*

Method of preparation and pH value of the extract	Number of deaths on days stated																	Total deaths
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Chloroform extract adjusted to pH 4.6	0	3	26	1	---	---	---	---	---	---	---	---	---	---	---	---	---	30
Precipitate obtained with chloroform (adjusted to pH 4.6)	0	1	1	0	0	5	2	1	5	1	---	---	---	---	---	---	---	16
Ether extract from medium after extracting with chloroform and adjusting to original pH value	0	0	0	0	0	1	1	(1)	2	2	7	(1)	5	1	(1)	(1)	9	28

[1] Records of the death rate were not made on this day.

As checks upon the experiments, extractions were made with ether and with chloroform from medium that had not been used for culturing. These extracts were added to diluted honey and fed to bees. All of this food was consumed, but none of the bees appeared to be poisoned since the death rate was normal. (Table 5 and fig. 5.)

TABLE 5.—*Daily death rate of bees in cages when given honey for food*

Investigator	Cage No.	Number of deaths on days stated																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Phillips	1	20	0	0	4	2	12	2	3	6	8	4	6	11	10	9	3	1	---	---
Do	2	1	0	4	28	12	6	2	5	4	9	6	6	8	2	3	2	2	---	---
Burnside	3	0	0	0	0	1	3	6	1	7	3	3	1	3	3	1	5	7	3	2
Do	4	0	0	0	0	0	0	[1] 1	1	2	(2)	5	3	2	0	1	1	(2)	2	4

[1] Three other check lots of 25 each showed that the first deaths of normally fed bees occurred on the sixth or seventh day.

[2] Records of the number of deaths were not made on these days.

TABLE 5.—*Daily death rate of bees in cages when given honey for food*—Contd.

Investigator	Cage No.	Number of deaths on days stated																		Total deaths
		20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	
Phillips	1	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	101
Do	2	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	100
Burnside	3	2	1	1	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	53
Do	4	2	5	5	(2)	(2)	6	0	5	11	10	4	2	1	5	1	3	0	1	83

[2] Records of the number of deaths were not made on these days.

The graphs of Figures 1 to 4 and Tables 1 to 4 show the rate of mortality obtained under varying conditions when fungous extracts mixed with dilute honey were fed to bees in cages.

The normal death rate for caged bees when given honey as food is shown in Table 5 and in the graphs of Figure 5. Graphs 1 and 2 of this figure were drawn from data obtained by Phillips (*19, p. 395–399*) on the death rate of bees in cages when given honey for

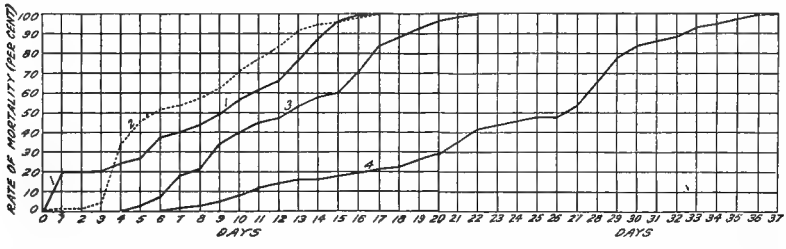


FIGURE 5.—Rate of mortality of lots of honeybees in cages when fed with honey. This graph represents normal death rates for bees kept in cages

food. Graphs 3 and 4 were drawn from data obtained by the writer while determining the normal death rate of bees in cages, as checks upon other experiments. These cages were kept in the laboratory on the opposite side of the room from the source of light. The bees were supplied with honey diluted with an equal volume of water. The temperature of the laboratory during the time that the experiments were in progress ranged between 23° and 27° C.

In view of the difference in death rate between the experiments of Phillips and those of the writer, when bees in cages were given honey for food, one is led to believe that there were important differences in the conditions of the experiments. Either the bees used by Phillips were not entirely healthy or the conditions under which his experiments were conducted were less favorable for caged bees.

RESULTS OF EXPERIMENTS WITH THE PENICILLIA

While studying the fungi associated with honeybees the writer (*5, p. 64*) found that species of Penicillium caused the mummification of bees more often than did any of the other forms. Bees mummified by Penicillia resemble closely those killed by Aspergilli. Attempts were made to infect healthy bees with cultures of Penicillium isolated from other bees. Adult bees in cages and in colonies were unaffected when spores of these organisms were fed to them. Adult bees and brood in colonies exposed to the cultures of Penicillium on

brood combs were also unaffected. Bees were attacked and killed, however, when the spores of an unidentified species of Penicillium, isolated from mycelium growing in a wound of a living bee, were placed in wounds made with a needle. The infection was at first localized in the wound, but soon spread to other parts of the body. Nothing further could be done with this species though the following members of this ubiquitous group of fungi were tested for pathogenicity: *Penicillium corylophilum* Dierckx, *P. cyclopium* Westling, *P. palitans* Westling, *P. expansum* Link sensu Thom, *P. commune* Thom, and *P. brevicaule* Saccardo sensu Thom.

Although the spores of the above species of Penicillium did not germinate within the digestive tract of living bees, they remained viable and often germinated soon after the death of the bee, causing mummification of the tissues. This probably accounts for the similarity of conditions observed in bees mummified by Penicillia with those conditions observed in bees killed by Aspergilli.

RESULTS OF EXPERIMENTS WITH THE MUCORS

Adult worker bees were inoculated with species of the genus Mucor which had been isolated from bees mummified by these organisms. They were unaffected in most of the experiments, though an occasional bee was attacked by one species, *M. hiemalis* Wehmer, when bees were caged on cultures, or when spores of this organism were mixed with dilute honey and fed to them.

Only an occasional bee was attacked in colony experiments in which most of the honey was removed from the hives and replaced with honey diluted with three volumes of a water suspension of spores of *M. hiemalis*. The brood in such cases was unaffected. The other species isolated from bees and tested for pathogenicity on bees in cages were *M. racemosus* Fresenius, *Rhizopus nigricans* Ehrenberg sensu Lendner, and *Syncephalastrum racemosum* F. Cohn. None of the bees were attacked by these.

SYMPTOMS PRODUCED BY OTHER FILAMENTOUS FUNGI

A considerable number of fungi belonging to genera other than those already discussed have been isolated from bees and tested for pathogenicity by feeding spores to bees, or by caging bees on the cultures. Among the fungi tested were *Fusarium negundo* Hubert and other unidentified species of Fusarium, unidentified species of Sporotrichum, *Cladosporium herbarum* Pers, *Hormodendrum atrum* Bonord, and *Myceliophthora inflata* Burnside. Only negative results were obtained in the experiments with these fungi. An unidentified species of Isaria, isolated from a sowbug, and *Metarrhizium anisopliae* (Metsch.) Sorokin attacked less than 10 per cent of the inoculated bees.

Other fungi have been isolated that were found vegetating within the digestive tract or tissues of sick bees. Although the observations made indicated that some of these were pathogenic to the bee, inoculation experiments were limited to those forms that appear on bees with considerable frequency.

RESULTS OF EXPERIMENTS WITH THE YEASTS

In the experiments with yeasts, ordinary baker's yeast, certain identified yeasts isolated from bees, and several unidentified species, were used. The specific identifications were determined by comparison of the morphological and cultural characteristics of the yeast with those given for these species by Guilliermond (*13*). Introductions through the alimentary canal were made by mixing cultures of yeast with the food, by caging bees on the cultures, and by wetting them with water suspensions of yeasts. Inoculations were made directly into the blood of bees through wounds made with a sterile needle. The foods with which the yeasts were mixed included dilute honey, 10 to 20 per cent sugar solution, and Laurent's medium for yeasts to which about 20 per cent of sugar or honey was added.

A dysenteric condition appeared among caged bees that were heavily inoculated by feeding on *Saccharomyces ellipsoideus* Hansen, *S. cerevisiae* Hansen, and on two cultures of *Mycoderma cerevisiae* Desm. and Hansen that differed in cultural characteristics. Uninoculated bees kept under conditions as nearly identical as could be obtained were not similarly affected. No effects were noticeable from feeding on *Saccharomyces apiculatus* Klöcker and one other unidentified, slow-growing yeast. The death rate among inoculated bees averaged higher than among the checks for the entire series of experiments, although the difference was so small that its significance is as yet uncertain.

When bees were heavily inoculated by feeding on *S. cerevisiae* and *S. ellipsoideus*, taken from cultures on potato slants, a condition resembling intoxication developed. Gas, which gave positive tests for CO_2, was formed within the ventriculus of the intoxicated bees. Recovery from this condition, however, occurred within three or four hours, and the death rate among the bees remained normal when the food containing yeast was replaced with pure honey.

Caged bees were unaffected when small quantities of yeast were mixed with their food, and bees in colonies were apparently unaffected when the only food available consisted of dilute honey or sugar sirup with which half a dozen yeast cultures were mixed.

Limited growth was observed to occur within the ventriculus of infected bees when their food consisted of dilute honey sirup or Laurent's medium. Growth never progressed farther, however, than the formation of one or two buds by less than 20 per cent of the cells. In about 3 or 4 days after bees were given the cultures most of the yeast had passed from the ventriculus into the rectum. Yeast cells within the contents of the rectum were found to be viable up to 10 days after being eaten.

When the inoculation with yeasts was made in the blood by punctures in the thorax, infection and death resulted in from 50 to 100 per cent of the individuals. With *Saccharomyces apiculatus* death resulted in about 50 per cent of the cases. Weakening and crawling of some of the inoculated bees was first noticed after 2 days, and death usually occurred in less than 24 hours after the first symptoms were apparent. Most of the inoculated bees that were infected died in from 2½ to 6 days after they were inoculated. A few died of disease after 8 days, although as a rule those that survived for 8 days after inoculation had not been infected.

Other yeasts isolated from bees, including *S. ellipsoideus*, *S. cerevisiae*, *Mycoderma cerevisiae* I, and *M. cerevisiae* II, three unidentified yeasts, as well as brewer's yeast, gave results similar to those with *S. apiculatus*.

The highest death rate was obtained with *S. ellipsoideus* and *S. cerevisiae*. With both of these organisms the first symptoms of disease appeared in from 24 to 36 hours at normal room temperature, although at 30° C. this time was shortened. The highest death rate occurred during the second to fourth day after inoculation, and a few bees died between the fifth and sixth days. With these two organisms from 75 to 100 per cent of the bees inoculated usually died from infection.

With *Mycoderma cerevisiae* I, *M. cerevisiae* II, and the three unidentified yeasts from bees, the death rate from infection was slightly higher than with *Saccharomyces apiculatus*. Infected bees rarely lived longer than seven days after the inoculations were made. From 50 to 70 per cent of the inoculated bees died as a result of infection.

Microscopical examinations were made of the thoracic muscles and blood of inoculated bees at intervals after inoculation. Yeast cells multiplied rapidly within the blood and at the time of death were present in such large numbers that the blood appeared milky. Although the organism was ultimately carried to all of the body divisions by the blood, the most abundant development seemed to occur on the muscle fibers of the thorax. Yeast cells seemed to multiply there while the blood flowed over them without altering their position.

In a few cases the infection appeared more or less localized in the region surrounding the point of inoculation. With *S. apiculatus*, small spindle-shaped to linear pockets filled with yeast cells were observed in considerable numbers between the fibers of the wing muscles and apparently between the sarcostyles near the surface of the muscle fibers. There were usually from 5 to 20 yeast cells in each of these pockets at the time death occurred.

Symptoms were readily discernible upon dissection of the bees after death. The blood was found to have lost its pale-brownish color and had become milky. At the time of death, the wing muscles were slightly less rigid than in normal bees. Later they became mummified and brittle. The drying wing muscles shrink away from the exoskeleton except where they are attached. One of the most distinctive symptoms of yeast infection determinable without the aid of a microscope is the presence of a chalk-white coating on the surface of the muscles after they have dried. The presence of numerous yeast cells within the blood and on the muscle fibers is readily established with the aid of a microscope. No external changes were observed by which bees dead of infection with yeasts could be distinguished.

DISCUSSION

These investigations have dealt with the fungi for which pathogenicity has been established and those that are closely associated with bees but usually do not parasitize them. All of these ordinarily reach their typical development as saprophytes under a wide variety

of conditions. Some of them occur regularly on brood combs under proper conditions for their growth, whereas others are rarely found there.

The specific descriptions have not been included, since they are obtainable in standard works on mycology.

The Aspergilli are widely distributed, and the various species develop under widely different conditions. They are most commonly met with as saprophytes growing upon rotting fruits and other food products in which there is a high concentration of sugars. During dearths of nectar, spores of Aspergilli may be gathered by bees with juices of fruit or other juices which contain sugar. They are often found on the brood combs under certain conditions of moisture which exist in weak colonies, or in hives in which colonies have died out during winter or spring. Wet extracting combs when stored in damp rooms may also be attacked by a number of the species. Many of the species of Aspergillus, however, reach their typical development under dry conditions. Grain in storage, dried fish, foods, and herbarium specimens are frequently attacked by these. Some forms are typical inhabitants of the soil. Still others are capable of becoming active animal parasites, attacking insects, birds, and mammals, including man.

Of the several hundred isolations of Aspergilli from bees affected by mycosis, more than half were yellow-green spored forms of the *Aspergillus-flavus-oryzae* group. The greater number of these in turn were of the *A. flavus* series with comparatively long conidiophores. This may probably be accounted for by their virulent pathogenic nature, their abundant spore production, and their widespread saprophytic growth on a variety of natural substances including extracting and brood combs. All of the organisms belonging to this group isolated from bees from the chief beekeeping sections of the United States and from Canada and Europe have been found to be pathogenic when tested. Tests with a considerable number of races, strains, or species of the group, obtained elsewhere than from bees, seem to indicate that there is considerable difference in the degree of virulence. Intermediate strains, all known as *A. flavus-oryzae*, have been isolated from bees on but few occasions. These organisms, when tested, attacked both bees and brood. Intermediate forms from other sources have also given positive results, but the death rate among bees was generally lower than with *A. flavus*. Other strains failed to attack bees under similar conditions.

A. oryzae was not isolated from bees, but at least one strain, *A. oryzae* (113 L), obtained from the Thom and Church collection, attacked a few bees in cages and an occasional larva when spores were shaken over a brood comb containing uncapped brood. It is only among the *A. flavus* series of this group, however, that pathogenic organisms are found that are of importance as causing bee diseases.

The deep-green strains, which produce acid most abundantly are the ones that attack bees most readily. On the other hand, the thickness of the spore wall, which in *A. flavus* is about twice as thick as in *A. oryzae*, may be a determing factor in protecting the spores from the digestive fluids of the bee until germination can take place.

Organisms belonging to the *Aspergillus fumigatus* group occur on adult bees (pl. 4, A) and less frequently on the brood. Tests for pathogenicity with *A. fumigatus* isolated from bees obtained from widely scattered sections of the United States have all given positive results. Cultures obtained from the collection of Thom and Church and from a prominent drug manufacturer of Detroit have been found capable of parasitizing bees. Negative results have never been obtained with *A. fumigatus*, but brood-comb cultures placed in colonies caused less damage than was caused by *A. flavus*. In cage experiments the mortality from the fungus has usually been 100 per cent.

Aspergillus nidulans occurs less frequently on bees than does *A. fumigatus*. Isolations have been made from adult bees from time to time and once from brood. Inoculation experiments with *A. nidulans* from bees and from other sources seem to indicate that a number of strains are pathogenic to bees. The death rate among bees inoculated with *A. nidulans* is lower than with *A. flavus* or *A. fumigatus*, and the bees frequently escape infection.

Aspergillus glaucus has been isolated from mummified larvae and from adult bees. In experiments with caged bees only a small percentage were infected, and cultures obtained from the Thom and Church collection failed to attack any of the bees. Brood was not attacked when spores were scattered over larvae from two to four days old.

The black-spored Aspergillus, *Aspergillus niger*, has rarely been found on bees, although isolations have been made from adult bees and from mummified larvae, and a few worker bees were attacked in inoculation experiments. Attempts to cause infection of brood by *A. niger* were not made. Such an extreme case as that described by Morgenthaler (*18*), in which the comb cells were filled with a loose, dark-brown or black powder and larvae shriveled, but with swollen edges, lay covered with this dust, has never been observed in these experiments. The brood combs of tightly closed hives from which the bees were removed have been observed to be completely overgrown with *A. niger*. No noticeable infection occurred, however, when these combs were given to strong colonies even though brood rearing was begun in them within a few days.

In one instance an organism identified as *Aspergillus ochraceus* appeared to have been the cause of heavy losses in a Connecticut apiary. Examination of samples sent to the writer showed that more than 75 per cent of the diseased bees were attacked by this organism. (Pl. 3, A.) A second lot from the same apiary yielded about 60 per cent of infected bees. Inoculation experiments with young cultures were unsuccessful, as neither bees nor brood were attacked. When year-old cultures that had thoroughly dried were used, all of the inoculated bees were attacked and killed.

It was found that young but apparently mature spores of this Aspergillus were not only unable to germinate within the alimentary canal but were killed after a short time. No growth occurred after the bees were killed, and the spores failed to germinate when placed on nutrient agar. Gorging with young spores was ineffective in stimulating an attack. During the culture of this organism the spores were observed to be at first thin walled with a gelatinlike

coating. Later on the spore walls became thicker and roughened while changes of a chemical nature result in hardening of the gelatinous covering. This additional protection in old spores from the digestive fluids of the bee may explain the ability of old spores to attack bees and the complete lack of this ability in young spores. After germination, the digestive fluids of bees appear to have no injurious effects upon the young mycelium.

Two other cultures of *A. ochraceus*, isolated from bees but differing in morphological and cultural characteristics from this form, failed to parasitize bees or brood regardless of the age of the spores.

A few species of Aspergillus, among them *A. versicolor*, *A. terreus*, *A. candidus*, *A. sydowi*, *A. sulphureus*, and *A. clavatus*, isolated from beehives or bees, failed to infect bees in cages. Colonies of bees, therefore, were not tested with these organisms. The mycelium of these species was superficial on bees, and the characteristic mummification, common in mycosis, was absent. If these species ever attack bees, it would appear that special conditions are necessary.

The Penicillia appear to have only a purely saprophytic relation to bees. The resemblance of bees mummified by any of a number of species of Penicillium to those dead of mycosis may be explained by the rapid saprophytic growth within the bee immediately after death.

The frequency with which the Penicillia occur on bees may be accounted for, at least in part, by their ubiquitous nature and by the fact that the spores are not quickly killed by the intestinal fluids. Viable spores in large numbers are usually present on the brood combs and equipment within the hive. When Penicillium spores gain entrance to the alimentary canal before death, they may grow saprophytically within the bee after death.

The only damage to bees for which the Penicillia are responsible is caused by their saprophytic growth within the hive. Since the conditions of moisture and temperature within strong, well-managed colonies rarely permit the growth of fungi on the combs, damage is generally limited to poorly ventilated hives, weak colonies, and combs in unoccupied hives.

Mucors have been found to occur commonly on bees, but the experimental evidence thus far obtained indicates that ordinarily they are not pathogenic. Sporangiospores may swell considerably or even bud once or twice in a yeastlike manner within the ventriculus without seriously injuring the bee. It was found by cultures from the ventriculus and rectum of inoculated bees that sporangiospores of most of the Mucors investigated were soon killed by the intestinal fluids of adult bees. Inoculated bees that later died of starvation were not attacked by the Mucor after death.

It appears, however, that, under certain conditions which as yet have been only partially determined, bees are parasitized by one or more species of Mucor. In the inoculation experiments with *Mucor hiemalis* an occasional bee died of infection. This species has often been isolated from mycelium found vegetating in sick bees and from dead bees.

The results of investigations with this Mucor will be given in another paper.

Inoculations with other filamentous fungi that commonly occur on bees have always given negative results; consequently the writer is

of the opinion that they rarely attack healthy bees. Occasionally fungi differing from those found to be pathogenic by experimental inoculation have been isolated from mycelium found vegetating within the digestive tract or tissues of sick bees. This would seem to indicate that the fungi observed could parasitize the bees. However, since no one species other than those already discussed was isolated more than two or three times, their pathogenic relation to bees was not extensively investigated.

The yeasts have been observed to occur with considerable constancy within the alimentary canal of bees. The number of cells present was usually small, and they were often limited to the contents of the rectum, but a considerable number of cases have been observed in which much of the contents of the honey stomach or of the ventriculus consisted of yeast cells. In most of these cases the cells were evenly distributed within the contents, and, although buds in various stages of development were present, it could not be ascertained whether growth had occurred within the bee. At one time yeast, in more or less rounded hard masses of the size of wheat grains, was found in the honey stomachs of more than 10 per cent of a lot of sick bees collected in November, 1924, at Ann Arbor, Mich. Three distinct yeasts, one of which was identified as *Saccharomyces ellipsoideus*, were isolated from the masses of yeast cells. It was thought possible that these yeasts had developed within the honey stomachs of the bees and were responsible for their abnormal condition.

A series of feedings with these yeasts and others, including *Saccharomyces apiculatus*, isolated from bees failed to justify such an assumption. None of the yeasts multiplied extensively within the alimentary canal, and the bees were not seriously affected by the presence within the digestive tract of large quantities of yeast. Masses of yeast cells, such as were found within the honey stomachs, did not form there within these inoculated bees. Such masses did form, however, but without apparent harm, within the ventriculus of bees that were heavily inoculated. In this case the yeast masses were doubtless formed as a result of the sifting out of the cells from the food contents of the ventriculus. Such a process could also occur in nature when bees feed upon partly fermented honey.

Yeasts have been found, often in large numbers, in the tissues of bees several days after their death. Among these, *Saccharomyces apiculatus* and *S. ellipsoideus* were recognized. A small oval yeast that was not identified was found in the thoracic muscles of about 50 per cent of a sample of nearly a hundred dead bees sent to the bee-culture laboratory from South Carolina. The gross symptoms of these infected bees resembled closely those of bees inoculated with yeasts by being punctured with a needle that had been dipped into a suspension of yeast cells.

Following these observations on dead bees, it seemed desirable to observe living bees to determine if yeasts are capable in nature of parasitizing healthy bees by gaining entrance into their blood. Although many apparently affected bees were examined, yeasts were never found vegetating in the blood.

Although it could not be determined whether or not the yeasts found in the muscle tissues and blood of dead bees had been the

cause of death, all of the methods of infecting other than the puncture method gave negative results.

In summing up the evidence from the experimental inoculations with yeasts and the observations on bees, it appears that ordinarily yeasts do not infect the blood or tissues of healthy, uninjured bees, although they are capable of becoming actively pathogenic when they gain entrance to the blood or tissues.

It has been shown that certain species of fungi are pathogenic to honeybees of all ages. Some of these attack adult bees in nature with considerable frequency and reach a degree of economic importance when conditions favor the production of large numbers of spores. Others attack bees so rarely under normal conditions that their importance as causative agents of disease is negligible, whereas still others that have never been found on bees in nature were found to attack bees when inoculated experimentally.

The greater number of fungus species that cause diseases of adult bees also attack the brood. This was found to be the case with most of the species or strains of Aspergillus used in these investigations. When inoculated experimentally with a pathogenic Aspergillus, brood is attacked and killed more quickly than are adult bees, though the loss of brood resulting from Aspergillus mycosis is much less than that of adult bees.

The frequency with which bees are attacked by fungi in nature appears to depend chiefly upon the virulence of the pathogenic species and upon their dispersion. Conditions that favor abundant growth of pathogenic fungi in nature are conducive to the spread of fungous diseases. The fact that brood is rarely attacked can probably be explained by the small probability that larval food will contain a sufficient number of viable spores to cause infection.

The pathogenicity of a fungus appears to be determined by the ability of its spores and mycelium to resist the action of the intestinal fluids within the digestive tract of bees.

Mycosis of bees reaches its greatest significance with adult workers. Throughout the active season an appreciable number of these are killed by pathogenic fungi, principally by the yellow-green spored Aspergilli. In one case about 40 per cent of a lot of bees that showed symptoms similar to mycosis yielded pathogenic species of Aspergillus. Usually, however, only about 5 per cent of the suspected bees were infected.

It is difficult to estimate the importance of mycosis among bees because adult bees generally die away from the hive, and, when the percentage remains small, larvae may be carried out soon after they become infected. The death rate from mycosis during the winter varies with conditions within the hive. In strong colonies under good wintering conditions it is negligible; in weak colonies, when pathogenic fungi have grown over the combs, mycosis may become an important factor in the dwindling and death of the colony.

In view of the fact indicated by this study, that fungous diseases of bees are not likely to assume the importance of either of the foul broods, methods of control have not been extensively studied.

Complete control would be difficult to attain, since worker bees may be infected while gathering sweet juices from fruits, food products, and many other natural and prepared substances, upon which

pathogenic fungi grow. Infection from this source, however, is not likely to become epidemic.

Infection from molded combs, equipment, and dead bees can be considerably reduced by care about the apiary. During the winter ample provisions should be made for the escape from the hives of metabolic water vapor. The bottom board should be cleaned of molded bees as early in the spring as weather conditions are suitable for manipulating the bees without danger of chilling them. Brood combs and extracting combs, when not in use, should be stored in dry rooms to prevent pathogenic fungi from growing on them.

In order to prevent the possible occurrence of disease, all badly molded combs and equipment used inside of the hive should be dipped for a few minutes in a 20 per cent solution of formalin in water or exposed to formaldehyde gas in an air-tight chamber for two or three days. Molded hive bodies, bottom boards, and covers which can not be dipped readily should be washed with the same solution.

SUMMARY

This investigation of the fungous pests of bees shows that parasitic fungous species occur on bees and brood, that they may cause quite virulent diseases, that some of these forms reported as pathogenic in Europe also occur in North America, and that there are additional pathogens not heretofore reported. Certain other mycological and biochemical data have been obtained and presented; and the effects of the invading fungi on the bees, their development, and the disturbances caused within the body of the bees and larvae, have been worked out in considerable detail.

The fungi shown to be pathogenic include species of Aspergillus and Mucor, and the Saccharomycetes. Several species of Aspergillus and one of Mucor are most important.

Members of the *Aspergillus flavus* series were shown to attack bees more frequently than other forms of Aspergillus, but *A. fumigatus* is also virulently pathogenic. In addition, *A. nidulans*, *A. niger*, *A. glaucus*, and *A. ochraceus* attack bees in nature.

Bees are attacked when spores of pathogenic fungi are taken into the alimentary canal. They can be artificially inoculated by mixing spores of the fungi with their food or by causing them to come in contact with mature cultures. Infection also results when spores are placed in the blood of bees by puncturing the exoskeleton.

The spores germinate within the food contents of the alimentary canal. The hyphae penetrate the wall, and under favorable conditions all of the softer tissues are attacked by the mycelium. The spores of nonpathogenic fungi do not germinate within the alimentary canal of healthy bees, but with some species they remain viable and germinate after the death of the bee.

Germ tubes were found not to penetrate the exoskeleton of healthy adult bees. They may be induced to penetrate the skin of larvae, but this rarely, if ever, occurs in nature.

Before death the gross symptoms of mycosis are not distinct from those of other disturbances in adult bees. At the time of death, or soon thereafter, positive diagnosis can be made microscopically from

the presence of mycelium within the tissues. Diagnosis may also be made from the post-mortem changes.

The action of pathogenic fungi upon the tissues of bees is both physical and chemical in nature. Tissues are penetrated by the developing mycelium and are digested by the fungous enzymes. One pathogenic fungus, *Aspergillus flavus* Ao5c, was shown to produce a transient toxic substance which is the cause of fatal poisoning in bees.

None of the species of Penicillium encountered attack healthy bees in nature. *Mucor hiemalis* attacks bees under proper conditions, but several other species of Mucor studied appear to be harmless.

Yeasts were found to be more or less constantly present in the alimentary canal of bees, although, except in extreme cases, bees are normally unaffected by their presence. A number of yeasts, however, including *Saccharomyces ellipsoideus*, *S. cerevisiae*, *S. apiculatus*, *Mycoderma cerevisiae*, and baker's yeast, are pathogenic when introduced into the blood of bees. Yeasts rarely if ever gain entrance to the blood of healthy bees in nature; consequently they appear to be of little significance pathogenically.

Filamentous fungi, differing from those investigated, have also been observed vegetating in tissues of sick bees, but inoculations have uniformly given negative results.

The observations made thus far tend to indicate that bees become infected from beekeeping equipment, dead bees, and molded combs, and from molded fruit and other substances that contain a high concentration of sugar.

Badly molded combs and equipment within the hives of infected colonies should be disinfected by being dipped or washed in a 20 per cent solution of formalin in water or by being exposed to formaldehyde gas.

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DISEASES OF BEES

BEING THE REPORT OF A CONFERENCE HELD AT ROTHAMSTED ON SEPTEMBER 26TH, 1936, UNDER THE CHAIRMANSHIP OF

SIR E. J. RUSSELL, D.Sc., F.R.S.
(Director of Rothamsted Experimental Station)

With contributions by

SIR E. J. RUSSELL, D.Sc., F.R.S.
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ROTHAMSTED EXPERIMENTAL STATION
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INTRODUCTION

By Sir E. J. Russell, D.Sc., F.R.S.

The Conference at which the following papers were read was the third of its kind held at Rothamsted. The Reports of the two previous ones proved so acceptable to beekeepers that the editions were soon exhausted ; a larger edition is being printed this time so as to avoid this possibility. This Conference is in several ways far more important than its predecessors : it gives the results of Dr. Morgenthaler's wide experience on the subject, which he himself came over to present ; it gives also the results of Scottish experience, presented by Dr. Morison, and of American experience, kindly contributed by Dr. Hambleton, who, although he could not be here in person, sent his paper on to be read and printed. Finally it sets out the results of an investigation made here on Brood Diseases of bees by Dr. Tarr, in general consultation with Mr. Morland and Dr. Williams, during the past three years. The work was made possible by the co-operation of the Ministry of Agriculture and the Agricultural Research Council on the one hand, with the Beekeepers' Associations working through the British Bee Keepers' Association on the other : the first time such collaboration has been possible, and a great tribute to the organising ability of the officers of the various associations concerned.

As a result of this work Dr. Tarr has been able to establish a clear distinction between American Foul Brood and European Foul Brood ; he has isolated and studied the organism (a bacterium) responsible for the former and is well on the way to clearing up the complexities of the latter ; he has shown that a third disease, the so-called Addled Brood, is very prevalent, and has worked out its cause and indicated a remedy.

The work has been supervised on the beekeeping side by the expert Bee Advisory Committee at Rothamsted and on the scientific side by Dr. Williams and by Dr. Schütze of the Bacteriological Department of the Lister Institute. All the experts who have examined the work agree that it is unusually good and that the results can be accepted as trustworthy.

It is hoped therefore that the investigations can be continued and extended to other diseases of bees, particularly of adult bees.

There is every reason why the work should go on. Interest in beekeeping is undoubtedly increasing. There are no definite figures, no census having been found practicable, but the increase is undeniable. The reason is clear : beekeeping is a very interesting activity for the amateur and a promising line for the professional.

It adds greatly to the pleasure of gardening ; it gives a new interest to the man who has retired from a busy life in the town to a quieter life in the country ; it is an indispensable adjunct to the growing of fruit—which is considerably increasing in this country. Further, it caters for a healthy and growing public demand ; for honey is a healthy food, supplying in a very agreeable form something not easily obtainable elsewhere, and which more and more people are learning to appreciate and desire.

Further evidence of the interest now being taken in beekeeping and in honey production is that a National Mark has been established for honey. Owing to certain difficulties of definition it was found necessary to carry on investigations into the properties of honey ; these have been started at Rothamsted under a grant provided by the Ministry of Agriculture and the Research Council. It is not unreasonable to suppose that these official bodies were influenced in their decision to bear the whole cost of this work by the fact that the beekeepers were already showing the reality of their own interest in the matter by providing funds for the study of bee diseases.

The Conference, having heard the papers, unanimously carried the resolution moved by Dr. Gregg and Dr. Thompson urging that the work should be continued. It was very gratifying to the Rothamsted Staff that two such well-known authorities should have thus supported their work. We at Rothamsted are prepared to carry on the work : it remains only for the Beekeeping Associations to do their share. With co-operation and good will, success is bound to come.

BROOD DISEASES IN ENGLAND: THE RESULTS OF A THREE-YEAR INVESTIGATION

By H. L. A. Tarr, Ph. D.

(Rothamsted Experimental Station, Harpenden, Herts.)

In the time available it will only be possible to summarize briefly the results which have been obtained since the inception of the brood disease research scheme at Rothamsted. In doing this it will be necessary to assume some knowledge of the common characteristics of the various brood diseases: so much has been said and written about them recently that this demand would not appear unfair. The fact that there is indeed a multiplicity of brood diseases in England must be emphasised. Statements to the contrary have been and are being made by individuals who occupy prominent positions in beekeeping in this country: such statements are definitely erroneous. The fact that there is a multiplicity of brood diseases is of considerable importance when one has to consider their treatment.

Since May, 1934, two hundred and five samples of diseased brood have been sent to the laboratory and the following diagnoses have been made: American foul brood, 104; Addled brood, 58; European foul brood, 13; Chalk brood, 11; American foul brood and Addled brood, 1; American foul brood and Chalk brood, 1; chilled or neglected brood, 4; spray poisoning, 1; Sac brood, 4; drone laying queen and decomposing brood, 5; and drone laying queen and Chalk brood, 3. Though it cannot be stated that these figures denote the actual proportion of brood diseases in England, they certainly are of value in that they give some indication of their distribution, the proportion being relatively constant from year to year. It is practically certain that the distribution of the different diseases could not have been foretold prior to the commencement of the investigation. So far most of the time has been devoted to experiments designed to determine the causes of the three most prevalent brood diseases, for without this knowledge it would be difficult to devise treatment. The results of experiments relating to the different diseases will be discussed separately.

American foul brood

The examination of numerous samples has led to a verification of the fact that the spores of *Bacillus larvae* are almost invariably present in apparently pure culture in the ropy remains and scales of larvae dead of this disease, and further that this organism causes the disease. A number of experiments have been carried out in order to determine the relationship of *B. larvae* to the cause of American foul brood. It has been found that disease is caused by feeding decaying larvae, dead of American foul brood, to bees of healthy nuclei even when the material is suspended in water and heated for twenty minutes at 85°C (185° F), the spores of the causal organism resisting this temperature readily. Heating under steam pressure at a high temperature killed the spores of *B. larvae*, and material so treated was no longer capable of initiating disease. After considerable trouble a culture medium upon which *B. larvae* grew and sporulated readily was evolved : most media upon which this organism grows readily will not support spore formation. Suspensions of the vegetative cells and of the spores of *B. larvae* were prepared upon this medium and were employed in a series of infection experiments.

Vegetative cells of *B. larvae* when introduced into healthy nuclei by feeding the bees, feeding the larvae directly, or spraying the bacteria over developing brood, have in no experiment so far produced disease. Thus in two different experiments, approximately 170,000 million and 80,000 million vegetative cells prepared on the same medium as that employed for obtaining spores, were sprayed over eggs and developing larvae of healthy nuclei and no disease developed. Toumanoff, working in France, obtained similar results. With spores of *B. larvae*, obtained from pure cultures of the organism, American foul brood was readily initiated, providing a fairly large dose (mass inoculum) was employed. It was also found that a very much smaller inoculum of spores was effective in producing the disease, when the developing brood of the nucleus was sprayed directly with them than when they were fed to the bees in syrup. In one series of experiments, in which the same spore suspension was employed throughout, nuclei in which the brood was sprayed with approximately 620 million or 62 million spores soon developed American foul brood, while the disease did not develop in a nucleus receiving only approximately 6.2 million spores. When the spores were fed to the bees in syrup instead of being sprayed over developing brood, disease resulted in nuclei receiving approximately 62,000 million or 6,200 million spores, but not in those receiving approximately 620 million or 62 million spores. Sturtevant, working in the United States, found that a colony of bees would not develop American foul brood unless it received at least 50 million spores of *B. larvae* fed to the bees in 1 litre of syrup, the spores used in his experiments being derived from scales of larvae dead of the disease. The above work confirms, in general, his results. The fact that the

limiting infective dose in Sturtevant's experiments was considerably smaller than that used in the above described experiments may be because *B. larvae* rapidly loses virulence following cultivation on laboratory media. It seems highly probable that a few resistant endospores become established in the guts of very young larvae, and that once established they resist the digestive processes until conditions which favour their development arise, while the less resistant vegetative cells of the organism are rapidly killed under identical conditions.

It is of interest that, in these experiments, relatively large doses of vegetative cells of *B. larvae* would not cause American foul brood to develop. If a method of keeping this organism in the vegetative stage could be devised, then the control of the disease in infected colonies might be simplified. Unfortunately the possibility of doing this seems rather remote.

The results obtained have shown that American foul brood is the most prevalent brood disease in England, that it is a distinct disease caused by a resistant spore forming bacillus, and that a mass inoculum of spores of the organism is required to initiate the disease. So far no attempts have been made to study methods of controlling this disease. The very nature of the disease makes the possibility of obtaining a simple chemical remedy an extremely doubtful one, and it is not proposed to encourage any false hopes in this direction. Practical measures of control are known and the value of these under the conditions which pertain to this country must be determined.

Addled brood

The fact that this complaint should occupy such a prominent position with reference to the total number of brood diseases was not foreseen when the investigation commenced. Should the treatment of this very prevalent disease be as simple as is indicated by preliminary experiments we may well be pleased that so much of the disease is of this type and not so-called " foul brood." Before discussing the results obtained in practical experiments it is essential that brief reference be made to the somewhat scanty pertinent literature.

Throughout the past few decades numerous references have been made in the German literature to " Eitaubheit," a disease of bees in which apparently normal, fertile queens lay eggs which never develop. There is no adequate equivalent in the English language for this name and it is best expressed as Addled egg disease, the eggs being known as "Addled eggs" (Taube Eier). References to the condition in which brood dies at some stage prior to reaching maturity have been far less numerous in comparison. As far as I have been able to ascertain the late Dr. Leuenberger was the first to describe Addled brood though he did not actually employ this term. In one case of Eitaubheit he observed that a small

number of eggs succeeded in hatching, but that the larvae which developed from them, a few of which were sealed over by the bees, died before reaching maturity. This seems to have been a mixed case of Addled eggs and Addled brood, the queen being responsible for the condition. In 1925 Anderson described cases of Addled brood in Scotland. These cases were marked by the fact that practically all the sealed brood died just prior to the time of emergence, but occasionally addled eggs (eggs which did not hatch) were noticed, and, of the bees which did succeed in developing, many could not fly. The disease could be produced in healthy colonies by introducing queens from affected stocks to them, while requeening affected stocks with normal queens always cleared up the disease. Moreaux, working in France, has also recently described a case of Addled brood. His paper appeared at about the same time as the 1935 report on brood diseases was issued from this Station. In the last named article the appearance of larvae in cases of Addled brood (" Uncertain ") was described in some detail. It appears that the numerous cases of diseased brood received at this Station in which at first no accurate diagnosis could be made, and which were temporarily designated " Uncertain," were, in reality, cases of Addled brood. Unfortunately there is not time to describe in detail the appearance of larvae dead of this disease. Normally the pupae or prepupae are attacked, the appearance of the dead brood being similar to that noticed in larvae which have died and undergone autolysis. Usually these larvae are almost, or quite, sterile bacteriologically. The results of a series of experiments which have been carried out in order to ascertain the cause of the complaint leave no doubt that it is, in effect, Addled brood. There is every indication that the complaint can vary greatly in severity. Thus in some colonies relatively few Addled pupae are seen, and such a colony may show few or no external symptoms of weakness. On the other hand some stocks may be badly affected, and become so weak that they store no surplus honey and may even fail to resist the winter. It seems fairly certain that the cases investigated by Anderson merely represented very severe Addled brood in which very advanced pupae were those chiefly affected.

In three years, fifty-eight samples of Addled brood have been sent in, representing about 28 per cent. of all the samples received. Preliminary experiments showed that the disease, unlike American and European foul brood, is not contagious : combs containing an abundance of affected pupae when placed in healthy nuclei never caused disease. During the past season queens taken from affected stocks have been obtained from certain beekeepers, and several of these have been successfully introduced to queenless nuclei. In every case in which such a queen was accepted, the nucleus concerned soon showed signs of Addled brood ; a certain proportion of the sealed brood produced by the queen, dying before reaching maturity. When the affected queen was removed from

such a nucleus normal sealed brood soon appeared, following the introduction of a healthy queen. It seems that the disease is not necessarily one of old queens, for queens mated in 1936 have been found to produce Addled brood. One experiment has shown that drone as well as worker pupae are affected; this might be taken to indicate that the queen is directly responsible for the trouble and that the drone plays no part. The fact that eggs and very young larvae, when inserted in an affected colony, have been observed to develop normally, supports the idea that the queen causes the malady and that it is not due to lack of attention of the larvae by the nurse bees.

Though the superficial cause of the disease is a defective queen, the fundamental cause remains to be determined. The defect may be hereditary, the queen possessing some "lethal factor," or the queen may suffer from some infectious disease or from some abstruse pathological abnormality. This remains to be determined. Since further experiments on the control of this disease are needed, it is hoped that beekeepers who experience the complaint will try requeening affected stocks and will notify this Station of the result. The importance of continued investigation can readily be foreseen, especially in view of the fact that the queen breeder must at all costs eradicate the disease from his apiaries.

European foul brood

At present it appears that this disease is not widespread in England, but the fact that it does occur, that it is highly contagious, that it causes more trouble than American foul brood in Switzerland, and that its cause has been in doubt has made it advisable to investigate it thoroughly. If more is known about it, then it will be easier to employ measures to prevent its spread should it again show signs of increasing.

Larvae affected with European foul brood, unlike those dead of American foul brood, exhibit a very varied bacterial flora, and this fact has greatly complicated the determination of the cause of the disease. The remarkable confusion which has existed with reference to its etiology, has been referred to in a number of publications. The results of preliminary experiments carried out here, led to the suggestion that European foul brood might not be a single disease, but that it was, perhaps, a mixed bacterial infection of the brood of weak stocks of bees. Further experiments carried out during the past season have shown that this hypothesis was erroneous, and that the disease is, in fact, a single one in which various modifications may occur.

White (1912-1920) working at the United States Department of Agriculture, concluded, on the basis of a large number of experiments, that European foul brood was a single disease caused by a lanceolate-shaped coccus organism which he termed *Bacillus pluton*. He was unable to cultivate this organism on any laboratory

medium. He also showed that a number of other bacteria, which occurred in larvae sick or dead of this disease (*Streptococcus apis, Bacterium eurydice, Bacillus alvei, Bacillus orpheus*), would not produce disease when introduced into healthy colonies of bees. Since *B. pluton* would not grow, his evidence that it caused European foul brood was based upon the fact that no bacterium cultured from sick or dead larvae would cause the disease, and that all attempts to show that a filterable virus was responsible for producing the disease failed. Superficially this evidence was rather unconvincing, and subsequently it aroused much criticism. Thus White's hypothesis has been contested by Lochhead in Canada, by Burnside at the United States Department of Agriculture, by Borchert in Germany, and formerly by myself. This confusion has undoubtedly arisen through the failure of all these workers to distinguish White's *Bacillus pluton* from the various species of "*S. apis*" with which it can readily be confused morphologically. The fact that *Bacillus pluton* does exist as a species distinct from "*S. apis*," and that it is practically certain that it causes European foul brood, is apparent when the following experimental results are considered.

Three to four day old, apparently healthy larvae in colonies badly affected with European foul brood frequently have numbers of small lanceolate-shaped cocci in their intestinal tracts. These cocci occur singly, or, more frequently, in pairs and short chains, and at this early stage in infection are present in pure, or apparently pure, culture in the gut of the affected larva. When the whole intestine is removed, with aseptic precautions, from such a larva, and attempts are made to culture this coccus from the whole gut or portions of it on media suitable for the growth of most bacteria occurring in larvae affected with brood disease, the coccus fails to multiply. Very occasionally no bacteria grow under these conditions, but more frequently other bacteria make their appearance, notably small rod shaped bacteria (*Bacterium eurydice*) and sometimes *Bacillus alvei*. These cocci correspond to the *Bacillus pluton* described by White.

Larvae which are obviously affected with European foul brood always contain *B. pluton*-like organisms in large numbers in their intestinal tracts, and in nearly every case large numbers of other bacteria are present simultaneously—the so-called secondary invaders. Among these secondary invaders, normally the first to appear is a small rod-shaped bacterium (*Bacterium eurydice*), but "*S. apis*" species also frequently appear at this stage. However, these "*S. apis*" species are by no means always present in affected larvae. When present "*S. apis*" can be distinguished morphologically from *B. pluton*, though this distinction is by no means a clear cut one, some measure of experience being necessary before the two species can be readily differentiated. Normally the "*S. apis*" type of cell is more spherical than *B. pluton*, the latter being

almost always lanceolate-shaped with definitely pointed ends. When " *S. apis* " is demonstrable microscopically in affected larvae it can always be cultured readily on a variety of substrates.

" *S. apis* " is not, as has hitherto been assumed, a single species, and already three different varieties have been isolated. Two of these appear to be identical with *S. liquefaciens* and *S. glycerinaceus*, which are well-known lactic acid bacteria. Though it may prove to be more convenient to speak of these species collectively as " *S. apis* " when they are found in larvae affected with European foul brood, the term is strictly incorrect, and for purposes of classification the species name should be dropped. The source of these species of lactic acid bacteria is uncertain, but it is likely that the bees come in contact with them when collecting water. Such species are commonly found in cattle faeces. The fact that " *S. apis* " does not exist as a distinct species makes it increasingly difficult to accept the theory that European foul brood is caused by a pleomorphic organism (Lochhead, Burnside), especially in view of other evidence given in this paper.

The intestine of a single larva recently infected with European foul brood, and containing *B. pluton* in apparently pure culture, will soon initiate disease in a healthy nucleus when it is emulsified in water and the resulting suspension is sprayed over developing brood. However, as in the case of American foul brood, a certain " mass inoculum " of *B. pluton* cells is necessary before disease can be produced in a healthy colony. In one experiment a fairly uniform suspension of *B. pluton* cells was prepared by macerating the guts of two recently infected larvae, in which this organism appeared in apparently pure culture, in 25 cc. of dilute phosphate buffer (pH. 7.2). When portions of this suspension were examined bacteriologically, no coccus shaped cells grew on the medium used, the suspension being practically " sterile " as regards bacteria capable of multiplying on this medium, only a few colonies of small rod-shaped bacteria appearing (probably *Bacterium eurydice*). The approximate number of *B. pluton* cells in this suspension was estimated using a Thoma haemocytometer slide. Three different healthy nuclei were inoculated on June 25 by feeding them 1, 5 and 10 cc. amounts of this suspension in 30 cc. of syrup. Within 18 days the two nuclei receiving the larger doses (approximately 980 and 490 million cells of *B. pluton*) showed European foul brood, while the nucleus receiving the smallest dose (approximately 98 million cells) showed no disease up to the beginning of September.

All attempts to initiate European foul brood in healthy nuclei, by spraying the developing brood with suspensions containing large numbers of the organisms occurring as secondary invaders in this disease (" *S. apis*," *Bacterium eurydice*, *B. alvei* or *B. para-alvei*), have met with failure. However, it has been found that the course of the disease can be profoundly modified by spraying cultures of certain of these organisms over the developing brood of nuclei

which have been recently infected with *B. pluton*. Thus *B. para-alvei*, *B. orpheus*, "*S. apis*," and another spore forming bacillus which was recently described, have all been introduced into recently infected colonies, and have been found in large numbers in the decaying larvae. The modification induced by spraying *B. para-alvei* was, as might be expected, similar to the "*Parafoulbrood*" disease described by Burnside. This would seem to prove fairly conclusively that this disease is merely one modification of European foul brood and not a distinct disease. A peculiar modification noticed in the laboratory was one in which a yeast or torula was the principal secondary invader, and the combs gave off a pronounced smell of fermentation. When *B. alvei* and *B. para-alvei* are the active putrefactive agents the odour emitted by the decaying larvae is particularly foul, but it is not nearly so unpleasant when "*S. apis*," *B. orpheus*, or the other spore-forming bacillus mentioned above, constitute the secondary invaders.

Numerous attempts to cultivate *B. pluton* have been made, but so far all have failed. The usual media employed in the cultivation of other brood disease bacteria and a number of specially prepared media (made from whole larvae, intestines of larvae, or royal jelly) have not supported growth, when used aerobically, anaerobically or at different pH values. Curiously enough attempts to cultivate *B. pluton* in the stomachs of 3-4 day old larvae which were fed this organism and subsequently starved several days at 33°C. (hive temperature), have also failed. *B. pluton* cells could readily be demonstrated in the stomachs of recently fed larvae, but these rapidly decreased in number, and in most cases practically disappeared, the larvae dying and autolysing, occasionally with no visible bacteria, but more frequently with some rod-shaped bacteria like *Bacterium eurydice*. All available evidence points to the fact that *B. pluton* is a strict parasite which will multiply only in the guts of young developing larvae. It seems probable that the parasite is introduced by the bee into the larva when it is very young, perhaps at the time of hatching, and that once established it rapidly multiplies in the intestine, probably being localised in the peritrophic membrane (White) where a delicate balance of nutrients favouring its growth is reached. It is possible that *B. pluton* will grow on living animal tissues *in vitro*, and it is hoped that this possibility may be tested. Conclusive proof regarding the cause of European foul brood can only be obtained when *B. pluton* has been cultivated, has been shown to cause the disease, and has been isolated from larvae thus infected.

Several attempts have been made to determine how European foul brood is carried by the bee. Bees usually remove larvae sick or dead of this disease by sucking the infected material from the cells, hence one would expect to find large numbers of disease bacteria in the intestinal tracts of nurse and house cleaning bees from infected colonies. This has been found to be the case, for

bees taken from brood combs containing large numbers of larvae sick or dead of European foul brood have quantities of bacteria in their rectal ampullae. Among these the secondary invaders found in European foul brood are usually most prominent, especially *B. alvei* and small rod-shaped bacteria, but *B. pluton* organisms are frequently present. European foul brood has been produced in a healthy nucleus by spraying an aqueous suspension of the gut contents of six such bees recently taken from an infected colony over the developing brood, but it is not known how long the parasite will remain alive in the gut of the bee. It is extremely doubtful if *B. pluton* multiplies in the digestive tract of the bee. All attempts to demonstrate *B. pluton* in the pharyngeal, mandibular or salivary glands of nurse or house-cleaning bees taken from infected stocks, either microscopically or by means of infection experiments, have failed. So far no evidence has been obtained which indicates that this organism multiplies elsewhere than in the gut of the young larva.

Queens from infected stocks have in no instance caused disease when introduced into healthy queenless nuclei. In these experiments the queen and 12 young worker bees were removed from the affected stock and were caged from 1-2 days with candy as the sole source of food prior to introduction. These results verify those obtained by Morgenthaler and his associates working in Switzerland.

European foul brood has been induced in healthy nuclei early in the brood rearing season by suspending in them combs containing large numbers of decomposing larvae artificially infected by feeding them pure cultures of *S. apis* or *B. alvei* and subsequently starving them for four days at hive temperature. These results have been obtained in each of two consecutive seasons. It has been found that when the disease is initiated in this manner it never appears as soon as in nuclei infected directly with *B. pluton* cells taken from the gut of a young infected larva, especially when this organism is sprayed directly on the eggs and young larvae. There seems to be a definite lag period, usually of about three weeks, during which no infected larvae are seen. It might be inferred from these experiments that *B. pluton* normally exists in colonies of bees waiting for suitable conditions to multiply, and that these conditions can be induced by putting into the colony very large numbers of decomposing larvae which have been artificially infected with secondary invaders found in European foul brood. This is only a suggestion which must be verified by further experiments. So far all attempts to induce European foul brood in healthy nuclei by " artificial weakening " (removing bees or sealed brood and giving a surplus of eggs and young larvae) have failed, but these experiments were not carried out early in the brood-rearing season. Normally European foul brood can only be induced readily in the early part of the brood-rearing season, and, unlike American foul brood, disappears, or tends to disappear, toward the close of the brood rearing.

A thorough study of the control methods employed in the case of European foul brood must be made. In two cases in which swarms from colonies affected with European foul brood were hived on fresh foundation in clean hives the disease did not reappear. In another case in which a swarm was hived on drawn comb the disease soon broke out again. It is not improbable that the shaking method will prove of value in eradicating European foul brood in very obstinate cases of the disease which have failed to respond to the usual requeening treatment.

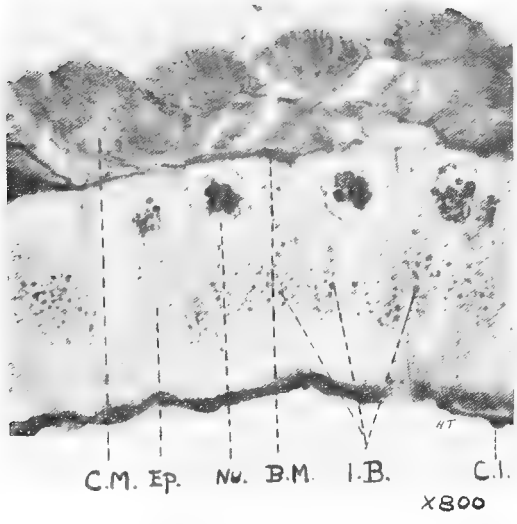

Fig. 1. Photomicrograph (×800) of a portion of a transverse section of the anterior end of the small intestine of a worker bee suffering from Bee Paralysis. The section was stained with safranin and methyl violet. B.M., basement membrane; C.I., chitinous intima lining the cavity of the small intestine; C.M., circular or transverse muscle fibre; Ep., epithelial cell with its nucleus Nu.; I.B., inclusion bodies which seem to be found only in bees suffering from Bee Paralysis.

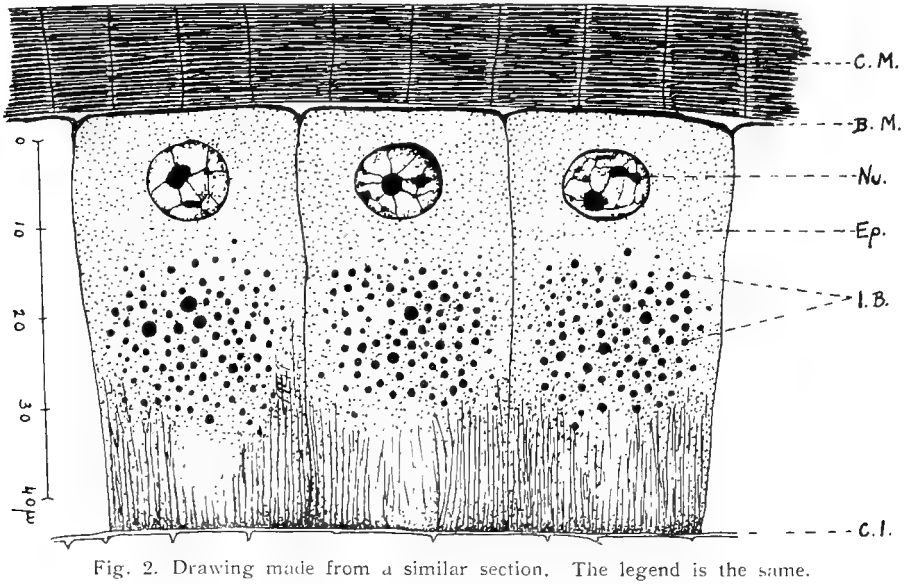

Fig. 2. Drawing made from a similar section. The legend is the same.

BEE PARALYSIS

By G. D. Morison, Ph.D.

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I have to apologise for the incompleteness of these notes, which is due to my not having had sufficient time to study the data at my disposal.

Definition: Bee Paralysis is a disease of adult workers, queens and probably drones, and it seems to attack all the common races of honey-bees kept in Great Britain. The affected stock dwindles slowly or more or less rapidly, owing to loss of adult bees. When the stock dwindles slowly, usually a percentage of only older (foraging) bees are affected. In a stock dying rapidly, foragers and younger bees are affected. Bees usually die away from the hive, but they may die scattered in front of the hives or even clustered in small numbers, resembling certain cases of acarine disease. The climatic conditions probably greatly influence the behaviour of bees outside the hive.

When a small percentage of bees is affected, some or all the diseased are hustled away from the hive by their seemingly healthy sisters. These diseased bees are lively with the abdomen not abnormally distended. They try again and again to enter the hive, but each time their healthy sisters forbid their entry, usually without attempting to sting them and often licking them as if to remove a substance from their bodies. The diseased bees lose their hairs in the struggles and they appear polished, darker and smaller than their sisters. In the end they may become almost hairless. They are often mistaken for robbers. Apparently they die from starvation and exposure outside the hive.

A small percentage of bees may be affected without the above mentioned behaviour occurring.

When a large percentage of bees is affected the stock is lethargic, the frames are not, or only slightly marked with faeces. The diseased bee moves its antennae normally; it flutters its wings considerably, but it cannot fly and it may not be able to right itself if turned on its back. The leg movements for walking are weak and the legs may tremble frequently, finally the bee dies lying more or less on its side with its legs tucked in or spread out in various ways. The mouth-parts are held rather extruded soliciting drink and the bee

continually tries to suck up fluids, but apparently without success. The bee attempts to clean itself or its neighbours with its mouth-parts. *It dies with the mouth-parts widely extended.*

" Respiratory " movements of the post-abdomen are variable, ceasing with loss of movement of wings and legs. The sting will often function on the stimulus of pressure shortly after other movements have apparently ceased. The post-abdomen is usually abnormally distended by the contents of the alimentary canal. The abnormal distention is usually due to :

1. Pale watery contents of the rectum,
2. Normal-looking but abnormal quantity of rectal contents,
3. Rectal contents normal in volume but proventriculus greatly distended with sugary solution.

The thorax and post-abdomen are usually rather polished through loss of hair which has probably been rubbed off by the diseased bees licking one another. The surface of the body may be coated with a very delicate film of sticky substance which, I think, is largely faecal in origin. This faecal matter seems to be passed out in very small droplets. It forms a good stratum for bacteria, which may easily contaminate smears of the blood of the bee. An obnoxious smell, often described as " fishy " may be associated with diseased bees. It may be due to secondary and tertiary amines in the faeces and it may be the chief reason for the eviction of the diseased bees by their healthy sisters.

To sum up, the chief characters of the disease are :—

1. Distended post-abdomen,
2. Trembling of wings and legs,
3. Distended mouth-parts and the bees die with them distended,
4. Exceptional thirst,
5. Loss of hair,
6. Fishy smell,
7. Inclusion bodies in cells of small intestine.

Periods of Disease.—The disease may exterminate a stock within a few weeks or months, but its course is affected by many factors and the stock may recover by itself, or with the conscious aid of the beekeeper. The individual bee seems to succumb to the disease, which may be found during any month of the year, though it is commonest during May-July. It may reappear after disappearing for some months.

Distribution in Great Britain.—Based on my records for the last ten years and including about 140 cases, the disease occurs throughout England and Wales and in Scotland at least as far north as Aberdeen, but it is much scarcer in Scotland than in England.

COMPARISON WITH OTHER DISEASES.—The disease is also called "Black Robber Disease," from the appearance and behaviour of the bees in certain cases. It may be the disease noted by Cheshire, who ascribed the cause to an organism he called *Bacillus gaytoni*. Unfortunately he did not publish a detailed investigation of the disease or the Bacillus. Certainly, abnormal numbers of bacteria are often present in the alimentary canals of diseased bees and they deserve study. Bee Paralysis resembles the diseases known as May-Sickness, Paratyphoid, Septicaemia, Schwindsucht, Schwarzsucht, Bee Paralysis in America and intoxication and some other diseases associated with fungus or yeasts in the alimentary canal. It may be the Schwindsucht or Schwarzsucht of writers in German, and it may be the Bee Paralysis of America, though Prof. Phillips, on seeing a stock affected with the disease, said that it was not American Bee Paralysis. The symptoms suggest that the disease is located in the alimentary canal and that later paralysis of the nervous system sets in.

INCLUSION BODIES.—The diagnostic characters of Bee Paralysis are not very satisfactory since they are shared by many other diseases. What one wants is a character peculiar to the disease—as for instance the presence of *Nosema* in the alimentary canal indicating *Nosema* infestation. As far as I am aware, no microscopic character diagnostic of the disease has hitherto been found. I now think that I have found such a character. It exists in the form of minute spherical or ellipsoidal bodies which I call "inclusion bodies," inside the cells of the anterior end of the small intestine.* The bodies measure 1-5 microns in diameter, the largest bodies occurring in the cells of the small intestine just behind the openings of the Malpighian tubes and they become smaller the further back they lie till they disappear at about the end of the first quarter of the small intestine. Large numbers are grouped together in each cell. They lie most abundantly between the nucleus and the inner wall of the cell. I have found these bodies in all bees that I considered afflicted with Bee Paralysis and not in bees suffering from other diseases, nor in healthy bees of different ages or at different stages of activity. They are not described by writers on the histlolgy and cytology of the alimentary canal of healthy bees.

What are these bodies ? I suggest that they may be "inclusion bodies" like those found in animals and plants suffering from certain virus diseases. If the suggestion is correct, Bee Paralysis is a virus disease, which may be diagnosed by these bodies in the cells of the fore end of the small intestine. Since the bodies do not occur in healthy bees they are not likely to be metabolic products of a normal bee. They do not appear in the cavity of the small intestine or in the rectum amongst the faeces or in the blood. On the whole, I think that the evidence is against their being bacteria, fungi or yeasts.

*Figs 1 and 2.

TECHNIQUE.—I have not yet succeeded in seeing these bodies in fresh tissue or in tissue which is preserved but not sectioned and stained, but this is due chiefly to the difficulty of freeing the cells from the thick layer of muscles and the cuticle between which they lie. The method I adopt is to fix the alimentary canal from the living bees in Bouin's fixative, then pass it through the usual reagents to embedding in paraffin wax. The sections are stained with safranin and methyl violet, resulting in the inclusion bodies being stained bright red by the safranin and clearly differentiated from the surrounding tissue which is stained various combinations of violet and red. Other fixatives and stains will demonstrate the bodies, but the staining is not so differential.

TREATMENT.—The most satisfactory treatment for Bee Paralysis is feeding with syrup (1-2 lbs. per B. S. frame of bees), unless a honey-flow happens to coincide with the outbreak of disease and the stock is strong enough to take advantage of the flow. A change of queen is desirable since there is some evidence that a diseased queen is able to transmit the disease to workers. The disease does not spread easily in an apiary. There seems no need to burn the equipment of a stock which succumbed from this disease alone, yet it seems wise to keep the equipment of an affected stock confined to the single hive.

In conclusion, Bee Paralysis is a field of research where many workers would find problems of scientific interest and practical importance.

BROOD AND ADULT BEE DISEASES IN SWITZERLAND

By Otto Morgenthaler, Dr.(phil.)
(Eidg. milchwirtschaftliche und bakteriologische Anstalt, Liebefeld bei Bern. Director: Professor Dr. R. Burri)

First of all I should like to thank Sir John Russell and the Bee Research Advisory Committee, who invited me to to-day's meeting, for the great honour of being able to speak to you. I know that this honour does not apply to me personally but to my country, and especially to my friend, Dr. Leuenberger, who died in March of this year, and who 28 years ago gave us a foul brood law and, for German-speaking Switzerland, a foul brood insurance, which up to the present has fully stood the test, technically and administratively, and has given beekeepers complete satisfaction. Since the English Beekeepers are also working toward a foul brood law and a foul brood insurance, I shall speak to-day principally about our experience with foul brood and only at the close shall I briefly touch upon our anxieties and our successes with diseases of adult bees.

Time does not permit me to go into detail, and this is not necessary, as Mr. Illingworth, two years ago, has given you an excellent account of bee disease legislation in Switzerland. So I shall emphasize only three points which to me seem important for the success of the Swiss method. I shall be very glad to give information about special points in the discussion.

The first important step was that we were able to convince our government of the economic importance of beekeeping. By so doing, bees were accorded the same legal protection as other domestic animals. Bee diseases were included in the federal animal disease law and now there are government funds for their control as for combating other animal diseases. The federal veterinary office gives yearly about 10,000 fr. for the control of bee diseases; that is to say, mostly for salaries to the bee inspectors for their work among sick colonies. The cantonal governments together give an equal amount for this purpose. The beekeepers do not give any contribution either to the federal government or to the canton. We find that this sacrifice on the part of the government is not too high if we consider the total expenditure for the control of all animal

diseases. The federal government contributes about 1,000,000 fr. annually for the control of all animal diseases. The contribution for bee diseases (10,000 fr.) is therefore only about 1 per cent.

The second important point is that the beekeepers, in spite of this government aid, have retained full autonomy in the control of bee diseases. Apiculture has its own peculiarities and needs which must be followed if one wants success. It is of little use to be a good veterinarian. To know all the diseases of the other domestic animals does not make one a good bee doctor. The first qualification for our bee inspectors is not that they be good veterinarians or good microscopists, but they they should be good beekeepers. They are trained in special courses for their position as bee inspectors. We can say that in Switzerland the matter is regulated thus : the government gives the money and the beekeepers do the rest. There is a mutual confidence whereby both parties are satisfied.

The government should not be drawn in more than absolutely necessary. For this reason our foul brood insurance is private, that is to say, it is the affair of the beekeepers' society. This requires first of all that beekeepers have a strong central organisation. This condition is fulfilled in Switzerland. Thus it was possible that, with the very small insurance premium of 5 centimes per colony, 100 per cent. of the value of the diseased hive could be paid out as an indemnity. Besides a reserve fund could be created which at present amounts to about 50,000 fr. This sum even enables us to meet unexpected situations.

Each local beekeeper's association has also a special fund for the control of bee diseases ; that is to say, chiefly for the sanitary inspection within its territory.

The third important point is absolute certainty of diagnosis. The whole control of a disease is impossible as long as there is confusion in the diagnosis. Our inspectors are not scientists, they might make a mistake in this matter. Anyone who has much to do with bee diseases knows how closely certain diseases and anomalies resemble each other externally. For this reason it is required that all diagnosis be made in the laboratory. The bee division of the Liebefeld Experimental Station performs this task as well as research work on still unknown bee diseases. It was founded by Professor Burri who, in 1904, was the first to give a clear bacteriological differentiation between the two kinds of foul brood. The bee division at Liebefeld (including a department for pollen analysis of honey) gives work at present to six people Its annual budget is about 50,000 fr. The division is not under the authority of the veterinary office but under that of the agricultural department. Still we do not find these 50,000 fr. disproportionate, since the expenditure of the agricultural department for agricultural experimental stations is about 1½ million francs annually. The expenditure for bees amounts to only the thirtieth part of this total.

With the aid of two Figures I should like to make you better acquainted with our experience with foul brood. The first shows

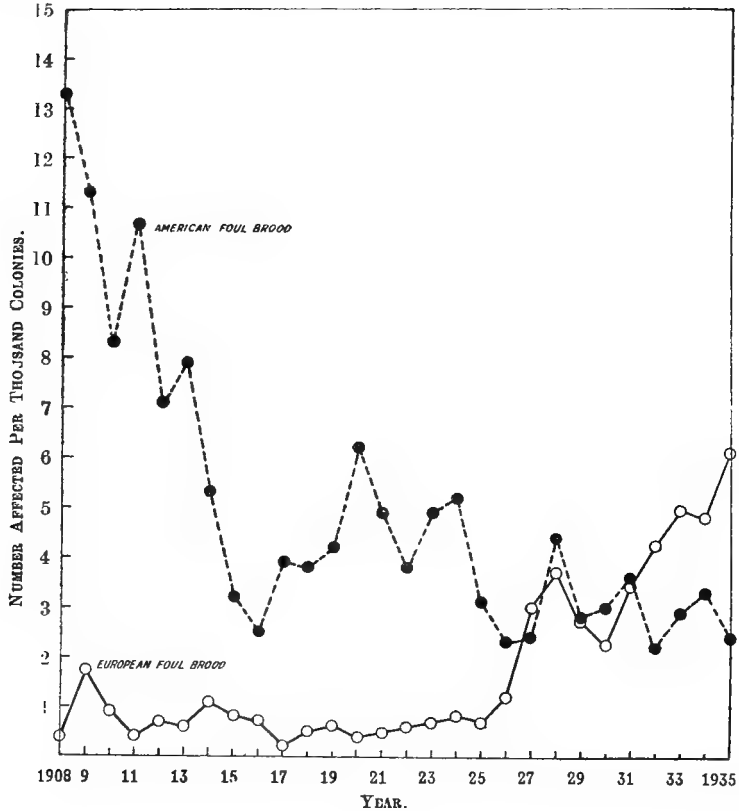

FIGURE 1.

the total number of cases of American and European foul brood from 1908 to 1935, taken from the reports of Dr Leuenberger. I am afraid you will be disappointed on examining this table to see that the success of which I have spoken has reference only to one form of foul brood, namely, American foul brood. We see a pronounced reduction of this disease from about 13 per thousand colonies in the year 1908 to about 3 per thousand in the year 1935. This is the splendid result of Leuenberger's organisation. The control method consists in burning the combs of a sick colony and shaking off the bees as an artificial swarm. The hive is disinfected with a flame and, what is most important, the apiaries of the entire neighbourhood are inspected repeatedly. In this way it has been possible to free large areas from American foul brood. If we have not yet succeeded in entirely banishing this disease from our country it is not the method which is to blame. I emphasize this because recently in America and in Germany certain workers

have criticized the artificial swarm method and have wanted to burn the bees also. In answer to them we can point out that with us the artificial swarm method has proved a complete success in 28 years of practice. It is not this method which is to blame for the fact that we still have 3 cases of American foul brood per thousand colonies, but human imperfection, which again and again prevents the clear regulations for complete inspection, and eradication being correctly carried out.*

The curve which shows the occurrence of European foul brood since 1908 runs in quite another way. This disease was formerly scarcely known in German-speaking Switzerland. But during the last few years it has begun to increase seriously, and is now already more than twice as prevalent as American foul brood. This increase of European foul brood during the period in which we have successfully treated American foul brood was very painful for us, and we had to conclude that our methods of fighting European foul brood were inadequate. What were our control methods and our ideas with respect to European foul brood?

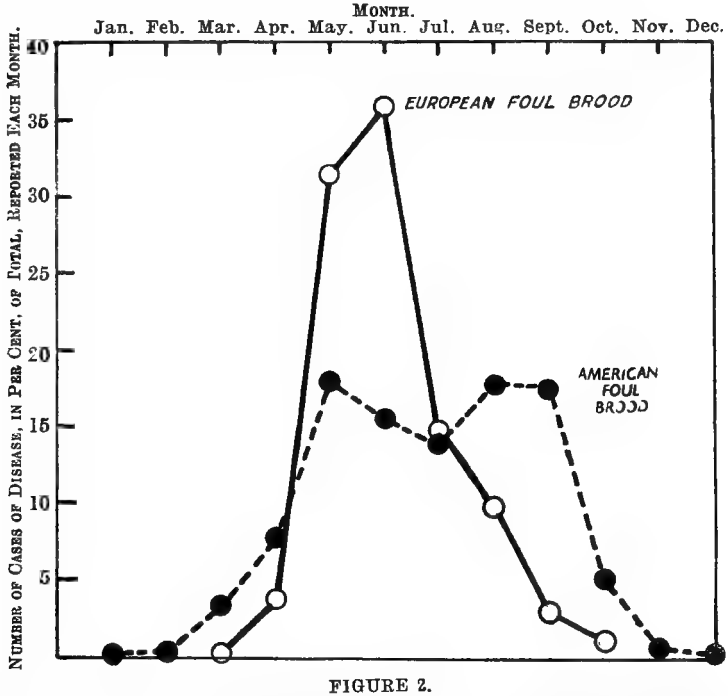

FIGURE 2.

* It is easy to prove that the shaking method, when carefully carried out, is really effective, but whether it is too complicated for official use in every country is a matter of controversy. We are of the opinion that this is really a question of the education of the beekeepers.

As we see, this disease played almost no rôle formerly and furthermore it was observed that it sometimes disappeared again spontaneously. We therefore did not bother ourselves much about it. We also became acquainted at this time with the opinion held in the United States, where on the basis of a very wide experience, European foul brood was represented as a deficiency disease, as a disease which only appeared where there were bad beekeeping methods and where the colonies, especially in spring, suffered from hunger, or for some other reason could not develop well. This hypothesis of a deficiency disease was also strongly supported by the very remarkable seasonal occurrence of European foul brood. I find this seasonal occurrence so interesting and so important for an understanding of European foul brood that I should like to discuss it briefly with the aid of Figure 2. You find here again American and European foul brood represented by two lines. The months are indicated above and directly under each month the number of cases of foul brood, in per cent. of the total for the year, observed in this month.† Let us first examine American foul brood. Naturally we have few cases in winter because few inspections are made then. But from spring until autumn one can always demonstrate the presence of this disease. We say that we can easily find American foul brood in a diseased colony at any time.

It is quite different with European foul brood. It begins slowly in spring, is often scarcely to be demonstrated in the first brood period in April, but increases rapidly, reaching a peak in May and June, then falls back again just as quickly. In autumn, when American foul brood is still very easily demonstrable, European foul brood has practically disappeared without any treatment, but will return again next May and June. We are surprised at the similarity between the seasonal course of European foul brood and that of Nosema infection. Nosema is also most pronounced in May, and apparently disappears in autumn, only to reappear the following spring.

We have no certain explanation for this remarkable seasonal occurrence of European foul brood, but it is understandable that one is inclined to find an explanation in the deficiency theory. May and June are also highly important months for normal bees. The time of greatest growth, of greatest breeding activity, of the mighty impulse for expansion of the bee colony is found at this time. It is a critical period. In the case of other living creatures and also in the case of man, one speaks of a growth crisis. This growth crisis of the bee colony coincides, unfortunately, all to often with a weather crisis, a setback in the weather in May. Instead of spring warmth and a rich flow of honey, hunger and cold invade

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†This figure shows all the cases of foul brood received at Liebefeld during the past 30 years. (About 1,500 cases of American and 1,200 cases of European foul brood.)

the hive, and then, even without the influence of bacteria, through undernourishment and lack of warmth alone, the colony can get into a wretched condition.

This temporal coincidence of the European foul brood peak with the growth and weather crisis in colonies has led to the obvious interpretation that European foul brood is merely due to insufficient nourishment and insufficient warmth ; that is to say, a deficiency disease. We do not deny that one can often observe cases where the disease after a good honey yield or after packing and artificial feeding has disappeared again. But recent years have shown us with all clearness that the nature of the European foul brood is not sufficiently characterized by the designation " deficiency disease." We have had several excellent years since 1930 and, in spite of this, the disease has spread. We have also often found a very marked European foul brood infection in vigorous colonies that had plenty of honey and pollen. We have not noticed that the race of bee has great influence on the appearance of the disease, for it is just as common with the black bee of German-speaking Switzerland as with the Italian bee of the canton Tessin and the hybrid bee of French-speaking Switzerland. All our experts and inspectors are now agreed that, although environmental conditions play an important rôle in the occurrence of European foul brood, we must not, however, disregard the significant part played by disease bacteria. And because we now attribute a more important rôle to infection than we have hitherto, we must also pay more attention to disinfection in its control. We are convinced, that failure in the control of European foul brood comes from the fact that we have tried to fight it only by indirect methods instead of by the direct removal of the infectious substance, as in the case of American foul brood. According to our observations it is not possible for the bees, even after dequeening, to remove the infectious material from the hive sufficiently. For the bacilli of European foul brood are not only present in the sick and dead larvae, but we also find them in great numbers under the cell cappings in cells which contained living and dead nymphs. It seems that the bacilli get into the cell with the excrements of the larvae before cocoon spinning. So we also burn the combs in the case of European foul brood and the experience of recent years leads us to hope that in this way we shall succeed in curbing the increase of European foul brood.

As we attribute a more important rôle to infection the great importance of bacteriological research becomes evident. We all know how involved the bacteriology of European foul brood is and what conflicting opinions the specialists hold to-day. However, I shall not enter further into such details here and only give expression to the great pleasure which it has afforded me to take a glimpse at the excellent and very important research of my colleague, Dr. Tarr.

As we see, of the two most important brood diseases one is conquered for the Swiss beekeeper, but the other still gives us much concern and requires further careful research. Conditions are exactly the same in the case of the two most important diseases of the adult bee, which I shall discuss in a few words :—When in 1922 acarine disease was discovered in our country, we were very fortunate to possess in the foul brood organization a weapon with which we could also control other bee diseases. Our foul brood inspectors became bee disease inspectors and to the animal disease law was simply added a new paragraph, " acarine disease." This immediate defence has allowed us, up to the present, to save the greater part of Switzerland from an acarine infection. But, in spite of the good organization, our campaign would probably have been hopeless if Mr. Frow had not come to our help at the right moment with his treatment. In extensive scientific and practical experiments we have tested this remedy and found that it actually exceeded the boldest expectations and, when used correctly, killed all mites. Swiss beekeeping owes Mr. Frow great gratitude for his discovery which he, in absolute unselfishness, offered the public for use. Acarine disease will soon be no longer a matter for public concern in Switzerland, since every beekeeper is able to protect his apiary from infection by correct application of this remedy. What remains for us to do in this matter is to get rid of certain disadvantages of the Frow treatment, principally the danger of robbing.

As far as the success of control is concerned, we can therefore compare acarine disease with American foul brood. On the other hand European foul brood has points of great similarity with Nosema disease, as I have already noted in my remarks relating to seasonal occurrence. Also, as yet an effective control method has not been found for Nosema disease. As with European foul brood, one knows much too little of its nature. We are glad that we have not included Nosema in the animal disease law. Before we can introduce a Nosema law or a Nosema insurance, we must study the disease itself further. With us the Swiss Nosema commission has taken up this task. In it the federal veterinary office, the entomological institute in Zürich, the bee division at Liebefeld, and the beekeepers' societies are represented. I think that our Nosema commission corresponds exactly to your Bee Research Advisory Committee.

In the Nosema question we have sufficient material for at least a days' discussion. You all know the remarkable phenomenon in which a bee colony which is 100 per cent. infected with Nosema can appear absolutely healthy externally. I shall not try your patience by naming all Nosema theories and should like here only to offer for discussion the theory which we hold at the present time. We believe that Nosema infection alone, in a well-managed colony and under good conditions, cannot cause great damage. The dreaded devastation which has hitherto been ascribed to

Nosema and which consists in a conspicuous weakening of the colony in spring, "spring dwindling," we believe to be the result of a mixed infection of Nosema (in the middle intestine) and of amoeba (in the malpighian tubes). It interested us very much to learn that Amoeba has also been found in Great Britain by Dr. Morison.

Before I close my survey of Swiss experience with reference to bee diseases, I should like to mention a special field of research with which we have been busy for some years. It concerns the the diseases of the queen. My colleague in Liebefeld, Mr. Fyg, who devotes himself especially to this problem, tells me that up to now, after an examination of about 500 queens, he has found 42 different abnormalities and diseases of the bee queen. We believe that queen diseases play a far greater rôle in practice than has hitherto been recognised.

According to our views bee diseases are not only a question of bad beekeeping methods and of bad environmental conditions. We have seen many cases in which bee colonies, with the best of care and under the best environmental conditions, suffered heavily from a brood disease or a disease of adult bees. Moreover, in the history of apiculture we have seen that the alarm over bee diseases did not originate with careless people and with bunglers, but on the contrary with the pioneers of practical beekeeping. They had realized that all their efforts to improve beekeeping were futile, as long as the diseases were not scientifically studied and as long as they were not conquered by special control methods.

Ladies and Gentlemen, Switzerland is a small country and our experience with bee diseases is based, therefore, only on relatively little material. But I should like to point out that with regard to climate, flora, beekeeping methods and races of bees we have very varied conditions which perhaps invests our conclusions with somewhat greater value. We are always very eager to learn the experience of other countries. Therefore I should like to take the liberty here, as I have done at former international congresses of apiculture, of pointing out the desirability of statistics on the occurrence of bee diseases in all lands. We were highly interested in the work which Mr. Morland has undertaken in determining the geographical distribution of foul brood in England.* I can show you here with the aid of maps the distribution of the four most important diseases in Switzerland.† We should be much better informed on many questions of bee pathology if we possessed such maps of all countries.

If, in conclusion, I should give the reason why Switzerland shows some successes in controlling bee diseases I believe I can say that

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* "Brood Diseases of Bees." Rothamsted Experimental Station Conference Report 18, 1934. (Out of print in separate copies).

† Maps showing the distribution of American foul brood, European foul brood, Acarine disease and Amoeba disease in Switzerland were exhibited.

the reason lies in the good relationship between practice and science. The beekeepers know that they cannot get further in the control of bee diseases without scientific help, and the scientists know that they cannot understand bee diseases if they do not also to some extent understand the life and treatment of healthy bees, viz. apiary practice. I should like to close my address by saying how gratified I am by the fact that this good relationship between science and practice, and with it the most important prerequisite for a successful control of bee diseases, also exists in the British Isles.

CONTROL OF AMERICAN FOUL BROOD IN THE UNITED STATES

By JAS. I. HAMBLETON, B.S.

(Principal Apiculturist, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture).

DURING the time that Dr. G. F. White, of the Bureau of Entomology, U.S. Department of Agriculture, was investigating the brood diseases of bees, about 1906, a survey was being conducted to determine the distribution of American foul brood in the beekeeping localities of the United States. It soon became evident that the disease was by no means localized but widespread throughout the country. Before this time some of the States had begun to realize the serious inroads that American foul brood was making.

As early as 1877 San Bernardino County, California, passed a bee-disease law, and 6 years later a State law was enacted in California. It is interesting to note that the State law specified that infected colonies be burned. The State of Michigan passed a bee-disease law of State-wide application in 1881 which also prescribed the burning of infected colonies. In 1897 Wisconsin appointed the first State apiary inspector, N. E. France, with headquarters in the State Capitol. Other States followed suit by enacting special laws and appointing apiary inspectors, and to-day practically every State has a bee-disease law or some administrative organization under which bee inspection is carried on.

These early inspectors did their best. Upon receipt of a call from a beekeeper, a gross diagnosis would be made in the apiary and instructions left as to how to treat. Burning had already been frowned upon as unnecessary and wasteful. In some cases, where the inspector felt so inclined, he would lend a hand in the unpleasant task of treating colonies by the so-called shaking method. The next day he would be responding to a call in some other part of the State. Although the inspector would cover a great many miles of territory during a season, the beekeepers who benefited by his visits were insignificantly few. His activities nevertheless filled an educational need, and soon every beekeeper was on the look-out for disease.

In the light of present knowledge it can be seen that from the very beginning the shaking treatment was unsatisfactory. Reduced

to its greatest simplicity, the method consisted merely in shaking the bees from an infected colony into a clean hive that contained frames having one-inch foundation starters. If there was little brood in the infected colony, combs and small amounts of honey were melted. Hives that contained much honey were usually salvaged. If many colonies in an apiary were infected, the brood combs were stacked on a queen-right but diseased colony and the brood was allowed to emerge, when this colony in turn would be treated, thus saving bees, wax, and honey. So-called improvements in this method came into use. Beekeepers established what were known as hospital yards, which were usually removed some distance from the regular apiaries. All diseased brood was allowed to emerge in the hospital yard. Other variations in the shaking treatment and in the methods of disposing of diseased material were advocated. One unique method was to place an infected colony in a tank, replace the cover with a clean hive, and drive the bees into it by slowly filling the tank with water. The theory was that the bees could be transferred with practically no disturbance and thus carry little contaminated honey into the clean hive. Various other contraptions and methods were used for transferring the bees from contaminated to clean hives. The shaking treatment, however, in some form or other continued to be used and the practice finally became well standardized.

In 1916 the State of Wisconsin again took the lead in the control of American foul brood. In full recognition of the contagious nature of the disease, and also of the inefficiency of a system whereby one inspector examined only the apiaries from which requests had been received, the area clean-up method was invoked, in which the State inspector with several assistants examined all the bees in a given locality, whether or not they were suspected of being diseased. Under this plan the same area would be re-inspected the second year, and while, owing to limited funds, some portions had to be neglected, the inspectors made a conscientious effort to do a thorough job wherever they went. The State of Texas also early adopted this system and organized a highly efficient State inspection service.

Most of the leading beekeeping States now follow some modification of the area clean-up plan, usually organized on a county basis. In several States the counties make definite appropriations for American foul brood control, and such funds are matched by grants from the State. The responsibility for control is thus placed to a certain extent upon the beekeepers of a county. A number of the large States expend as much as $25,000 to $30,000 annually for the control of American foul brood.

The next phase in the control of this disease consisted in the strengthening of statutes relating to inspection work, and several States wrote into their laws the prohibition of entry of bees on combs. In other words, only package bees could enter a State. Other States permitted the entry of bees on combs provided they were accom-

panied by a certificate issued by a responsible State official stating that the apiary from which they came was free of disease. Some States regulate the movement of bees within their own boundaries, making it necessary, for example, for a beekeeper wishing to move colonies to an out-apiary to receive a permit from the State inspector. In some places the sale of used beekeeping equipment is only allowed upon its being accompanied by a certificate showing freedom from disease. With respect to these provisions there is no uniformity in the laws of the various States. Some are very strict and some lenient. Some States have strict apiary laws, but appropriate no funds for enforcement; consequently, the beekeepers receive little or no State aid. They fight their own battles as best they can and blame their neighbours for maintaining nuisances in the way of sources of infection.

In the application of area clean-up methods where the shaking treatment or some variation of it was the sole method of control or eradication, State apiary officials found they were making little headway and, in spite of vigorous efforts, the disease would reappear even in areas in which control measures had been applied for several consecutive years. It was evident that better methods of control were necessary, and thus was reborn the application of fire to diseased colonies.

The tenaciousness with which beekeepers treasure old combs is well known. Even combs composed mostly of drone cells are discarded with great reluctance, and to melt well-drawn-out combs of worker cells, only a few of which are diseased, or which have been used only in the supers of a diseased colony, requires almost superhuman will power. In 1922 Dr. J. C. Hutzelman, of Glendale, Ohio, came to the rescue of such beekeepers. Dr. Hutzelman, who was a practising physician, had had some training in bacteriology; consequently, when he advocated the use of a solution containing 20 per cent. formalin and 80 per cent. alcohol for disinfecting foul brood combs, the beekeeping fraternity immediately took notice. This happened during the days of national prohibition, when the average citizen could not buy grain alcohol to be used in making his own solution. Also the Hutzelman solution was patented. Stories of the success of formalin-alcohol for saving combs appeared in the bee press, and soon many experimenters were trying other concoctions, the principal one being 1 part formalin to 4 parts water. The University of California and the Department of Agriculture tested both the formalin-alcohol and the formalin-water solutions and found that it was possible to sterilize combs with either, provided the utmost care was taken in the preparation of the combs for treatment and in the subsequent handling of the combs.

The use of disinfectant solutions caught the fancy of beekeepers, and many practical experimenters also entered the field of research. Some concluded that, if formalin-water and formalin-alcohol

mixtures were satisfactory, pure formaldehyde in vapour form would be better. Large metal-lined fumigating rooms holding thousands of combs were constructed. To do a thorough job some beekeepers shook not only diseased but healthy colonies and subjected all combs to the gas ; they thus started again the following year with all their equipment thoroughly sterilized. Package bees from the South were placed on this clean equipment. However, these beekeepers failed to take into account the poisonous nature of formaldehyde, and the fact that, when combs containing films of honey are exposed to formaldehyde gas the honey absorbs it in lethal amounts. Even though well-aired combs may give no odour of formaldehyde, the honey continues to hold the bitter chemical. Naturally, bees placed on such combs died, and many losses were incurred through the use of formaldehyde vapour. In using the water-formalin solution this trouble was not encountered, since the water in the solution usually dissolved any remaining honey.

The next solution to be advocated was chlorine. This chemical was allowed to bubble through tanks of water in which combs were immersed. Although chlorine can be had only in metal cylinders and is an extremely dangerous gas to handle, nevertheless beekeepers tried it and successes were reported with this, as with all other solutions and methods that have been mentioned.

The use of disinfectants, however, met with indifferent success in the hands of beekeepers, for it failed to check the disease to the satisfaction of State officials from the standpoint of using public funds in the most efficient manner. It merely gave them an added reason for resorting to the burning of infected colonies.

When burning was first advocated, the bee journals were full of articles pro and con, mostly con. It was pictured as a wanton, unethical method of dealing with the disease ; the shaking treatment had been used for years and was as good as when first advocated. Nevertheless, some of the braver State apiary officials felt that nothing less than burning should be employed. To enter a person's premises and destroy his property by fire, however, was another matter, and in many cases, instead of the kindly welcome of a beekeeper, the inspectors found themselves face to face with an armed antagonist.

The State of California finally won support of enough beekeepers to amend its statute in such a manner as to specify that all diseased colonies should be burned. The matter was carried to the courts, and after a bitter struggle the practice was upheld as being constitutional.

The burning treatment usually consists in killing the bees with cyanide and burning all bees, combs, frames and honey in a pit at least 18 inches deep. This pit is afterwards filled in, and the hive, including the bottom board, brood chambers, supers and inner and outer covers, is sterilized by thoroughly scraping and washing with lye or strong soap or by scorching with a gasoline torch.

Most progressive beekeepers in the United States are now in favour of burning. Many would not resort to the old shaking treatment or the use of disinfectants. As a matter of fact, a beekeeper can use any method he sees fit. It is only the colonies found infected at the time of the inspector's visit that must be burned. It is not uncommon to find apiaries that have had an intermittent history of disease for as long as 50 years, their first complete freedom not coming until 3 or 4 years after application of the safe and economical method of burning and burying.

At this point it is well to go back and draw a parallel with respect to developments that have taken place in scientific research on American foul brood. The participation of the Federal Government with respect to this disease has been confined to research. After Dr. White had worked out the life history of the causative organism and had given it the name *Bacillus larvae*, progress was slow and little was added to our knowledge of the disease until Dr. A. P. Sturtevant reported the results of his work dealing with the development of the disease in relation to the metabolism of *B. larvae*. This research explained why the gross symptoms of the disease were so uniform; the organism simply would not grow in a medium of high sugar concentration. This in turn explained why larvae of feeding age seldom fell victims to the disease; it was not until after the feeding period and the beginning of quiescence that the sugar content of the gut fell low enough to enable the spores of *B. larvae* to germinate. The organism then did its work quickly. As a result, in American foul brood the diagnostic features are extremely regular, in great contrast to the symptoms encountered in European foul brood.

Just previous to the publication of these results, Dr. Hutzelman announced to the beekeeping world the success of the formalin-alcohol method for disinfecting combs. Out of the tests that the Department of Agriculture and some of the State universities made of the Hutzelman and various other solutions came the recommendation for the use of formalin-water solution for disinfecting combs, and this mixture was found to be fully as efficacious as formalin-alcohol, if not more so. A great many beekeepers used both solutions, and many samples of treated combs were submitted to the Department of Agriculture for sterility tests. Judging from these, the results were fairly satisfactory; yet in apiaries in which disease seemed to be carefully handled, American foul brood continued to recur. In fact, the general failure of these disinfectants in the hands of beekeepers helped to encourage the adoption of the more drastic method, namely, that of burning.

One of the State experiment stations, after some preliminary research, recommended the use of chlorine. Again many treated combs were sent to the Department of Agriculture for sterility tests, and at first practically all samples appeared to be sterile. The odour of chlorine, however, clung tenaciously to the treated combs. It

was then suggested that enough chlorine might be retained in the treated scales of American foul brood to inhibit growth while in culture. When the treated scales were washed in distilled water the spores germinated surprisingly well, whereas there was no growth in cultures from unwashed scales. Consequently all samples that were received for testing were washed, and it was found that the same thing occurred with scales that had been treated with formaldehyde, though to a less extent ; that is, formaldehyde, even in small doses, appeared to delay the germination period of the spores. Cases varied considerably, but, in some, germination would not take place for 30 days. Therefore, before a definite diagnosis could be made on any treated sample, it was deemed advisable to keep cultures in incubation for 30 days before pronouncing the sample sterile.

The placing, by some of the States, of embargoes on the shipment of bees on combs suggested to other States the imposition of embargoes on honey, specifying that only honey accompanied by a certificate showing that it had been produced in disease-free apiaries would be allowed to enter. Whether there was a modicum of retaliation in advocating such action or whether it was just misguided faith, in view of the amount of American foul brood in the United States, the time was not ripe to insist upon the certification of honey. The Department of Agriculture looked upon honey certification as inimical to the welfare of the industry, maintaining that the marketing problems did not warrant this additional burden unless the certification of honey did serve its avowed purpose, namely, constitute a worthwhile disease control measure. Dr. A. P. Sturtevant was therefore assigned the task of ascertaining the part played by commercial shipments of honey in the dissemination of American foul brood. At the outset it was conceivable that the spore content of honey from infected colonies would vary considerably. The spore content of honey from the brood chamber would most likely be different from that produced in the supers. Moreover, the honey from a lightly infected colony would have a smaller spore content than that from a heavily infected one. These conceptions in turn presented for answer the question : What constitutes the minimum infectious dose or inoculum for American foul brood ?

Dr. Sturtevant, working at Laramie, Wyoming, where colonies can be completely isolated and where there are no so-called wild bees, fed healthy colonies different numbers of spores suspended in sugar syrup. Briefly, he found that the dividing line was in the neighbourhood of 50 million spores fed in a liter of sugar syrup, for when a smaller number was used most of the experimental colonies did not develop the disease and above that point most colonies did develop typical American foul brood. Various subsequent tests indicated that the minimum infectious dose must be close to this point.

The next step was to ascertain the spore content of commercial honeys. Samples of bottled honey were procured from shops and groceries in the principal cities of the United States. Filtration and microscopical examination of 212 samples showed the presence of spores in 8 per cent., or 17 samples. These 17 samples were fed to healthy colonies and positive American foul brood developed in only one. Other series of samples have been examined since, with similar results. It was found that the rôle of commercial honey in the spread of American foul brood had been grossly exaggerated.

During the course of this work Dr. Sturtevant also found that the number of spores in culture affected the time of germination. With a large inoculum the germination period was normal, but with progressively smaller doses it would be delayed as much as 30 days.

Laws requiring the certification of honey still remain on the statute books of certain States, but they are for the most part not enforced. The day may come when the United States will be ready to require honey certification as a clean-up measure, but we are yet too far from the goal to use such a measure.

The possibility that there may be strains of honey bees that show some degree of resistance or immunity to American foul brood has attracted many beekeepers. No doubt this is born of a great desire to have a better weapon than chlorine, formaldehyde, or fire. American foul brood is one of the most costly items with which beekeepers in the United States have to contend. A beekeeper operating an apiary in a locality where American foul brood exists, scarcely dares to perform any colony manipulation without keeping in mind the possiblity of finding the disease. Authentic cases have been found in which colonies have cleaned up the disease, and in the course of experimental work it is not uncommon to find colonies that can be inoculated only with difficulty. There is also the example of Italian bees being superior to black bees in ridding themselves of European foul brood. Consequently, there is some basis for thinking, or hoping, that a disease-resistant strain may be found and perpetuated. The United States Department of Agriculture, in co-operation with several of the State agricultural experiment stations, is outlining a series of experiments to delve into the matter, and a number of outstanding specialists have been enlisted to help with the work. Not the least of these will be geneticists, since breeding will be one of the important features of the investigation. Whether or not a resistant strain is found, or a strain possessing physiological immunity or such characteristics as will enable it to maintain itself free or partially free of the disease, makes little difference. Even if such ends are not attained, much good is bound to come from these experiments, as the complete story of American foul brood is yet to be told.

DISCUSSION

MR. L. ILLINGWORTH (Cambridge) gave the following brief account of the proposed Foul Brood Insurance scheme.

On July 8th, 1936, a Society named BEE DISEASES INSURANCE, LTD., was registered Under the Industrial and Provident Societies Act, with Dr. A. L. Gregg as chairman, Dr. F. Thompson and Messrs. B. C. Berkeley, C. W. Bowell, W. E. Hamlin, J. E. Swaffield, and C. Wilkinson as Directors, and myself as Secretary.

A circular letter describing the aims and objects of the Society has been sent to every Beekeepers' Association in Great Britain.

It is too early to predict what response the Associations will make to this invitation to join the scheme, as most of them will not hold meetings to consider the question before the autumn or winter. The Barnet B.K.A. has definitely decided to come in, Cambridge and District will almost certainly do so, but Cornwall, Norfolk, Warwickshire, and Worcestershire cannot see their way to join, or propose to take no action at present.

The scheme is quite frankly an imitation of the Foul Brood Insurance system initiated by the late Dr. Leuenberger, which has proved such a success in German Switzerland, having now been in operation for nearly 30 years. The small committee appointed to draw up the scheme has carefully considered what modifications are necessary for Great Britain, and has prepared bye-laws and regulations covering every department of the Society's activities, as well as providing for the representation of the subscribing Associations, so that each may have a voice in the working of the scheme. These will be laid before a meeting of delegates from the Associations for approval or modification, to be held during the coming winter as soon as it is known which Associations will join.

This is all the information about the progress of BEE DISEASES INSURANCE, LTD., that can be given at present.

In the moment or two that I have left it may be well to outline the main features of the scheme and to answer one or two objections. For fuller information I must refer you to the registered rules of the Society and to the circular letter already mentioned.

BEE DISEASES INSURANCE, LTD., is not founded for private profit. After providing a reserve fund sufficient for all eventualities all further profit, as well as interest on that fund, will be used to promote bee disease research, or returned to the Beekeepers'

Associations in the form of grants, as the Directors, with the approval of the representatives of the Associations, may decide.

Subscribing Associations will be required to take up five shares of £1 each for every 100 members only 4/- per share being payable the first year. It is hoped that it will be unnecessary to call up any more money on the shares, so there will be no annual payment. Thus Associations will only be asked to pay £1 for every 100 members.

In addition there will be an Annual Premium of 1d. per colony of bees (spring count), minimum 6d.

Some Associations seem to think this is more than they can afford. It is difficult to see how the annual premium can be less than 1d. The chairman of one B.K.A. has offered to pay the 6d. minimum for all the members the first year. Where an Association has a number of very poor members cannot this excellent example be followed ?

As to the initial payment on the shares, even this has been criticized in some quarters. £1 per 100 members the first year only, does not seem very much to ask for, as the Society must have some working capital to start with. If beekeepers really want foul brood control will they not be willing to make some slight sacrifice to obtain it ? Surely, if an Association has no funds to pay for the shares, there is one beekeeper in a hundred who would give £1, or eight who would be prepared to give 2/6 to get control of foul brood.

Let no Association say that there is little disease in its territory and therefore it has no need of foul brood insurance. This is a national scheme. Only by receiving a large number of premiums from areas where there is little disease can we hope to weather the first few difficult years, and set the scheme on a firm financial basis. You can help to make it a success now. If it fails it will be many years before another plan is tried. Your county may not always be as free from disease as it is to-day. You may one day be glad of the help BEE DISEASES INSURANCE, LTD., can give you.

DR. A. L. GREGG stated that the research on brood diseases which was being conducted at Rothamsted had reached a critical stage, and that there was every hope that, if it could be continued, some tangible result might be reached. The beekeepers would, he said, be very foolish were they to let slip the opportunity of continuing this research. He proposed the following resolution: " That this Conference considers that the Beekeepers' Associations should continue to provide the financial support required to ensure the continuance of the foul brood research at Rothamsted."

The motion was carried.

MR. LINDLEY (Gloucester) said that he felt that the work on brood diseases was extremely useful and on behalf of the newly formed Honey Producers' Association offered the sum of five pounds a year for three years in support of the work. (This contribution has since been increased.)

Mr. W. Herrod-Hempsall (Ministry of Agriculture), said that he had listened with great interest to the papers, especially to the excellent one by Jas. I. Hambleton, chief of the United States Bee Culture Laboratory. He believed that we might eventually be compelled to adopt the plan now being followed in that country. When visiting the United States he had an opportunity to see the terrible ravages caused by foul brood. This occasion gave an opportunity to address a solemn word of warning to beekeepers in the British Isles. During the last three years foul brood had spread to an alarming extent, indeed, in some districts, it is endemic and a serious menace to the industry. He stated that he had inspected apiaries in which all the colonies were affected with brood disease. In England, Scotland and Wales to-day there are thousands of bee colonies affected with brood disease.

He went on to say that in the annual reports of beekeepers' associations it is not unusual for the statement to be made that : " no brood disease is present in the county." Such statements, made in good faith, are misleading because one of the troubles we are faced with is that many beekeepers are obsessed with the idea that the presence of disease in their apiaries is a disgrace and a reflection on their management. Consequently, when an outbreak occurs, they conceal the fact from the association officials. One must also take into account the large proportion of beekeepers who are not members of an association, and from whom no information is available as to the condition of their colonies. He, personally, begged beekeepers, when in doubt about the health of their colonies, not to be reticent, but to obtain the advice of a competent person immediately. Rothamsted provided such advice.

The following note from Colonel Howorth, C.M.G., of Devon, was read as he was unable to attend : " My original communication on the use of the Frow treatment for acarine disease was published in the *British Bee Journal*, June 7th and 28th, 1928 ; since then I have distributed some 30,000 leaflets describing my method. I know of only one instance in which complete extermination of the mites was not effected (I am satisfied with nothing less than 100 per cent. mortality among adult mites and their eggs, although this entails some risk to the bees.) Mr. Frow's method differs from mine in that he applies the treatment from above the bees, whilst I, holding that the temperature is always lower and more equable on the floor board, apply the remedy there. He also uses a smaller dose and permits treatment during the brood-rearing season, whilst I use a larger dose and place a section rack or shallow below the brood-chamber, and have found that the brood of the bee suffers during the treatment."

The following notes with reference to " Bee Paralysis " have been added since the Conference.

Brother Adam, O.S.B. (St. Mary's Abbey, Buckfast.)

Bee Paralysis can cause serious loss to the beekeeper. In cases where this disease has been allowed to develop unchecked I have seen colonies perish outright, the dead bees accumulating within the hive between the combs to a depth of six inches.

The most conspicuous symptoms of paralysis are that the affected bees become denuded of hair, possess a distended abdomen, present a glossy black appearance and are bereft of all power of locomotion. They seem listless and can but feebly flutter their wings. When in this condition the diseased bees are evicted, to perish outside their home, by the still healthy members of the colony. The decomposing bodies, as they accumulate in front of the hive, give off a most obnoxious odour.

Paralysis attacks bees principally during the early part of the autumn. However, minor infestations may occur at any season of the year.

Disinfectants, even if used in strong solutions, impart not the slightest relief to colonies affected by this disease, whereas dusting the bees with flowers of sulphur has never yet to my knowledge failed to effect a complete cure. A handful of sulphur, sprinkled over the bees and the tops of the brood-combs, and the same dose repeated a fortnight later, will cure the worst case of this disease.

Apparently certain strains of bees are practically immune to this form of paralysis. On the other hand some strains seem most susceptible to it.

Very little is known of the causative agent of bee paralysis. According to the findings of a Swedish investigator, G. Turesson, paralysis is caused by poisons produced by certain moulds or fungi. By feeding a colony a solution of honey containing such toxins he found that the bees developed paralysis within three to four days and finally succumbed to its effects.

The disease, supposedly caused by *Bacillus gaytoni*, to which Cheshire referred, whilst similar in some respects to paralysis differs substantially from this malady. In both diseases the affected bees present a shiny black appearance. In paralysis, however, the bees have distended abdomens and are deprived of practically all power of movement, whereas in the other disease, according to Cheshire, the bees are " undersized " and found " running " upon the ground.

The disease mentioned by Cheshire appears to be identical to the Schwarzsucht or Waldtrachtkrankheit so common on the Continent. E. Zander distinctly states that bees afflicted with this complaint possess a contracted abdomen. On the Continent this disease occurs chiefly when bees work on the pines, buckwheat, or heather. Instances of this trouble have come under my observation on Dartmoor. The affected bees were undersized, hairless and

jet-black in appearance. After about ten to twenty days all the diseased bees present in a colony seem to die suddenly, or, perhaps, are killed off by the healthy members. They invariably perish suddenly and their dead bodies are found lying outside the hive. But bees that die of this malady do not give off any perceptible odour.

There is a form of paralysis, that is quite distinct from the one first mentioned, which at times causes serious depletion in the strength of colonies. The symptoms of this malady resemble acarine disease in that the affected bees are deprived of the use of their wings. Bees suffering from this kind of paralysis appear to be normal in every respect except that their wings are paralysed and often also dislocated. The disabled bees, mostly young ones, leave and run away from their home and eventually die of exhaustion. They do not form clusters on the ground as in the case of Acarine. The malady comes on suddenly, generally early in July, and again vanishes after a week or ten days. This form of paralysis is no doubt identical to the " Disappearing Disease " that at one time caused such serious losses to many apiarists in the U.S.A.

With the exception of the malignant paralysis all the other diseases mentioned are of transitory duration and affected colonies recover without the application of any remedial measures.

Dr. Tarr (Rothamsted). It is by no means certain that Bee Paralysis is a single disease, and, as can be seen from Brother Adam's remarks, there is some confusion as to exactly what is implied by this term. Whether such diseases as Waldtrachtkrankheit, Bee Paralysis and Black Robber Disease (Black shiny bees) are distinct complaints cannot be stated with certainty until their causes have been definitely determined. At present there is no accurate diagnostic feature by which these diseases can be distinguished, though the work accomplished by Dr. Morison may provide a useful clue. It may transpire that there are several virus diseases of the adult bee which differ slightly from one another, or that there is a single disease modified by the presence of various bacteria. Until practical experiments have been made, any statements regarding the etiology of these diseases can only be speculative. In this connection it is of interest that Dr. Morison states that Dr. Phillips, of Ithaca, believes that American Bee Paralysis is not identical with the disease occurring in this country. Many beekeepers state that Black Robber Disease or Bee Paralysis can be cured by requeening the affected colony. However, the success of this method cannot be considered as proven, and the reason for it is not known because there is, as yet, no definite indication that the queen carries the disease.

In 1933, Burnside, working in the United States, published a paper entitled " Preliminary Observations on Paralysis of Honeybees." He concluded that the disease is mildly infectious, and often

disappears of its own accord. Extracts prepared from bees affected with Paralysis in certain cases caused infection in healthy bees, but porcelain filtrates were not, in his experiments, able to cause infection. Burnside (1928) also described an apparently new disease of the adult bee: a septicaemia caused by an organism which invaded the blood of the bee, namely, *Bacillus apisepticus*. These findings show that much remains to be discovered with respect to some of the diseases of adult bees, and that a large number of such diseases may exist.

STATE OF NEW YORK
DEPARTMENT OF AGRICULTURE AND MARKETS
BERNE A. PYRKE, Commissioner

Circular 429

DISEASES OF THE HONEY BEE AND THEIR CONTROL

A Profitable Apiary

1931

B. D. VAN BUREN
DIRECTOR, BUREAU OF PLANT INDUSTRY

DISEASES OF THE HONEY BEE AND THEIR CONTROL

BY

A. C. GOULD, *State Apiary Inspector*

Introduction

The diseases of bees are widely spread throughout practically the entire state; few sections have escaped the ravages of one or more of these. Diseased colonies and equipment have been shipped over the state. Also, diseased colonies become weakened and are robbed out, and disease is thus spread from community to community. As a result, foulbrood has become a menace not only to the beekeeper but also to the fruit grower, who suffers from a lack of bees for pollinization purposes.

There is keen competition in the field of beekeeping, as in other branches of agriculture. The margin of profit is small, and the success of commercial beekeeping in many localities, and the future prosperity and progress of the bee industry, are dependent in a large measure upon the degree of control over bee diseases.

The beekeepers of the state, realizing the seriousness of the disease menace to the beekeeping industry, and feeling the need of protection, succeeded in securing the enactment of the Bee Disease Law, which provides for the inspection of bees for the purpose of discovering and eradicating contagious or communicable diseases of bees. The Department of Agriculture and Markets, in carrying out the provisions of this law, has sought to aid the beekeepers in their fight against foulbrood by instructing them in the diagnosis of diseases and in assisting them in discovering and eradicating them. Owing to a lack of adequate funds, the inspection work has been somewhat limited, but disease has been kept well under control, and in many localities a decided reduction has been made in the percentage of disease. However, the measure of success in this work is largely dependent upon the cooperation and help of the beekeepers.

The beekeepers of the state suffer an annual loss of many thousands of dollars as a direct result of the ravages of bee diseases, of which American foulbrood is the most destructive. The loss in honey crop, bees, and valuable equipment is great, but perhaps the cost of the increased labor occasioned by the disease is almost as great as the direct loss.

Not only has the loss in bees, equipment and labor greatly affected the beekeeping industry, but the discouragement of beekeepers, due to American foulbrood, has perhaps had an even more depressing effect upon the industry. The most discouraging aspect of the situation is the beekeeper's helplessness to guard against the disease. He can do nothing to prevent its spread to his apiaries when once it has gained a foothold in the locality.

Due to the fact that the disease is widely spread over the state, and that it is likely to make its appearance at any time, in the apiary of the small beekeeper as well as in that of the commercial honey-producer, it is essential that every beekeeper know how to diagnose and treat it. Eternal vigilance is the price of success, and only the careful and watchful beekeeper can hope to keep his apiaries free from disease.

It is not the purpose of this circular to give a scientific discussion of bee diseases or a detailed description of the various symptoms, but rather to give a brief discussion of the characteristics, diagnosis and treatment of the various bee diseases, with a view toward helping the beekeeper in diagnosing and treating them. Those who wish more detailed information should secure bulletins from the Department of Agriculture at Washington.

Diseases of Bees

The diseases of bees can be divided into two general classes; those affecting the adult bees and those affecting the brood. The diseases of adult bees in this country are not generally considered very serious and comparatively little is known concerning them. On the other hand, the brood diseases are far more destructive, and considerable study has been made of them, which has given us valuable information as to their nature, manner of spreading and methods of control.

BROOD DISEASES

There are three diseases of the brood of bees with which the beekeeper should be familiar; namely, American foulbrood, European foulbrood and sacbrood. Of these, American foulbrood is by far the most destructive, and the most difficult to control. European foulbrood often spreads rapidly throughout the apiary and causes great destruction, but is more easily controlled than American foulbrood. Sacbrood seldom causes serious loss, but it is sometimes confused with American foulbrood.

AMERICAN FOULBROOD

American foulbrood is the most deadly and the most difficult to control of all the brood diseases. Although it often spreads slowly, when it once gains a foothold in the apiary prompt treatment is necessary if it is to be eradicated. It may spread to other colonies, and as these become weakened they are likely to be robbed out and thus infect the entire apiary. The infected equipment also must be treated, as the spores are very resistant and will live in old combs and equipment for an indefinite period of time.

American foulbrood is caused by a spore-forming bacterium, *Bacillus larvae,* which enters the young larva during the feeding period, where it lies dormant until conditions become favorable for its growth. When the sugar content of the larva or pupa becomes sufficiently reduced, the spores germinate and the organisms

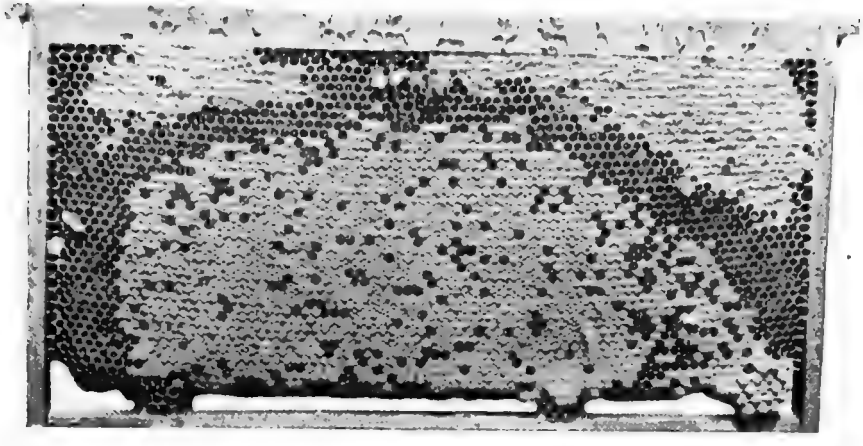


FIG. 1. This illustration shows a normal comb of healthy brood. Note how compactly the brood is placed, with very few unsealed cells intervening.

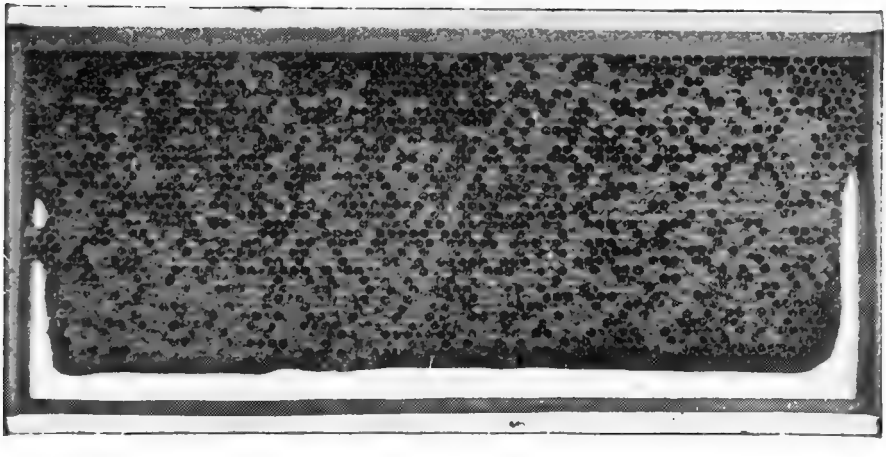


FIG. 2. This illustration shows a comb containing American foulbrood in an advanced stage. Note the spotted appearance of the brood caused by scattered sealed cells. (Courtesy of the A. I. Root Co.)

multiply rapidly, the larva soon showing signs of being affected. This usually takes place soon after the larva is capped.

The majority of the infected larvae die in the late larval stage, though some do not die until after transforming into the pupae. Usually the first indication that the larva is diseased is a sunken and slightly darkened appearance of the capping. The bees soon begin removing this capping. First a tiny pin-hole perforation is made, which is enlarged until finally the entire capping may be removed. The diseased larva first shows a slight brownish discoloration and begins to lose its normal shape. As the disease advances the color becomes darker and the decaying mass passes through all stages of brown, variously described as chocolate, mahogany and coffee color. The larva sinks down to the lower side of the cell, presenting a flattened, melted-down appearance. The back end of the dead larva is slightly elevated against the bottom of the cell, while the front end flattens down on the lower side of the cell, though in the earlier stages it may be slightly rounded at the front and somewhat elevated. The larvae lie in a fairly uniform position, stretched out on the lower sides of the cells. This characteristic aids in distinguishing American foulbrood from European foulbrood, in which latter disease the dead larvae are found in all shapes and positions.

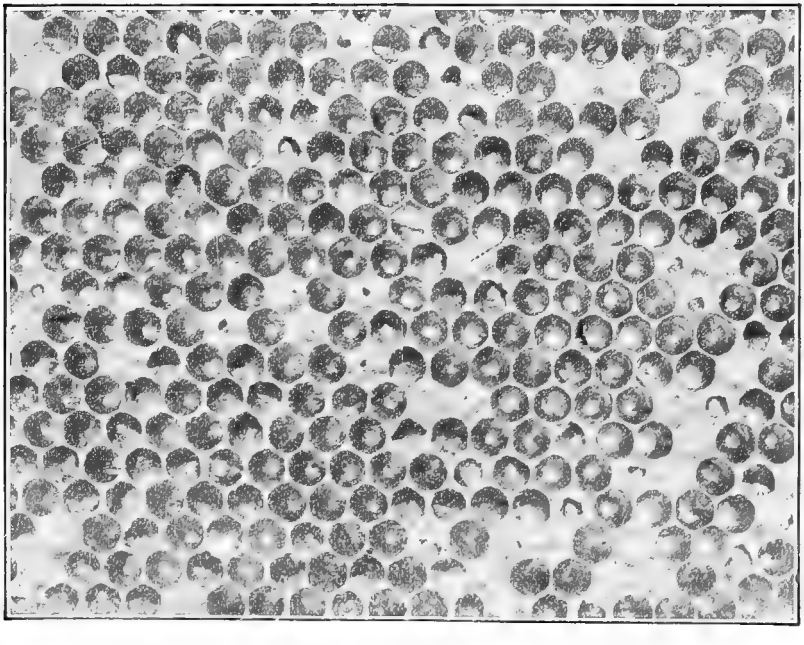
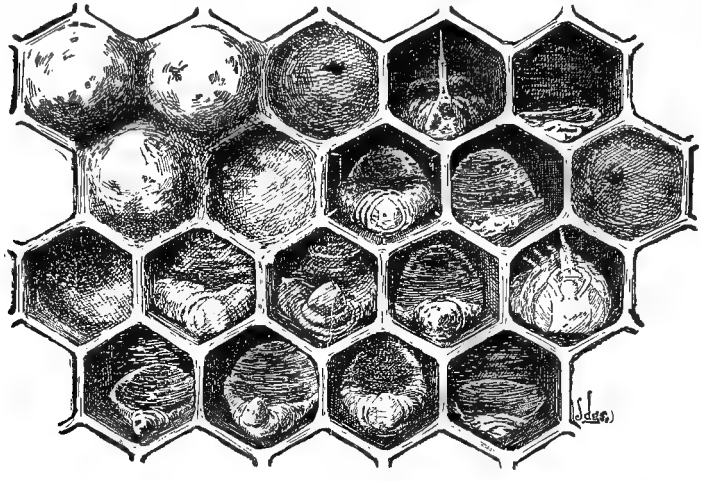


FIG. 3. This illustration shows a comb containing sacbrood.

In the later stages the remains of the infected larvae become dried down to scales, which are more or less firmly attached to the lower walls of the cells. When the larvae become dark colored and flattened down in the cells they usually become gluey and stringy. If a toothpick is inserted into the decayed mass and



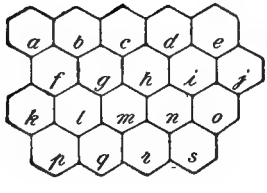


FIG. 4. American foulbrood: a, b, f, normal sealed cells; c, f, sunken cappings, showing perforations; g, sunken capping not perforated; h, l, m, n, q, r, larvae affected by disease; e, i, p, s, scales formed from dried-down larvae; d, o, pupae affected by disease. Three times natural size. (E. F. Phillips, Farmers' Bulletin No. 1084, U. S. Department of Agriculture.)

withdrawn, a long silky thread is drawn out, often two inches or more, which, on breaking, snaps back like a rubber band. This ropiness is one of the chief characteristics of the disease. However too much dependence should not be placed on this ropiness as it is not pronounced in some stages. When the disease is well advanced there can be detected a very pronounced odor similar to that of heated glue. In milder cases and in the earlier stages this odor is not always present.

After the head and legs have formed the pupa may die of the disease. Such a diseased pupa passes through the same stages as does the diseased larva and finally dries down to a scale. In the case of the dead pupa the tongue will occasionally be found projecting upward to the top of the cell. This is a certain indication of American foulbrood.

Chief Characteristics

1. The larvae are attacked after being capped.
2. The dead larvae lie flat on the lower side of the cell in a uniform position.
3. There is a decided ropiness at certain stages.
4. The color is mahogany or coffee brown.
5. Pupae are sometimes found with the tongue extended to the roof of the cell.
6. The scales adhere tenaciously to the cell-walls.

Treatment

There are two general methods of treatment, with various modifications, which as a rule are recommended; namely, the burning method and the shaking method. The shaking method can be carried out successfully by a careful and experienced beekeeper, but extreme care is required or the disease may be spread instead of being eradicated. Bees loaded with infected honey may drift into neighboring colonies during the shaking process and thus infect these nearby colonies. There is also danger of diseased honey being exposed or dropped on the ground where bees will get at it. The burning method is generally recommended, especially where there are few diseased colonies, as it is much easier, safer and in many cases much cheaper than the shaking method.

In order to eliminate American foulbrood it is necessary to destroy or remove the germs which cause the disease. These germs are very resistant to disinfectants and will live for years. Whatever method of treatment may be used it should always be borne in mind that the germs may be present in the honey, the old combs, and the old hive and other equipment used on the infected colony. Therefore, in order to make the treatment effective all this material must be destroyed or thoroughly disinfected, or kept where no bees can gain access to it.

Burning. Care must be exercised in burning, if it is to be effective. The first thing to do is to kill the bees. This can best be done by taking about a tablespoonful of Cyanogas (Calcium cyanide) on a hive-tool or a slip of paper and inserting it into the entrance of the hive. The bees will be dead in a few minutes. If there are bees near the entrance, place a newspaper in front of the hive to catch any dead ones that may fall to the ground. If the hive is bee-tight, sulphur may be used to kill the bees. Close the hive entrance, leaving an opening large enough to insert the nozzle of the smoker. Then get a good fire going in the smoker, put in a small handful of sulphur, insert the nozzle into the entrance of the hive and force smoke in until the bees are all dead.

Gasoline may also be used to kill the bees. Remove the hive-cover and place a burlap sack over the top of the hive, being careful to see that it fits down snugly. Close the entrance carefully. Now pour about a pint of gasoline over this sack, allowing it to run down between the frames. Replace the cover and let the hive

remain a few minutes until the bees are all dead. Care must be exercised in burning as the gasoline is inflammable. When bees are killed with gasoline or sulphur great care must be taken to see that the hives are bee-tight, or the bees may rush out and enter neighboring hives carrying the infection with them.

Next dig a hole in the ground about two feet deep and large enough to contain all the diseased material. The size will depend upon the amount of material to be burned. It should be of such size, however, that the remains can be buried at least one foot below the surface. Get a good fire going in the hole, using plenty of wood and kerosene. Carry the hive to the hole and set it down on the loose dirt or on some gunny sacks or paper spread on the ground. Take the frames out and place them on the fire or stand them around the edges, being careful not to smother the fire. Do not scatter honey or dead bees around. Many of the bees will exude a drop of honey on the tongue. This honey may be infected with disease germs. Scrape the inside of the hive, cover, the bottom board and burn these scrapings. After the burning is completed, any papers or gunny sacks used should be put on the fire. Care should be taken that all dead bees on the ground are burned or covered with earth. When the material is well burned down, cover with at least one foot of earth.

Caution. Cyanogas is poisonous and the fumes are deadly. Handle it with care and do not open the container in a closed building.

Do not allow any bees to escape during the killing process. Bees that come in from the fields are probably not dangerous, but bees that escape while the colony is being destroyed may carry infection.

The inside of hives, covers, and bottom boards should be scorched until they are a dark mahogany brown. This can best be done with a blowtorch.

Shaking Method. Treatment may be successful if carefully done by an experienced beekeeper. The method known as the shaking treatment is generally recommended. It is usually preferable to carry out this treatment during the middle of the day, though some prefer to do it late in the evening. If the colonies are close together it is sometimes advisable to shake late in the evening and cover up the neighboring colonies during the shaking process to prevent bees from the infected colonies entering healthy ones. It should be done when some honey is available in the fields so that the bees will not be inclined to rob. The procedure is as follows:

1. Dig a hole in the ground about two feet deep and large enough to accommodate all the infected frames and combs. Get a good fire going in this hole.

2. Set the infected colony to one side and in its place put a clean hive filled with frames of foundation or one-inch starters with no empty comb. In front of this hive spread two or three thicknesses of newspaper.

3. Remove the combs from the infected colony one at a time and shake or brush the bees onto the newspaper in front of the empty

hive. Each frame when shaken should be quickly put upon the fire. When all frames are shaken, pick up the empty hive and bottom board and jar the bees off on the paper.

4. Thoroughly scrape the inside of the infected hive-body, cover and bottom board, burning the scrapings in the fire. Gather up the newspaper in front of the hive and burn. After the fire has burned down, cover it with at least a foot of earth.

As soon as the queen is in the hive a queen-trap should be placed in front of the hive, or a queen excluder inserted between the hive-body and bottom board to prevent the queen leaving should the bees attempt to swarm out. This trap or excluder should be removed in three or four days.

The fact should always be borne in mind that shaking is a very dangerous process and is not generally recommended, especially in the apiary where there are healthy colonies. By attempting to save a few dollars' worth of old combs and equipment the bee-keeper often loses several times that amount by infecting healthy colonies.

Suggestions:

1. Be careful that no honey is spilled on the ground or exposed in any place where bees can get at it.

2. The old hive-body, cover, bottom board and supers from a diseased colony must not be used until they are thoroughly disinfected. This may be done by carefully scorching with a blow-torch or by boiling in a strong lye-water solution.

3. After treating diseased colonies, the hands, hive-tool, smoker and everything else which has come in contact with the diseased honey should be carefully washed in hot soapy water. The washings should be disposed of where bees cannot get at them.

4. Do not shake diseased colonies on drawn combs or frames of honey. Bees carry diseased honey with them and this will be stored in the empty comb. Later it will be fed to the brood and the disease will reappear.

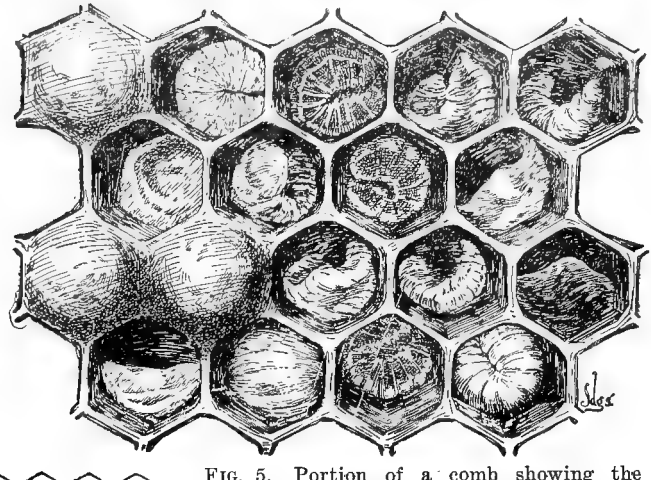
5. Care should be taken to prevent bees drifting into neighboring colonies during the shaking process. If the colonies are close together it may be advisable to move the diseased colonies to a new location at least three miles distant, a few days before treating. It is perhaps safer to move the healthy colonies to a new location and to treat the diseased ones in the old location.

Where there is a large number of diseased colonies it is sometimes advisable to save the infected combs and honey; however, it must be remembered that this is extremely dangerous and requires great care or the entire apiary may be reinfected. During the process of extracting the diseased honey, the extractor, honey house, supplies and other equipment may become infected by coming in contact with diseased honey. As long as there is a pound of diseased honey or a diseased comb left around, there is danger. Few honey houses are bee-tight. Robber-bees may gain access to the infected material and reinfect the apiary.

Diseased combs may be disinfected by the use of Hutzelman's solution or the water-alcohol or water-formalin solution. This treatment is not being used as much as formerly and it is doubtful if it pays unless there is a large number of combs to be treated. Formalin is very disagreeable to use, quite expensive, and care must be exercised in order to be successful. It is not generally recommended for the small beekeeper, since considerable equipment is necessary and many cases of failure have been reported. The method usually employed is to extract the honey, being careful to uncap every cell containing honey. If the water-formalin treatment is used, the cappings of the brood must also be removed. The combs are then soaked in water for twenty-four hours. The water is then removed and the combs are soaked in the formalin solution for forty-eight hours. This solution is then extracted from the combs and they are set out in the open to air for several weeks before being given to the bees.

EUROPEAN FOULBROOD

During the late nineties, European foulbrood swept across the State of New York and caused great losses to the beekeepers, as little was known of its cause or methods of control. While it still



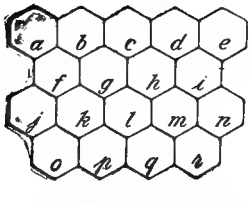


FIG. 5. Portion of a comb showing the effect of European foulbrood upon the larvae; a, j, k, normal sealed cells; b, c, d, e, g, i, l, m, p, q, larvae affected by disease; r, normal larva at age attacked by disease; f, h, n, o, dried-down larvae or scales. Three times natural size. (E. F. Phillips, Farmers' Bulletin No. 975, U. S. Dept. of Agriculture.)

causes considerable loss in some seasons and in some localities, it has lost its terror for the progressive beekeeper. He has learned how to control it by practicing better beekeeping methods. European foulbrood spreads through the apiary much more rapidly

than does American foulbrood, but it is less destructive and easier to control.

European foulbrood is a bacterial disease caused by *Bacillus pluton,* though other organisms are present. The larvae are attacked at an earlier stage than in American foulbrood. They may be affected as early as the fourth day and most of them die before being capped. The dead larvae take on a yellow color which later becomes brownish. These dead larvae are often coiled up in the bottom of the cells, and white tracheal lines may be seen radiating outward from the center. However, one of the chief characteristics of this disease is the irregularity of shape and position of the larvae. They may be twisted in any shape or form and may be in any position in the cell.

As decay continues the dead larvae dry down into dark scales. These scales do not adhere to the cell-walls as tightly as do the scales of American foulbrood. They can be removed easily. It often happens that considerable numbers of larvae die after being capped. These capped larvae, dead from European foulbrood, may be confused with American foulbrood. Some of the larvae that die at this stage may lie flat on the floor of the cells, but the majority of them occupy various positions in the cells and may assume most any shape. When they apparently lie flat, a close inspection will often reveal a slightly twisted position. At certain stages a ropiness may be present, but the rope is shorter and more granular than that of American foulbrood, and does not snap with a rubbery consistency, on breaking, as does that of American foulbrood. When the larva dries down it has the consistency of dead burned rubber. The color is dark, and at the rubbery stage may be almost black. In the early stages little odor is detected, but in the later stages of bad cases, a foul repulsive odor is present.

Treatment

Since European foulbrood is primarily a disease of weak colonies and of black bees, the best method of treatment is preventive measures. Black colonies should be requeened with a vigorous strain of Italians. All colonies should be kept strong by better beekeeping methods. This will usually serve to keep the disease well under control. However, in some seasons and in some localities the disease gives more trouble. Weak colonies should be united. In bad cases it may be necessary to take away some of the badly diseased combs or separate them from the brood-chamber by means of a queen excluder.

SACBROOD

Sacbrood is an infectious disease of the larvae, caused by a filterable virus. The larvae are usually attacked soon after the cells are capped. At first the larva becomes yellowish, then a grayish mottled to brownish color. The front end is often darker, sometimes even black. The dead larva usually lies on its back and often the front end is pointed and extended toward the roof of

the cell, something like a sled runner. It does not adhere to the cell-walls as does American foulbrood, and often can be removed whole without breaking the skin, the contents sinking to the bottom, giving it a sac-like appearance. The contents of the diseased larvae have a granular, liquid appearance.

Treatment

Sacbrood seldom causes serious loss and usually disappears without treatment when the honey-flow comes on. In bad cases it is sometimes advisable to requeen with a vigorous young Italian queen. Badly diseased combs may be placed above an excluder.

ADULT DISEASES

Isle of Wight Diseases

The Isle of Wight disease is caused by a mite (*Acerapis woodi*) which enters the trachael or breathing tubes of the bee. This disease has caused serious loss to beekeepers in Europe, but it is not known to exist at the present time in the United States.

Nosema Disease

Nosema disease is caused by a protozoan parasite, *Nosema apis,* which is found in the alimentary tract of the bee. The stomach of an infected bee become somewhat swollen and takes on a whitish color. Some bees may become weakened and die as a result of the disease. Nosema is widely distributed over the United States but it seldom causes any serious loss.

Paralysis

Paralysis is the name usually given to a peculiar sickness of adult bees as a result of which they die off in large numbers. The abdomen is often swollen and has a greasy appearance. The badly affected bees have a shaky, trembling motion and are unable to fly. Little is known of the cause or treatment of this disease.

Dysentery

Dysentery is a digestive disturbance of bees which results from poor stores and long winter confinement. In serious cases the inside of the hive and the entrance become spotted and great numbers of bees die. The condition usually disappears if the bees get a good flight. The best safeguard against dysentery is good winter stores and adequate winter protection.

NOTES

Never feed honey from an unknown source. It may be diseased. Sugar sirup is safer.

Do not exchange frames from one colony to another if you have any disease in your apiary.

Never expose honey in your apiary, as it may cause robbing.

When bees have their first flight in the spring, reduce the size of the entrances of all weak colonies. If any colonies are dead, carefully close the entrances and carry them to the basement or into a bee-tight house. A diseased colony may die during the winter and be robbed out in early spring, thus infecting your entire apiary.

Diseased honey may be made safe for bees by adding an equal amount of water and boiling in a closed vessel for at least one-half hour. Do not use a double boiler.

Do not purchase colonies of bees unless you are certain that they are free from disease.

It is not safe to buy bees on combs. Combless packages are safer.

It is not safe to purchase old combs, second-hand hives and equipment, or second-hand honey cans. Disease germs will live in such combs and equipment for years.

It is not safe to attempt to disinfect combs and equipment by the use of new methods or solutions which have not been thoroughly tried out by careful investigators and found to be effective.

After handling a diseased colony, clean your hands, hive tools and any other implements used, washing them with soap and water.

Do not use a solar wax-extractor if there is disease in your apiary.

Never winter a diseased colony. It may die during the winter and be robbed out, or bees may drift into other hives carrying the disease with them.

Do not permit diseased colonies to remain in your apiary. Slow robbing may be going on most any time without your knowledge.

Every beekeeper should inspect his colonies several times each season, keeping a careful watch for the appearance of disease.

In case of doubt as to the kind of disease present send samples of brood to the United States Department of Agriculture, Bureau of Entomology, Bee Culture, Washington, D. C.

The spores of American foulbrood will remain alive in boiling water for 10 minutes.

The spores of American foulbrood will remain alive in honey for years.

Spores of American foulbrood will remain alive in honey or diluted honey at 212 degrees Fahrenheit for one-half hour or more.

Spores of American foulbrood will remain alive in old comb and equipment for years.

Spores of American foulbrood will resist ordinary disinfectants such as 10 per cent formalin, 1-1000 mercuric chlorid or 5 per cent carbolic acid for hours or even months.

There is no conclusive evidence that drugs fed to bees are of any value in the treatment of American foulbrood.

LAW RELATING TO BEES

(Chapter 166 of the Laws of 1925, as amended)

ARTICLE 15

BEE DISEASES

§ 173. **Eradication of bee diseases.** The commissioner may cause inspections to be made of apiaries in the state for the discovery of infectious, contagious or communicable diseases. He may also cause investigations to be made as to the best method for the eradication of diseases of bees, and he may plan and execute appropriate methods for the eradication of such diseases.

The commissioner shall have access to all apiaries, structures, appliances or premises where bees or honey or comb used in apiaries may be. He may open any hive, colony, package or receptacle of any kind containing or which he has reason to believe contains any bees, comb, bee products, used beekeeping appliances, or anything else which is capable of transmitting contagious or infectious diseases of bees.

§ 174. **Keeping of diseased bees prohibited; existence of disease to be reported.** No person shall keep in his possession or under his care any colony of bees affected with a contagious or infectious disease. Any person who knows that any bees owned or controlled by him are affected with any contagious or infectious disease shall at once report such fact to the commissioner, stating all facts known to him with reference to said contagion or infection.

If any inspection made by the commissioner or his duly authorized representative discloses that any apiary, appliances, structures, colonies or comb are infected, the commissioner or his duly authorized representatives may give the owners or caretakers of such infected property instructions for treating such infection. If, in the judgment of the commissioner or his duly authorized representatives, it is necessary, he may order such owner to completely destroy the infected apiaries, colonies, structures, appliances or combs, or any such part of them as, in his judgment, he may deem best for the eradication or control of said infection. Said owner or person in charge shall carry out said instructions or orders within ten days after a date which shall be specified in said instructions or orders.

If said owner or person in charge refuses or neglects to carry out such instructions or orders within said period of ten days, the said commissioner or his duly authorized representative may apply

said treatment or may, in his discretion, destroy such infected property. No damage shall be awarded to the owner for the loss of any infected apiary, bees, hives, apiary appliance, or bee product destroyed under the provisions of this section or any regulation or order made in pursuance thereof.

Persons keeping bees shall keep them in hives of such construction that the frames may be easily removed for examination of the brood for the purpose of determining whether disease exists in the brood.

No person shall expose in any place to which bees have access any bee product, hive or other apiary appliance in such manner that contagious or infectious diseases of bees may be disseminated.

§ 175. **Transportation of bees and bee material.** No person shall transport any colony of bees, used comb, used beekeeping material, or live bees on comb, which is infected with or has been exposed to bee disease, unless it be within the beekeeper's own premises, or unless it be by permit from the commissioner.

Every shipment of a colony of bees, used comb, used beekeeping equipment, or live bees on comb into this state from another state or foreign country, shall be accompanied by a certificate of freedom of disease from an official of such state or foreign country recognized by the commissioner, and shall be held in quarantine at destination until inspected and released by the commissioner. Every transportation company upon the receipt of such shipment shall immediately notify the commissioner thereof, giving the name and address of the consignor and consignee.

§ 175-a. **Assessors shall list and report owners of bees: Special provision for Nassau county.** Assessors shall file with their annual assessment-roll a list of the names and addresses of all owners of bees within their respective districts. The provisions of this section shall not apply to the county of Nassau. The police department of such county shall annually prepare a list of the names and addresses of all the owners of bees in each town in such county including villages and file the same on or before July first in the office of the town clerk of such town.

SPRAYING AND DUSTING FRUIT TREES

Beekeepers in many sections of the state have suffered a considerable loss of bees due to the spraying of fruit trees with poisonous arsenicals while in blossom, or to the careless broadcasting of poisonous dusts. Because of these conditions many beekeepers are removing their bees from the vicinity of orchards. This has resulted in heavy losses to the fruit growers due to the lack of bees for proper pollinization of the blossoms. The fruit growers are waking up to this situation and are using more care in the application of poisonous sprays and dusts. In many cases they are paying the beekeepers to move their bees back to the orchards.

The beekeepers can help to improve conditions by calling the attention of fruit growers to the necessity of bees for pollinization purposes and to the danger of destroying bees and other pollinating insects by the careless use of poisonous sprays and dusts. Poisonous dusts, if applied when there is a wind, may drift for considerable distances and fall upon the flowers in neighboring fields. The bees collecting pollen and nectar from these flowers are themselves killed as well as the brood to which the poisonous material is fed.

Not only is it unnecessary to spray trees with arsenicals while in blossom, but it may even be injurious to the tender blossoms and prevent the setting of fruit.

The following is a copy of the law of New York State relating to spraying fruit trees when in blossom:

PENAL LAW

§ 1757. **Spraying fruit trees with poison.** Any person who will spray with, or apply in any way poison or any poisonous substance, to fruit trees while the same are in blossom, is guilty of a misdemeanor, punishable by a fine of not less than ten dollars ($10) or more than fifty dollars ($50) for each offense.

RULES AND REGULATIONS IN RELATION TO WEIGHTS AND MEASURES

The following rules and regulations heretofore adopted by the Department of Agriculture and Markets are in full force and effect:

(1) **General regulations.**

(a) Variations in all commodities unless otherwise stated in the regulations shall be as often above as below.

(b) All markings on containers must be on the top or side and must not be covered or obscured in any way.

(c) In cases of food, if the quantity of contents be stated by weight or measure, it shall be marked in terms of the largest unit contained in the package; for example, if the package contains a pound or pounds and a fraction of a pound, the contents shall be

expressed in terms of pounds and fractions thereof, or of pounds and ounces and not merely in ounces.

(d) Whenever markings are prescribed by the regulations to be in type of various sizes or kinds, writing or other method of marking will be permitted, provided it is equal in clearness and conspicuousness to the method of marking prescribed.

(e) In connection with the weight, measure or numerical count, no qualifying word, phrase or clause shall be used; a statement such as "minimum," "not less than," "average," "when packed," or a statement that the contents are "over" a certain amount, or a statement that the contents are "between" certain limits, is not permissible.

(f) Contents shall not be stated by numerical count, unless the commodity so sold is in definite units.

(g) Markings may be in terms of the metric system, anything in these regulations notwithstanding, where the commodity so marked is customarily bought and sold by metric weight or measure.

(h) The quantity of liquid commodities shall be stated in terms of liquid measure, unless such commodity is customarily bought and sold by weight, in which case the quantity should be stated in terms of weight.

Regulations for Honey

Section comb-honey sold in New York State need not have the weight stamped on the sections, whether it be in cartons or otherwise.

If more than six sections are sold in a case or other container the number of sections in the case or container must be marked on the outside.

If comb-honey is to be sold outside of the state the net weight must be marked on each section.

Honey put up in tin may be marked either in terms of gallons, quarts, pints, one-half pints, etc., or in terms of pounds and ounces.

Honey put up in glass may be marked either in terms of quarts, pints, half-pints, etc., or in terms of pounds and ounces.

Packages containing three ounces or less avoirdupois or two ounces or less fluid measure, need not be marked.

Address all inquiries concerning bee diseases to the Bureau of Plant Industry, Department of Agriculture and Markets, Albany, New York.

Copies of this circular may be obtained from the above address.

The Federal Government issues a number of bulletins and circulars relating to bees and beekeeping. A list of these may be obtained by writing to the United States Department of Agriculture, Bureau of Entomology, Washington, D. C.

If you wish to move or sell bees write to the Department of Agriculture and Markets, Albany, New York, for a permit.

ONTARIO AGRICULTURAL COLLEGE

BULLETIN 112.

FOUL BROOD OF BEES

BY

F. C. HARRISON, B.S.A., PROFESSOR OF BACTERIOLOGY.

ONTARIO DEPARTMENT OF AGRICULTURE,
TORONTO, ONT., DECEMBER, 1900.

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AGRICULTURAL COLLEGE AND EXPERIMENTAL FARM.

FOUL BROOD OF BEES.

By F. C. Harrison, Professor of Bacteriology.

Historical Resumé.

In all probability the first definite reference to foul brood is by Aristotle (1), who mentions an inertness which seizes the bees, and causes a bad smell in the hive. He also suggests that bees are liable to become diseased when the flowers on which they work are attacked by blight. Bee dysentery causes a bad odor as well as foul brood; but in the former disease the spotting and consequent smell are usually outside the hive.

Columella (2) mentions a bee pestilence and an annual distemper which seizes the bees in spring. Pliny (3) writes of a disease of bees, but as he uses the same term as Aristotle he has probably copied it from the latter author.

Schirach (4) in 1769 was the first writer to name the disease "Foul Brood." He says that "it is dangerous and a most destructive disorder to the bees, a genuine plague when the complaint has reached a certain stage. The cause can be attributed to two sources—1. The putrid (or tainted) food with which the bees feed the larvæ for lack of having better. 2. By the mistake of the queen bee, in misplacing the larvæ in their cells, head upside down. In this position the young bee, unable to get out of its prison, dies and rots away." Further, Schirach clearly distinguishes between foul brood and chilled brood, and mentions the fact, that putrefaction follows the death of the brood from frost, but in this case "it is only an accident and not a disease."

The remedy Schirach recommended was to remove all diseased combs from the infected hives and to keep the bees fasting for two days, after which they are furnished with other cakes of wax, and a suitable remedy given, "as a little hot water mixed with honey, nutmeg and saffron, or a syrup composed of equal parts of sugar and wine, seasoned with nutmeg." Thus, as Cowan (5) remarks "we had given us nearly 130 years ago, a method of cure almost identical with what is by some claimed as new to-day."

Tessier (6) about the same time as Schirach, says, that when the larvæ die in their cells it causes an infection in the hive which makes the bees sick. It is then necessary to drive away or sometimes move the bees from the hive, and to take care to fumigate the infected hive if it is going to be used again. It is necessary, in order to avoid the same inconvenience, to take out the parts of the comb that may be moulded by reason of the dampness. Duchet (7), who wrote on bees in 1771, does not mention any disease that can be certified as foul brood, but he describes bee dysentery.

Della Rocca (8), Vicaire-General of Syra, an island in the Levant, relates with much detail the history of an epidemic of foul brood, which caused great destruction in the island during the years 1777 to 1780. Della Rocca describes very minutely the symptoms, destruction and mistakes that were

made in attempting to combat the disease. "The disease," he says, "manifests its presence by defects in the combs filled with brood, and which only contain a putrid mass; instead of the bee pupæ there is only rottenness in the cells, which, however, being capped always preserve a healthy appearance. If these cells are broken open, a blackish liquid flows out, which spreads the infection through the hive. This disease only manifests itself in cells which contain a nearly mature larva or a capped one. The bees themselves remain in good health, and work with the same activity, but their numbers decrease daily. This disease, however, was not so general in a hive but that a small portion escaped; some new bees emerged, but in too small numbers to supply the daily losses Thus a hive attacked by this scourge will perish from scarcity of population. At first it was not noticed that this disease was epidemic, and the hives emptied by death of the bees, were filled with fresh swarms, and these contracted the same disease and perished. Yet another mistake was made. The remains of the hives that were lost were taken into the streets of the town to expose them to the sun, in order to extract all the wax, and the bees from the neighborhood sucked up the honey, caught the disease, and communicated it to other hives, and all, without exception perished in a short time. The epidemic, having reached the island, spread everywhere and the mortality among the bees was general, either from eating infected honey, or from stopping up the infected combs, or from the bees nourishing their brood on infected honey." Della Rocca criticizes Schirach's statement regarding the misplacement of the larvæ by the queen as a cause of the disease, because "everybody knows that the queen has nothing else to do but deposit eggs. These are then cared for and nourished by the bees, and when the larva is nearly ready to change into the pupa, the bees close the cell, and every position which is given the larva depends on their good pleasure and not on the queen's." Della Rocca himself thinks that "some pestilential blight had without doubt corrupted the quality of the honey and the dust from the authers," and recommends "burning everything without pity, as there is no other resource when the disease is well established, as the pest is without doubt the most terrible in the natural history of bees."

Neither Wildman (9), Keys (10), Woolridge, Needham (11), Rhein, Reaumur (12), or other authors about the same time (latter end of the 18th century) mention this disease.

Bevan (13) names the disease "Pestilence," and also quotes Schirach's name "Foul Brood," and says regarding it, that the "Pestilence has been attributed to the residence of dead larvæ in the cells, from a careless deposition of ova by the queen . . . it has also been attributed to cold and bad nursing, that is, feeding with unwholesome food."

Nothing further of note appears in bee literature till the year 1860, when Dr. Leuckhart (14) writes that he was formerly of the opinion that foul brood was caused by the same fungus (*Panhistophyton ovatum*) which is noticed in a disease of the silk worm, but now after observation and experiment, is quite certain that the disease is caused by neither vegetable nor animal parasite. He also notes that the term "foul brood" is applied to a number of diseases affecting bees.

Molitor Mühlfeld (15) recognizes two forms, one contagious and the other not contagious, and thinks that the only cause of contagious foul brood is a fly (*Ichneumon apium mellificarium*) which lays its eggs on the young larvae of the bee.

A discovery of note was Preuss's (16) in 1868. He contradicts Mühlfeld's statement about the fly, and states that foul brood cells can be detected by

the sunken cap. With a microscope magnifying 600 diameters, he found small dust like bodies with a diameter of $\frac{1}{500}$ m. m. belonging to the genus *Cryptococcus* (Kutzig) and called the specific form *alvearis*, likened it to the fermentation fungus (*Cryptococcus fermentum*) and thought that the last germ gained access to the young bee and changed to *Cryptococcus alvearis*. He notices that many experts lay the cause of the disease to fermenting food but the larvae are easily contaminated by the fermentation fungus which is always present in the air. He also mentions the enormous rapidity with which the fungus multiplies and gives an elaborate calculation of the number that might be found in a cell containing a deceased larva.

As might have been expected, Preuss's statement aroused considerable discussion at the meeting of German bee-keepers, a short while after the publication of his paper.

Vogel (17) expressed doubt as to whether *C. alvearis* was the real cause of foul brood or only a consequence of the disease, but on the whole agreed with Preuss.

Wiegand (17) agreed with Preuss's theory, and in giving his experience said that the disease was introduced into his apiary through honey brought from a distance.

Pollman (17) believed that the disease was introduced by feeding honey from Havanna, where, when extracting the honey, both brood and honeycomb were mashed up and the honey pressed out.

Dr. Leuckhart (17) agreed with those who thought the disease due to a fungus, but discredited the supposition that it was the same as the fermentation fungus mentioned by Preuss, and rather thought it was related to the silk worm fungus and that most of the brood diseases ending in death were called "foul brood" while they were really something else. He believed that the fungus was present in the eggs of the queen when laid.

Geilen (17) believed that the disease came from the putrefying remains of animal bodies, upon which the bees alighted.

Mühlfeld (18) again in 1869, presented his former views, and also those of Preuss's and gave directions for maintaining the health of bees. He recommended the boiling of the honey, and a use of carbolic acid in the strength of 1:100 or permanganate of potash 1:300 as disinfectants.

Lambrecht (19) thought that foul brood was caused by the fermentation of the bee bread.

Hallier (19) considered it no specific disease, but thought it was probably produced by different fungi.

Cornallia (20) proved contrary to the above and found a fungus, which he thought developed foul brood. He called it *Cryptococcus alveaxis* and used carbolic acid, potassium permag., and lime water as disinfectants.

Fisher (21) advanced a new foul brood theory in 1871, which somewhat follows the view of Liebeg regarding the silk worm disease and plant diseases. According to this theory, the predisposing cause was insufficient nourishment, especially short stores for winter and spring. Shortage of pollen supply was the next cause. Fisher tried to prove his views by the practical experience of bee keepers and explained that the first result of repeated and continued feeding was an increase in the production of bees; and a consequent disproportion between brood and brood feeders arose, which should be looked upon as another cause of foul brood. The disease, he said, might be lessened or exterminated by applying means to reduce the production of brood, as the removing of the queen and the area which the brood occupied. Foul brood is probably the cause of a quantitative dearth of nourishment and a consequent degenera-

tion of the bees. The appearance of fungous growths was only a secondary matter.

Schonfeld (22) infected several hives with foul brood and when it had fully developed he took a comb of the rotten brood to the Physiological Institute at Breslau, and had it submitted to a microscopical examination by Drs. Cohn and Eidam (22). This examination showed that in every dead larva, and in each foul broody cell, whether the contents were yet white and fluid or brown, tenacious and ropy, there were to be found long oval bodies, which Preuss called "micro-cocci." "Close to and among them, Cohn was the first "to find, with the most powerful of the five microscopes that were used, a "countless number of slender pale rods, joined together, and which he at once "identified as bacteria of the genus *bacillus*. The length of a single rod was "about 6 micromillimetres, but many of them were two and three jointed, so "that these foul brood bacteria microscopically resembled the authrax bacteria, "though of course they were different physiologically and in the manner in "which they acted as ferments."

It is not surprising when we remember the state of bacteriological knowledge in 1870, that Preuss should have mistaken micrococci for the spores of a bacillus. In 1885, Cheshire & W. Cheyne (23) confirmed Cohn's conclusions and demonstrated that the disease, foul brood, was caused by a bacillus to which they gave the name of *Bacillus alvei*; and they worked out the following requirements of the causal relation of this bacillus to the disease, usually spoken of as Koch's rules:

1. Constant association of this germ (*B. alvei*) with the disease in the larvae of bees.

2. Isolation of the germ from the diseased larvae, and study of the same in pure cultures on various media.

3. Production of the characteristic symptoms of the disease by inoculation of pure cultures.

4. Discovery of the same germ in the re infected larvae. Re-isolation and growth on various media, comparable to that previously isolated.

The infection brought about by Cheshire was accomplished by spraying a particular part of the comb with a culture of *B. alvei* in milk. This part and no other became affected with foul brood. Adult bees were also infected by feeding them with these cultivated bacilli. The experiments of Cheshire and Cheyne convinced everyone, and since that date *B. alvei* has been generally regarded as the causal agent in the production of this disease.

Dickel (24) wrote in 1888 that several species of bacilli were able to produce foul brood. There was one form of the disease which affected the unsealed brood, and another which affected the sealed brood; and even a third, a mixed form, which seemed to be most malignant.

Klamann (25) supported Dickel's researches, but stated that it was not necessary to count more than two kinds of the disease, as there were certainly several other microbes which contributed to the ruin of the hive. Klamann stated that he found seven and was persuaded that he would be able to isolate an even greater number of bacteria from the diseased larvae. It seemed to him certain that *B. alvei* was the most virulent, and that this germ alone was to be considered the cause of foul brood.

Symptoms of the Disease.

The disease principally affects the larvae. In a healthy comb the young larvae lie at the bottom of the cells, curled up in the shape of the letter C and in color are of a pure pearly whiteness, plump in appearance, with full

skin. If examined when the disease is just developing, the affected larvae are usually found to have changed their position. They are no longer curled up, but lie extended in the cell, or move about unnaturally. The bees themselves may at this time or subsequently show symptoms of the disease by a kind of inertness or inactivity which seizes them. As the disease progresses, the young larvae lose their plump appearance, become flabby and die. Then a process of decomposition begins, which is shown by their yellowish appearance. This yellow color turns brown, and if touched with a pin or needle at this time or later, a portion of the putrid mass may be pulled out in a long, ropy, tenacious string, due "to the chitinous aerating sacs and tracheae which do not undergo decomposition at all; and these remaining cause the peculiarity referred to" (26). This ropy mass gradually dries down to the bottom of the cell, leaving nothing but a brown scale which adheres to the wax.

As a rule, the bees do not remove larvae dead from this disease. Instead they become quite inactive, lose their desire to fly, are often seen fanning at the entrance of the hive, which in many cases emits a bad smell. A phase of the disease, which some authors have described as being a different form, is that in which the larvae die after the cells have been capped over. These cappings become darker in colour than those of the healthy brood; then become indented or sunken, and lastly become perforated with irregular holes. By putting a needle into any of these cells the same ropy mass, before described, may be drawn out. If an examination is made from the juice of the larvae at different stages of the disease, the bacillus may be detected; but spores do not form till after death has occurred. The ropy mass contains large numbers of spores, as does also the dry scale.

According to Cheshire (26), the bees themselves become diseased. In a number of cases he obtained the bacillus from the blood of bees from infected hives. Hilbert's examination in 1875 led him to declare that the mature bees in infected hives were liable to be affected. Some writers contradict the statement that the bees themselves are affected by the disease; but they lose sight of the fact that the bees do not die in the hive, but leave it sometime before death occurs.

The queen may become infected. Cheshire (26) demonstrated the presence of bacilli in the ovary of a queen; but he did not make cultures therefrom. W. G. Smith (27) reported that a queen sent to Cheshire and examined by him contained *B. alvei* in both of her ovaries. McKenzie (28) examined five queens from infected hives and succeeded in obtaining bacilli from the ovaries of three. He thinks that their presence there is accidental, as in the case of a queen from a badly diseased hive he was unable to find the bacillus, whilst in a six weeks old queen from a hive in which there were only a few diseased cells, he succeeded in finding it. A queen sent by T. A. Govan (29) to Cowan, the editor of the British Bee Journal, was examined, and *B. alvei* was found in the ovaries. F. F. Ward (30) removed a queen from a diseased hive and placed her in a strong, healthy stock, "which speedily became a mass of corruption." This operation was subsequently repeated with a like result.

I have also myself examined seven queens from diseased hives, and in three cases have had no difficulty in finding the bacillus, and have obtained typical cultures therefrom. The method of examination employed has been the same as that used by McKenzie. The upper surface of the bee is sterilized and cut longtitudinally, and all the internal organs except the ovaries are removed. The surface of the ovaries is then sterilized and a hot needle plunged into the centre and allowed to stay there until it is cold, when

it is withdrawn and used to inoculate agar cultures. According to Cheshire (26) the bacilli are found in the eggs. In one examination he says he counted nine bacilli from a half-developed egg taken from the ovary of a queen. McKenzie (28) thinks that this statement requires confirmation, as he was not able to find any infected eggs.

I have myself examined a very large number of eggs at various times. In these examinations three different methods were employed : 1.—The eggs were taken from the cells in which they were laid with sterilized forceps and washed in corrosive sublimate, 1 : 500 crushed and placed on agar plates. In many cases typical growths of *B alvei* developed from eggs thus treated ; but as it might be maintained that the eggs were laid in cells previously infected with *B. alvei*, and that the momentary immersion in corrosive sublimate failed to kill all the spores that were upon the exterior, the next lot of eggs 2.—Were crushed between cover-glasses, a small portion transferred into agar, and the remainder on the cover-glass stained by Gram's method. In several instances the bacillus was found in the crushed egg, and in every case the cultural test confirmed the microscopical examination. Again, as this method also might be criticized for the reasons above stated—(3) eggs were imbedded in parraffin and serial sections made and stained by Gram's method No cultural tests were made ; but in a few eggs of several hundreds sectioned a bacillus corresponding in its morphological characteristics to *B. alvei* was found. All the eggs examined were taken from hives more or less affected with the disease.

In view of these facts, I am of the opinion that the eggs of bees from diseased hives may in some instances be infected.

Chilled Brood.

Chilled brood is sometimes mistaken for foul brood ; but the appearance of the former is very different from that of the latter. In the case of chilled brood the larvae turn grey ; afterwards the colour darkens, and in the final stages of decomposition it becomes black. No ropiness develops.

A number of writers in various bee periodicals have mistaken chilled brood for foul brood, or they have stated that chilled brood turns to foul brood ; but Schirach, as long ago as 1769, clearly distinguished between the two, and McKenzie (28) also performed several experiments in refutation of the statement that if chilled brood is allowed to putrify foul brood may arise *de novo*. He endeavoured to isolate *B. alvei* from chilled brood, but without success. Again, he killed perfectly healthy brood by chilling, and infected some of the cells from a pure culture of *B. alvei*. The chilled brood were allowed to putrify in a moist chamber for several months and examined with the same results, viz : that in the cells in which *B. alvei* was placed it was to be found, but not in any others I have also performed similar experiments and they fully confirm McKenzie's contention. So far *B. alvei* has not been isolated from chilled brood in any stage of decomposition. Canestrini (31) described a case which was in all probability chilled brood and not an infectious malady ; but his inoculation experiments failed to establish the pathogenicity of the bacillus, which morphologically resembled *B. megatherium*.

Geographical Distribution.

It has been thought that the disease varies in different countries, that foul brood as it occurs in England is different from foul brood in America ; but as no bacteriological evidence has been produced in support of the con-

tention, it is not necessary to argue the question. I have examined diseased larvæ from Canada, from Europe (France, Switzerland, Austria, Germany, Italy and England), Cuba, and 13 States of the Union, ranging from New York to California and from Michigan to Florida, and have succeeded in isolating *B. alvei* from all of them. It is true that some of the cultures show certain differences, but they have not been sufficiently pronounced to constitute even a well marked variety of the species. The pathogenicity of the bacillus no doubt varies in different countries; of that we have abundant evidence, and the possible explanation is given by Bertrand, who thinks that where bees have been kept for many years the disease has existed for a long time and remains in an endemic state; but there has been produced in these countries a race of bees which have acquired a relative immunity, which considerably diminishes the effects of the disease, and enables apiculturists to treat it more easily. In new countries into which the disease has been introduced it rages with great virulence, and remedies giving good results in the older countries are worthless in the new. As an example of this statement, we have the different methods of treatment used in Canada and in Europe.

Bertrand (32) reports the disease as being present in every country in Europe. Benton (33) says that he has never met with the disease during the six years he has kept bees in the Orient. Della Rocca (7) described a terrible epidemic in the Levant in 1780. Bovill (34) says that he has never seen it in Cyprus. In Africa, Feuillebois (35) reports it in Algeria, and Bochatey (81) in Tunis. In Australia it is present in all the colonies, and especially so in New South Wales (36) and South Australia (37). Brickwell (38) reports that New Zealand is full of the disease.

The Organism.

Bacillus Alvei, Cheshire and W. Cheyne, 1885, from the larvæ of bees suffering from the disease known as foul brood, la loque (Fr.) and faul brut (Ger.).

Morphological Characteristics.—In form the organism is a slender bacillus, with ends slightly pointed and rounded. "In the larval juices it is about $\frac{1}{7,000}$ of an inch in length and $\frac{1}{20,500}$ in breadth. On agar the bacilli vary considerably in size, averaging $\frac{1}{7,260}$ inch, some as small as $\frac{1}{10,000}$ inch, and others as large as $\frac{1}{5,000}$ inch. When they have attained the latter size, division of the rod seems to begin. They are always somewhat pointed at their ends. Their average breadth is $\frac{1}{30,000}$ inch, ranging from $\frac{1}{35,000}$ to $\frac{1}{25,000}$ inch (23). Klamann (25) states that a clear space often appears in bacilli with pointed ends. From agar cultures 24 hours old, at 37° C., the bacilli average 4 μ in length and 1.0 μ in breadth. On gelatine cultures, grown at 22° C., they are somewhat shorter. They grow singly, but occasionally form chains of various length.

Stains.—With the ordinary aniline stains the bacilli colour rather badly—Eisenberg (39) and Klamann (25). The best stains are methelene blue and methyl violet. The bacilli accept Gram's stain, but the spores are not colored by it. I find the most satisfactory stain is methyl violet.

Capsule.—No capsule has been demonstrated by Welch's method.

Flagella.—The bacilli are actively motile and possess a single flagellum at one pole. The motility of the bacillus is quite pronounced in fresh cultures obtained from bouillon, agar and gelatine. The flagella stain by Pitfield's, Loefflers's and Van Ermegen's method.

Spore Formation.—Spores are formed by the bacillus, and are large

oval bodies averaging in length $\frac{1}{12,000}$ inch, and in breadth $\frac{1}{23,700}$ of an inch. On agar the spores are arranged in long rows, side by side, and are greater in diameter than the cells from which they are derived The earliest appearance of spore formation takes place in 41 hours, at 36° C. (Cheyne), but in some cases it is even sooner. The spores are formed in the centre of the rod, and the formation occurs as follows: The rod begins to swell and become spindle-shaped. Occasionally the swelling is more marked at one end than in the centre. The spindle-shape increases in size, and the centre of the swelling gradually ceases to take the stain. The capsule of the spore is apparently formed within the rod and is not merely the outer part of the rod. In three or four hours the rod is seen to have almost or completely disappeared, although parts of the faint outline of the ordinary bacillus may be noticed.

Germination of Spores — Under favourable conditions the beginning of the germination of the spores takes place in about three hours. The spore loses its oval shape, becomes elongated, and is soon seen to burst through the spore capsule. It then presents the appearance of a short rod, with a pale envelope embracing one end. The rod gradually leaves the spore capsule, and then goes on multiplying as a full grown bacillus. According to Eisenberg (39), the spores are decolorized by the tubercle bacilli stain, but preparations may be obtained by using the Ziehl-Neilsen stain and alcohol for decolorization. The spores also stain by the method of Neisser.

Polymorphism.—Variations in size and shape may be brought about by growth in acid media, or in media containing different sugars. These variations occur also in the same culture, subjected to exactly similar conditions of growth.

Involution Forms.—Abnormal forms are especially abundant when the bacillus is grown on blood serum; peculiar Y-like forms and clubbed shapes are of common occurrence, and relatively few spores are found.

Biological Characters.

Bouillon —"In meat infusion at the temperature of the body, they grow rapidly, causing muddiness and, after a few days, a slight but not tenacious scum" (23). In bouillon, with a reaction of + .08 (57), at 37° C., there is a slight turbidity in 14 hours, especially noticeable when the tube is shaken. In 24 hours, the liquid is uniformly turbid, with a very fine sediment. In 48 hours, the turbidity increases and a pellicle commences to form. Reaction of the culture at this time, +.07. After 96 hours the broth is clear, with a pellicle, white, rather massive, and somewhat tenacious. There is also much sediment. Reaction, after 10 days' growth,—neutral.

Glycerine Bouillon —Media with original reaction of +.08. At 37° C., the bouillon becomes slightly turbid in 12 hours, and quite turbid in 24, with a fine, whitish pellicle on surface, which does not extend to the sides of the tube. If the culture is shaken, the pellicle deposits in flaky masses. The reaction is +1.2. In 36 hours, the turbidity clears, leaving the media bright, with a smooth, thin, tenacious, and white pellicle on the surface. In many cases the pellicle becomes very wrinkled and greasy-looking. At the end of 8 days, the reaction is +2 2, and the bouillon is several shades darker in colour, but quite clear. The reaction after 14 day's growth is +4.2. At 22° C. the same changes occur but growth is slower. The bacilli are relatively less numerous than in bouillon and are slightly shorter and thicker.

Glucose Bouillon.—With a reaction of + 2.0, at 37° C., the broth is more turbid than plain bouillon after 14 hours' growth; and in 24 hours, the

sediment is heavy, and turbidity very marked, but no pellicle. In 48 hours. the media is opaque and cloudy, and the pellicle is beginning to form. In 96 hours the broth is less cloudy, but the sediment is heavier, and a white, thick pellicle is formed. It is often wrinkled, but not quite so much so as that on the glycerine broth. Reaction of broth after 10 days' growth, +4.6. The bacilli are occasionally clubbed and y-like forms may occur. They average 5 μ in length and may be slightly curved.

Lactose Bouillon.—With a reaction of +1.06, at 37° C., the growth resembles that of plain bouillon for the first 24 hours; but at the end of 48 hours, it is more turbid. In 96 hours, a tenacious pellicle forms, less massive than that on Glucose broth. Reaction after 10 days' growth, +2.4. The bacilli average 3.5 μ in length.

Saccharose Bouillon.—With a reaction of +1.0, at 37° C., the turbidity and sediment are heavier than any of the other bouillons. In 48 hours the broth is quite opaque and whitish looking. A heavy sediment is then present and pellicle formation is just beginning. In 96 hours, the cloudiness is about the same, but there is an increase of sediment and the pellicle is thin and membranous. Reaction of media after 10 days' growth, + 4.04. The bacilli average 5 μ in length.

Gelatine Plates.—At 22° C. in 24-36 hours, the colonies are small, round, oval, or lozenge-shaped, with peculiar projections or shoots from one end of the colony, giving it a pear-shaped, or tadpole-like appearance, according to the amount of development of the projection. In many cases, several of these outgrowths occur from different portions of the colony. By placing a cover glass on the surface of the gelatine and using objective 7, the bacilli may be seen moving around and around the colony and to and fro along the projections. At the end of 48 hours, the colonies are larger. Fine processes or projections are shooting out into the gelatine in all directions, forming peculiar figures in circles or club-line forms. "It is impossible," says Cheyne, "to give a proper idea of the appearance of the growth. The forms assumed are the most beautifully shaped I have ever seen; but they are very numerous, always retaining the tendency to form curves and circles." After a time the gelatine is liquefied and the beautiful appearance of the colony is destroyed by the liquefaction of the gelatine.

These peculiar shaped colonies are most typical when the germ is taken from the diseased larvæ. After prolonged cultivation on various kinds of media, there is a tendency for the colonies to become round, and the peculiar branching forms are not seen in such numbers. The composition of the gelatine also seems to make a difference in the appearance of the colonies. In gelatine containing 12 per cent. gelatine the processes are not so long. The same effect may be brought about by using more peptone in the composition of the media.

Gelatine Tubes.—In stick cultures at 20° C. growth occurs all along the line of puncture. On the surface, delicate branching or ramifying growth occurs in three days. These outgrowths soon run together and the gelatine is liquefied, first around the line of puncture, and in 5 days extends over the whole surface. The growth in the depth of the gelatine occurs as a whitish streak all along the needle track; and from this, numerous shoots and growths branch out into the gelatine in all directions, giving a haziness to the appearance of the gelatine, which then begins to liquefy. If the inoculation is a heavy one, the shoots are coarse and may have club-shaped extremities, and from these swollen ends fresh shoots may start. Cheyne obtained the most characteristic growth in gelatine containing 3 per cent. of peptone, as well

as 10 per cent. gelatine. The whole tube is liquefied in from 2-4 weeks' growth. The liquid becomes yellowish in color and gives off a peculiar odor. Klamaunn states that in gelatine acidified with lactic acid the growth is slow and long threads are formed.

Gelatine Streak Cultures.—In gelatine streak cultures the appearance is very similar to what one sees in stick cultures. The bacilli first grow along the line of inoculation; and then throw out shoots into the surrounding gelatine, producing the appearance noted in the stick culture. The bacilli move to and fro along the channels of liquefied gelatine.

Agar plates.—On agar plates at 37° C., the colonies at the end of eight hours are small and burr-like, with spines protruding in all directions, giving the colony the appearance of a sea-urchin. In some cases the projections are from one side or end. At the end of 12 hours, the colonies have well-defined projections, visible to the naked eye. The colonies in the depths of the agar are more spiny, the processes being much shorter. On agar plates streaked with a light inoculation, most beautiful forms occur. The growth of the bacilli spreads over the surface and branches repeatedly, giving the appearance of seaweed. This appearance is distinctively characteristic; and as the growth is very rapid, this method commends itself for making a quick diagnosis of the presence of the bacillus in larvæ supposed to be diseased.

Potato cultures.—On potatoes the growth differs considerably, according to the reaction and age of the potato. Sometimes a brownish wrinkled growth forms, which gives off a peculiar odor; at other times a dryish yellow layer appears. "The bacilli grow very slowly indeed at 20° C." (Cheyne 23.) Even at 37° C. they grow slowly.

Milk.—In milk at 37° C., coagulation of the casein occurs in three days. The milk becomes yellowish and gives off a characteristic odor. After several week's growth, the curd is digested and a whey-like fluid remains.

Blood serum.—On blood serum at 37° C, the growth is rather slow and polymorphic forms are common. "Very long filaments are formed" (23). These long forms may be from 5 to 10 times as long as the average bacillus growing on gelatine, and consist of single cells. The filaments are often wavy or twisted and of unequal thickness. The extremities of the long, bent rods are often clubbed; and y-forms are numerous. Spores are formed very sparingly, and the blood serum is liquefied.

Synthetic media (Uschinsky).—In Uschinsky's medium no growth occurs; but if the medium is neutralized, good growth ensues. The bacilli occur in threads and a pellicle is formed.

Dunham's Solution. The bacilli are small when grown in this solution; No threads form; but there is a slight indol reaction after nine days' growth.

Relation to Free Oxygen. Cheyne states that the germs grow most rapidly on the surface of agar and arrange themselves side by side; and they produce spores in this position after a few days' growth. Eisenberg (39) says nothing under the head of aerobiosis. Howard (40) writes that, "It grows best under anaerobic conditions; is a facultative aerobe; grows under the mica plate; and in the presence of oxygen the growth is slight and slow." Howard also states that under anaerobic conditions it emits a foul odour resembling that of foul brood. It will be thus seen that Cheyne and Howard do not agree on this point. The former author also says that the characteristic odour is given off under aerobic conditions, whilst Howard states that this smell is emitted under anaerobic conditions. Further, Cheyne states that the bacilli grow with great rapidity on the surface of agar, whereas

Howard obtains his best growth under the mica plate, which does not give complete anaerobiosis. Howard's conclusions are thus at variance with Cheyne's, and my own results fully corroborate those of the latter author.

Howard states that the vitality of the spores of *B. alvei* is destroyed when exposed to atmospheric air from 24 to 36 hours. In making his experiments he took sterilized road dust and mixed it with the dry foul brood masses from several cells which were previously dissolved is distilled water. The mixture was worked dry, and spread on sheets of paper, and trial cultures were made immediately and at intervals of every twelve hours for three days; and according to his results no growth occurred after 36 hours In giving these results, Howard does not state whether he exposed the spores to sunlight or diffused light; nor does he mention the age of the dry foul brood masses, which he used from several cells. These are points of considerable importance, for as everyone knows the disinfecting power of direct sunlight is much greater than diffused light, and the vitality of the spores from foul brood masses of different ages varies considerably. This, I may add, has been clearly shown by some of my experiments, subsequently described. In my experiments, the spores obtained from a pure culture on the surface of agar, were spread on cover glasses and placed in a glass chamber, so arranged that a current of air was constantly circulating over them. This chamber was exposed to the ordinary light of a room with six large windows, and a cover glass was taken out every 24 hours and tested, to see if the spores would grow. This experiment was continued for one month and at the end of that time the spores still germinated rapidly. In another experiment, spores spread on cover glasses were exposed to a very diffused light, simulating as far as possible the amount of light which would enter a hive. Cover glasses were taken out from time to time and transferred to agar, in order to ascertain if the spores were alive or not. The experiment was begun two years and four months ago and from the last cover glass taken and placed upon the surface of an agar plate a copious and typical growth of *B. alvei* was obtained. Further, thin strips of filter paper, plunged into a bouillon culture and allowed to dry, were threaded on a wire suspended in a wire basket and so exposed that the air could freely circulate around them in the ordinary light of a room. Trial cultures were made at intervals, and at the expiration of 6 months the spores from the paper germinated when the strips were placed on the surface of agar.

Again, a drop of bouillon containing spores was placed in a sterile tube and allowed to dry; and at the expiration of 124 hours (36 of which were in sunlight at a temperature varying from 30°-37° C) sterile bouillon was added. The tubes were then placed in the incubator, and in less than 24 hours a good growth of the germs had taken place.

From these experiments it will be seen that the results are directly at variance with Howard's statement, as they go to show that the vitality of the spores of *B. alvei* is not destroyed by exposure to atmospheric air, with or without sunlight, for even a much longer time than 24-36 hours.

With regard to the aerobiosis of this bacillus, good growth has been obtained in an atmosphere of hydrogen by Novy's method. Buchner's method also gave good results. The growths in the various media are very similar to those produced under aerobic conditions, but with this difference, that the surface growths are, as a rule, whiter in the hydrogen atmosphere. In illuminating gas (water gas) no growth occured; but the spores were not destroyed by the action of the gas; for when the gas was let out of the Novy jar, good growth ensued on all cultures. In acetylene gas, a restricted

growth occurred. In fermentation tubes, growth occurred both in the open and in the closed arm of the tubes. No gas was formed, the bouillon in the closed arm was uniformly turbid. Thus *B. alvei* is a facultative anaerobe.

Production of Alkali In ordinary bouillon a slight amount of ammonia is formed. Control bouillon did not give the Nessler test. In glycerine and the sugar bouillons, there is no trace of ammonia. Cheyne's cultures are faintly alkaline, both before and after inoculation in meat infusion. Klamann states that ammonia is produced.

Acids formed A varying amount of acid is formed. All the sugar bouillons give an acid reacton.

Formation of Pigment. On potatoes, a yellowish growth is produced; on all other media, the surface growth is white.

Development of odours Cheyne states that gelatine cultures give off an odour of stale, but not ammoniacal urine, or what may be better described as a shrimpy smell; and this peculiar odour has been formed by Cheshire to be distinctive of diseased larvae. Klamann and Howard both state that a peculiar odour resembling that of the diseased larvae may be noticed in artificial cultures.

The Effects of Desiccation. I have already noticed, under the head of "Relation to Free Oxygen," that the spores of *B. alvei* have considerable vitality in withstanding desiccation. My experiments prove conclusively that the spores are extremely hard to kill by desiccation and in this respect resemble those of authrax, which are known to resist thorough desiccation for a number of years. One experiment which shewed this characteristic was as follows: An agar plate completely covered with a typical growth of *B. alvei* was allowed to dry out completely, and was left exposed to the ordinary light of the room for 7 months, and at the end of that time, a portion of the film was scraped off with a knife, placed on suitable medium and incubated, with the result that a typical growth immediately ensued.

Spores on cover glasses were exposed to September sunlight (Latitude 43) for varying periods of time, and growth occurred after 4, 6 and 7 hour's exposure. The age of the spores varied from 5 days to 18 months; and spores 3 months old were not killed by 7 hours' exposure.

Thermal Relations.

Maximum for Growth. The maximum for growth is about 47°C. At 44°C., good growth occurs; but at 50°C., growth ceases. Experiments on maximum for growth were performed on germs isolated from a number of different places, and little or no difference was noticed in their behaviour when incubated at the temperatures mentioned.

Optimum for Growth. The optimum for growth is about 37.5°C. for all media except gelatine. This has been determined by Cheyne & Eisenberg (39). On gelatine the best results are, of course, obtained from higher temperatures; but as 10% gelatine melts at about 24°C., 22°C cannot be exceeded.

Minimum for Growth. Cheyne says that the bacilli do not grow below 16°C. I have, however, occasionally obtained growth at 14°C. on the surface of agar; but it has been extremely slow. The spores will not germinate at this temperature. No difference, under this head, is apparent in germs obtained from different countries.

Thermal Death Point. This is a very important matter, because in the heating of wax and honey from colonies suffering with foul brood, it is necessary to know the temperature that will destroy spores and thus prevent the infection of other bees; and unfortunately a considerable discrepancy exists

in the results of experiments to determine the thermal death point of the bacillus, accounted for in part by the different methods used by different investigators.

McKenzie (28) found the thermal death point by suspending silk threads saturated in a beef broth culture of *B. alvei* containing spores. The threads were allowed to dry, and introduced into melted wax, and left therein for a definite time, at a fixed temperature. At the end of that time, the thread was introduced into melted agar and thoroughly shaken so as to separate the wax from the threads. The cultures thus made were rapidly cooled, and the tubes placed in the incubator at 37°C. The following are his results :

At 100°C. for	¼	of an hour,	growth.
" "	½	" "	growth.
" "	1	hour,	growth.
" "	1½	hours,	growth.
" "	2	"	growth.
" "	3½	"	no growth.

At 90°C. for	½	hour,	growth.
" "	1	"	growth.
" "	2	hours,	growth.
" "	3	"	no growth.
" "	4	"	no growth.

A temperature of 50°C. did not destroy the spores in 24 hours. These experiments were repeated with the same results, which results were criticised by Corneil (28), who claimed that the heat to which the bacteria were exposed in melted wax was not moist but dry heat, and consequently that the wax had to be heated to a high temperature and for a long time in order to destroy the spores. According to the testimony of two prominent foundation makers, the wax during the refining and purifying process reaches a temperature of quite or nearly 100°C. for a short time. During the sheeting, however, it does not reach a temperature much above the melting point, say 79°C. Two other foundation makers, Dadant & Hunt (41), state that, in refining, the wax is heated for some time to 100°C., and is kept liquid for 24 hours; so McKenzie thinks that if these temperatures are reached in the making, there is little danger of foul brood from comb foundation, as the specific gravity of bacteria in the melted wax is so great that throughout the process of manufacture the bacteria tend to fall to the bottom. Sternberg (42) states that the spores require for their destruction a temperature of 100°C. for four minutes (determined in 1887); but there is no statement as to the age of the spores. In Howard's experiments (40) tubes of liquid gelatine containing spores of *B. alvei* were placed in an open vessel of boiling water and allowed to remain therein for a definite time—" in all probability the water did not reach boiling point "—and trial cultures were made at stated intervals, with the following results :

After	15	minutes—	growth.
"	30	"	"
"	45	"	"
"	50	"	no growth.
"	60	"	"

His trial cultures were on potato and gelatine ; but no statement is made regarding the age of the spores, where they were from, or the temperature at which they were incubated. It is, however, evident that they were not given the most favourable conditions for growth.

I have myself performed the following experiments on the thermal death point of the spores :

Method. Test tubes containing bouillon were placed in boiling water. Three loopfuls of culture were introduced into each of the tubes ; and tubes,

withdrawn from the boiling water at stated intervals, were cooled and incubated.

Results 1. Spores from a seven months old culture in bouillon were killed at a temperature of 100° in 1 hour and 20 minutes.

2. Spores from a $2\frac{1}{2}$ months old culture on agar were killed in two hours and a half.

3. Spores from agar nine days old,—slight growth after 2 hours and 45 minutes ; no growth after three hours.

4. Spores 14 days old and 21 days old,—in each case after two hours boiling, one of the duplicate tubes formed a growth ; another after $2\frac{1}{2}$ hours, whilst the remainder had no growth. All were killed in 3 hours.

I used also fine capillary glass tubes. A suspension of the spores in water was drawn up into sterile tubes, which were then sealed at both ends, The tubes were placed in boiling water and withdrawn at stated intervals. The contents of the tubes were then introduced into agar, which was incubated at 37°C. ; and great care was taken to have a suspension of the spores by filtering them through glass wool.

The results were : With a temperature of 98°C. (about the boiling point in this locality), spores from a 7 days' old culture on agar were killed in $2\frac{3}{4}$ hours ; and spores from agar 9 days old were killed in 3 hours.

Another experiment was made to determine the thermal death point in honey. The honey was of two kinds, clover and buckwheat. The former had a specific gravity of 1.042 at 60°C. and contained 0.057% of formic acid, while the latter had a specific gravity of 1.040 at 60°C. and contained 0.170% of formic acid. The spores used were from agar three weeks old, and three methods were followed :

1. Silk threads with dry spores thereon ; 2. Test tubes containing honey with a heavy inoculation of spores ; 3. Capillary tubes containing a suspension of spores in distilled water. The spores used were not filtered through sterile glass wool, as it seemed desirable to imitate as far as possible the conditions met with in infected honey. The following are the results :

1. *Silk threads with dried spores, from an agar culture two weeks old.*

Time.	Temperature.	Result.
15 minutes.....	115°C.	growth.
30 "	113 "	"
45 "	115 "	"
60 "	113 "	"
1 hour 15 minutes ...	114 "	"
1 " 30 " ..	115 "	"
1 " 45 " ..	115 "	"
2 hours	114 "	"
2 " 15 minutes...	116 "	"
2 " 30 " ..	115 "	"
2 " 46 " ..	115 "	no growth.

2. *Tubes containing honey and spores mixed together.*

30 minutes.....	115°C.	growth.
45 "	114 "	"
60 "	114 "	"
1 hour 15 minutes ...	114 "	"
1 " 30 " ..	114 "	"
1 " 45 " ..	115 "	"

2 hours	115°C	.....growth.
2 " 15 minutes ...	116 "	..... "
2 " 30 " ..	115 "	.....no growth.
2 " 45 " ..	115 "	..... "

3. *Capillary tubes with spores in distilled water.*

30 minutes.....	114°C.	..... growth.
1 hour	114 "	..... "
1 " 30 minutes....	114 "	..... "
2 hours	114 "	..... "
2 " 15 minutes ...	115 "	..... "
2 " 30 " ..	115 "	..... "
2 " 45 " ..	115 "	.....no growth.

The temperatures were taken in a large vessel containing 10 pounds of boiling honey. The experiment was repeated, using buckwheat honey instead of clover and with like results.

Relation to Light. A few experiments were made to ascertain the behaviour of spores toward light. Coverglasses spread with spores and dried, were exposed to bright sunlight during the month of February. The exposure was in the open air and the glasses were on black tile. The temperature varied from—12° C. to—22° C After exposure, the glasses were placed film side downwards on agar plates, and then incubated at 37° C.

	Time.	Result.
Results—3 hours sunlight.		Abundant growth in 16 hours
6 " "		" " " "
9 " "		" " " "

These experiments were repeated in September, when the outside temperature varied from 24° to 30° C., with the result, that there was growth after 4, 6, and 7 hours' exposure.

Agar plates exposed after inoculation showed great differences. For instances, spores 21 days old was killed by 5 hours' exposure, whilst plates made the day after with spores 2 months and 21 days old, required 7 hours' exposure. Spores 10 days old showed no growth after 5 hours' exposure; and spores 5 days old, no growth after 6 hours' exposure. From a large number of determinations, the average length of exposure necessary to kill spores within the above range of temperature was found to be 5 hours.

Vitality on various media. The cultures seem to live longer on agar than in liquid media. The vitality of old gelatine and bouillon cultures seems to be lessened by the products of the bacilli growing in these media. The spores taken from these sources have also decreased resisting power.

Effect of growth on reaction of media. Ordinary bouillon becomes slightly more alkaline as growth proceeds, the presence of ammonia being detected by Nessler's reagent; but control bouillon does not give the reaction. In bouillon, with the addition of glycerine and various sugars, the acidity of the media is increased, but more in the case of glucose broth than in any other. In these experiments accurate titration was made with phenolphtalein as indicator. Cheyne tried the reaction, "making the infusions faintly alkaline, and after the growth of this organism in it, it is faintly alkaline."

Sensitiveness to Antiseptics and Germicides. This subject is taken up in connection with the chemical remedies used for the disease.

Pathogenesis. Besides being pathogenic to the larvae of bees, Cheyne has inoculated two mice and one rabbit with spore-bearing cultivations with-

out effect. "Half a syringeful of a spore-bearing cultivation injected into the dorsal subcutaneous tissue of each of two mice resulted in the death of one of them in 23 hours, while the other seemed unaffected. In the case of the mouse which died, the seat of injection and the neighbouring cellular tissue was found to be very œdematous; but no microscopic changes were apparent in the internal organs. Numerous bacilli were found in the the œdematous liquid, as also a number of spores which had sprouted; and there were likewise a few bacilli in the blood taken from the heart. This was proved by cultivation as well as by microscopic examination. On examining sections of various organs no morbid changes were found, and only a few bacilli were seen in the blood vessels. A syringeful of the same culture was injected into a guinea pig; and the animal died 6 days later, with extensive necrosis of the muscular tissue and skin; and cheesy looking patches were distributed through it, but there was no true pus. On making sections of the necrosed tissue, numerous bacilli, apparently *B. alvei*, were seen; but there were also other bacilli and micrococci. No micro-organisms were seen in the internal organs. It thus remains questionable whether the necrosis was due to *B. alvei* or not, more especially as I have since injected three guinea pigs subcutaneously with spore bearing cultivation, but without effect.

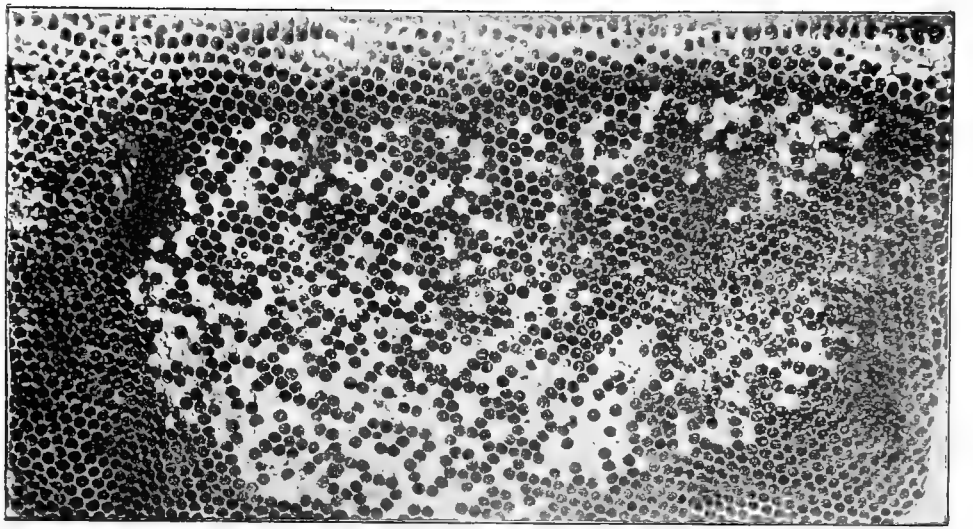
"The effect of feeding flies with material containing spores results in death of the flies, and bacilli were found in its juices as shewn by the microscopic examination and cultivation. Cockroaches were not killed" (28).

Fly blow larvae fed for three days on spores were not killed. With regard to the prevalence of the disease amongst wild bees, very little can be found on this subject in bee literature, but a correspondent of the *British Bee Journal* (43) found the disease among wild bee larvae in a tree, recognising it by the smell from the entrance and also from the appearance of the brood in the combs. The correspondent remarks that this tree had probably in former years been the cause of a great deal of trouble to neighbouring bee keepers. In all probability the disease is present among the various varieties of wild bees and wasps. Knight (54) mentions an epedemic among wasps in 1807; Kirby & Spence (55) another in 1815; and Bevan (13) one in 1824; but in none of these cases was any positive evidence given to show the epidemic was foul brood.

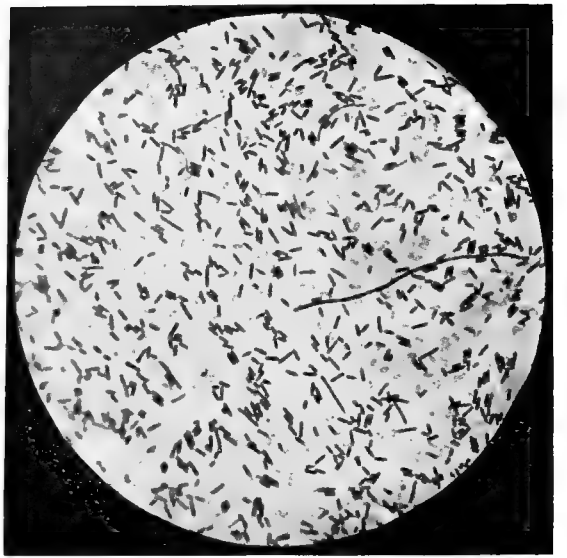
Economic Aspects.

Losses. Della Rocca (loc. cit.) in 1790 stated that the whole of the bees in the Island of Syra were carried away during 1777 to 1780 by the disease. Dzierzon (46) relates his losses from the disease. In 1868 he lost his entire apairy of 500 colonies from it. In Switzerland, the disease, at times, is extremely bad. Bertrand's apiaries have suffered severely, and the German papers make constant reference to its devastation. In England, Cowan (4) thinks that the "only visible hindrance to the rapid expansion of the bee industry is the prevalence of this pestilential disease which is so rapidly spreading over the country as to make bee-keeping a hazardous occupation"; and again, (47) "So rapidly has foul brood spread by contagion that in one season, unless precautions are taken, a whole neighborhood may become seriously infected, and the chances of successful beekeeping seriously imperilled, if not utterly destroyed.

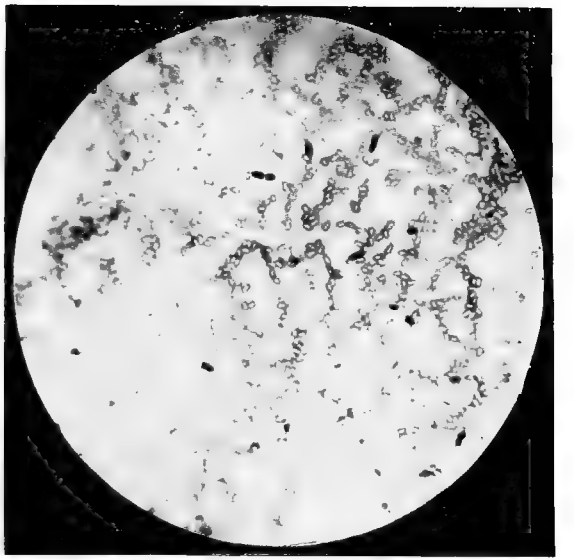
The committee on the Beekeeping Industry and Foul Brood in the United Kingdom, report that the destruction of stock by foul brood and the



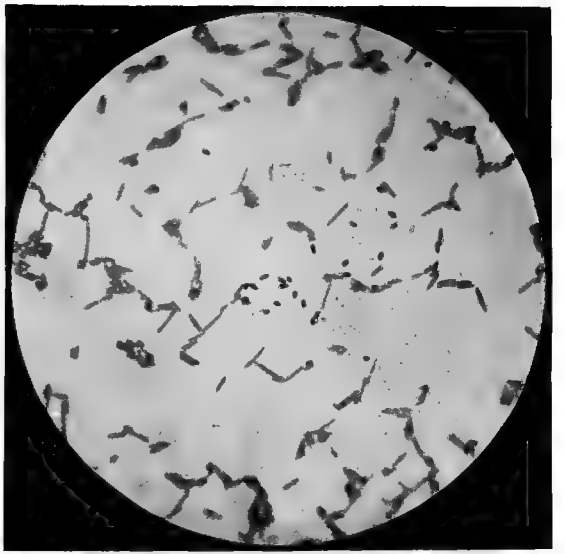
A diseased comb, (after N. E. France), showing sunken and darkened cappings. Also many cells with holes in the cappings.



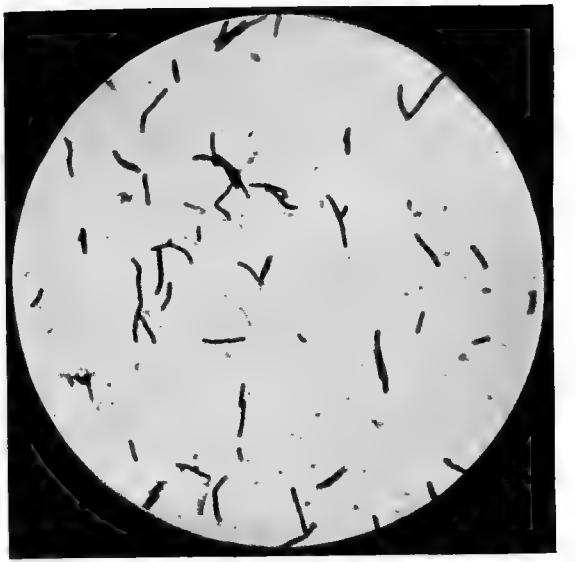
B. alvei and spores x 1000, from gelatine 7 days old at 20° C., stained with methyl violet. (Original.)



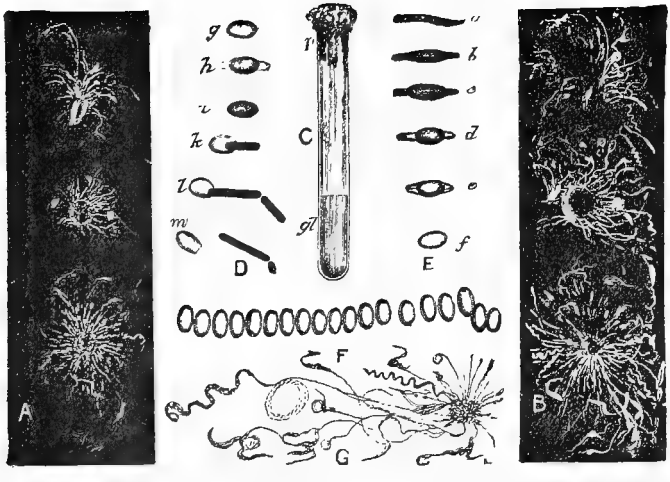
Spores of *B. alvei* x 1000, from agar 3 weeks old at 37° C., stained with methyl violet. (Original.)



B. alvei and spores x 1000, from agar 10 days at 37° C., stained by Moeller's method. (Original.)



B. alvei x 1000, from blood serum 7 days old at 37° C., stained with methyl violet. (Original.)



Cultures of *B. alvei* (after Cheyne). A. Colonies on the surface of gelatine (6 diameters). B. The same colonies 24 hours later. C. Culture tube; gl. gelatine; p. cotton wool plug. D. Spore becoming bacillus (1800 diameters.) E. Bacillus becoming a spore. F. Spores in line, taken from a gelatine culture. G. Colony developing.

discouragement arising therefrom is one of the two influences that retard the development of the bee industry.

In the United States, serious harm has been done, but no definite statistics can be cited. The disease causes great losses and several States have enacted laws for the prevention of the disease, making it a legal offence for a person to keep in his apiary a colony of bees affected with foul brood.

In Canada, the Ontario Foul Brood Inspector (56) reports in the years 1890 1892 inclusive, 622 apiaries inspected and 2,395 cases; in the years 1893-1898, 527 apiaries inspected and the disease present in 212, or about 40 per cent.

In New Zealand and Australia, the disease is looked upon as being very wide spread. It will thus be seen that wherever bees are kept, serious losses are caused annually by this disease.

Natural method of Infection. With regard to the natural methods of infection, a good deal depends on the natural predisposition of the bees to disease and the state of health of the colony. Weak, sickly, or badly nourished bees are as a rule the most susceptible. We must also remember that germs themselves vary in their ability to produce disease. As in diphtheria, we may get a light or severe type of the disease; so also in foul brood, we may have a light or a severe attack; but the facts demonstrating the variability of this capacity are not well known; I have, however, noticed that after prolonged cultivation of *B alvei* in which more than 30 transfers have been made, and the bacteria with spores have been given to bees in syrup, the virulence of the germ has seemed to be considerably impaired. In one case the colony experimented with was rather weak, was confined to the hive all day, and allowed flight only in the evening, and the spores were given in large quantities in syrup every day, nevertheless it was several weeks before the disease established itself, and then only in a light form So we may have mild or severe epidemics and the liability to take the disease may be increased by chilling the bees or otherwise unfavourably modifying their metabolism; and in all such cases, they succumb more easily to the disease than when in a normal, healthy condition.

With regard to the manner in which the disease is carried from hive to hive, Chesbire (26) thinks that the larvae are most usually affected by the antennae of the nurse bees, and also that the tramp of the bees frequently detaches numbers of spores, which fly about in the air and settle here and there, often where they take effect. I think that in comparison with other diseases which are air borne there is usually not very much danger from this cause in the case of *B. alvei.* The spores are generally found in very sticky surroundings, which, even if dry, serves to fix and keep them *in situ.* Chesshire also states that he has not found the bacillus from honey or pollen in infected hives. This statement, however, is directly contradicted by the experience of practical bee-keepers and others. I have myself repeatedly found *B. alvei* in capped honey cells, and in the pollen masses found in diseased hives, the examination in the former case having been made by removing the capping with sterilized forceps and plunging a heated platinum needle into it and then putting the needle into melted agar, from which plates were poured, cooled and incubated.

Probably the chief method of carrying the disease from one hive to another is by the bees from healthy hives robbing colonies that have become weak and diseased. In such cases the robbers carry with them the germs of the disease. There is likely nothing to be feared from using wax foundation from the regular makers; for, as we have already stated, the wax, in the

process of making, is subjected to a temperature sufficiently high to kill any spores that may be present.

I may add that I found spores of *B. alvei* in two samples of wax sent me by R. F. Holtermann of the *Canadian Bee Journal*, but both samples were from hives which were very badly infected with the disease.

In 1897, about ten pounds of wax was infected with large numbers of spores grown upon agar. The wax was cut up into small pieces, and heated at a low temperature, only just sufficient to melt it; and as McKenzie (28) had shown that the spores settled to the bottom, the wax was vigorously stirred from the time the spores were added until it had set again. The wax, thus infected, was sent to Holtermann for foundation-making. He manufactured it by the usual process of melting and gave the foundation made from it to bees, and no foul brood developed in the colony supplied with it during the years 1897 and 1898. The probability is that the spores are fixed in the wax, and are thus unable to infect the bees.

Healthy bees may pick up spores of *B. alvei* from flowers previously visited by diseased bees; wasps, which are noted robbers, may also carry the disease, and thus infect a locality.

The very large traffic in bees and bee-keeping supplies where agriculture is carried on, probably favors the spread of the disease. In fact, many instances are cited in bee journals of infection carried from one locality to another by the importation of bees and bee supplies.

Persons manipulating diseased hives and then examining healthy ones may be the means of spreading the disease. The practice of using a knife for cutting out diseased comb and then using the same knife for work amongst healthy comb (which I have seen done) is by no means wise, as the spores may thus be transferred from diseased to healthy hives. Cowan (4) observes that beekeepers who have not succeeded with their bees in consequence of foul brood have been known to sell by auction hives in which the bees have died. In such cases the purchasers are usually beginners who have no idea of the danger they are incurring.

Conditions favoring the spread of the Disease, Besides the weak or badly nourished condition in which bees may be, and lack of other hygenic conditions which favour the spread of this disease, great humidity in winter is said to be favourable and probably great heat is also conducive. (45.)

Predisposition of Varieties. No definite statements can be made as to the predisposition of various races to this disease. Quinby (49) says that black bees are more subject to foul brood than Italians. Aspinall (51) also affirms that common bees are more liable to the disease than Italians, but de Layens (47) states that Italians are more easily infected than black bees. (See also page 17.)

Remedies.

Three remedies have been tried:

1. Stamping out.
2. Starvation.
3. Treatment by chemicals: (a) by feeding chemicals in food; (b) by putting certain chemical substances into the hive and allowing them to evaporate at the temperature of the hive. This latter method may be regarded as rather preventitive than curative.

1. *Stamping out Method.* By the stamping out method all affected bees, combs and frames are destroyed, and the hives thoroughly disinfected. Cowan (4) thinks that if foul brood were under government inspection, and all cases promptly dealt with by destruction, the disease could be stamped out. The British Bee-Keepers' Association has asked the Board of Agriculture to secure legislation on this line, because it thinks that in this way the trouble would be removed and the industry would receive an impetus which would benefit bee-keepers, farmers and fruit growers.

The earliest advocate of this system was Della Rocca (18), who maintained "in extreme cases that it was necessary to burn everything without pity, as there was no other resource." Since Della Rocca's time, this method has been frequently resorted to in severe cases that would not yield to treatment either by starvation or by the use of chemicals; but to have any lasting effect, it would have to be universally carried out, and would involve the difficult question of compensation.

2. *Starvation Methods.* The starvation method was first proposed by Schirach (3) who advised that the combs be removed and bees allowed to fast during two days, and then be placed upon clean new comb, and fed on a syrup prepared with a little hot water mixed with honey, nutmeg and saffron.

Since Schirach's time different modifications of this method have been made, and it has been largely used in the United States and Canada, whilst in Europe treatment by medicated syrups has been more in vogue. In 1879 L. C. Root (58) gave his approval to this method, but he advised that the bees be confined in a cool, dark place for 24 hours, in order that all the honey which they carried with them might be consumed, and that the bees be then put into a hive filled with healthy comb or foundation and the condemned hive scalded with boiling water and thoroughly scraped. At a later date McEvoy (44), the Ontario Provincial foul brood inspector, introduced another modification and has himself described his method as follows: "In the "honey season, when the bees are gathering freely, remove the combs in the "evening and shake the bees into their own hives; give them frames with "comb foundation starters on and let them build comb for four days. The "bees will make the starters into comb during the four days and store the "diseased honey in them, which they took with them from the old comb. "Then in the evening of the fourth day take out the new combs and give "them comb foundation to work out, and then the cure will be complete. "By this method of treatment all the diseased honey is removed from the "bees before the full sheets of foundation are worked out. All the old foul "brood combs must be burned or made into wax after they are removed "from the hives, and all the new combs made out of the starters during the "four days must be burned or made into wax, on account of the diseased "honey that would be stored in them.

"All the curing or treating of diseased colonies should be done in the "evening, so as not to have any robbing done or cause any of the bees from "the diseased colonies to mix and go with bees of sound colonies. By doing "all the work in the evening it gives the bees a chance to settle down nicely "before morning and then there is no confusion or trouble.

"This same method of curing colonies of foul brood can be carried on at "any time from May to October, when the bees are not gathering any honey "by feeding plenty of maple syrup in the evenings to take the place of the "honey flow.

"It will set the bees robbing and spread the disease to work with foul "broody colonies in warm days, when bees are not gathering honey, and for that "reason all work must be done in the evenings, when no bees are flying.

"Where the diseased colonies are weak in bees, put the bees in two, "three or four together, so as to get a good sized swarm to start the cure with, "as it does not pay to spend time fussing with little weak colonies.

"When the bees are not gathering honey, any apiary can be cured of "foul brood by removing the diseased combs in the evening, and giving the "bees frames with comb foundation starters on. Then, also, in the evening "feed the bees plenty of sugar syrup, and they will draw out the foundation "and store the diseased honey which they took with them from the old combs; "in the fourth evening remove the new combs made out of the starters and "give the bees full sheets of comb foundation and feed plenty of sugar syrup "each evening until every colony is in first-class order.

"Make the syrup out of granulated sugar and put one pound of water to "every two pounds of sugar, and then bring it to a boil As previously "stated, all the old combs must be burned or made into wax when removed "from the hives, and so must all the new combs made during the four days.

"The empty hives that had foul brood in them do not need any disin-"fectant in any way. I have handled many hundreds of colonies in the Pro-"vince of Ontario and cured them of foul brood without getting a single hive "scalded or disinfected in any way, and these colonies are cured right in the "same old hives."

McEvoy positively states that "No colony can be cured of foul brood by "the use of any drug. All the old combs must be removed from every dis-"eased colony and the hive got away from the bees before brood rearing is "commenced in the new clean combs."

Howard (40) is most emphatically opposed to the drug treatment. "I regard," says he, "the use of any and all drugs in the treatment of foul brood as a useless waste of time and material, wholly ineffectual, inviting ruin and total loss of bees. Any method which has not for its object the entire removal of all infectious material beyond the reach of both bees and brood will prove detrimental and destructive and surely encourage the recurrence of the disease."

A. I. Root (45) says that "The starvation plan in connection with burning the combs and frames and boiling the hives has worked best in treating foul brood. It never reappeared after such treatment, though it did in all cases where the hives were not boiled, thus confirming the theory or fact of spores."

These two authors, therefore, go further than McEvoy in both advising the disinfection of the hives.

McEvoy (56), however, admits that his method as described above cannot be used for every case. His reports frequently refer to burned colonies; and he acknowledges that his method does not always cure. In 1890 he used the expression, "600 cases of foul brood and over 360 cured"; and again in a subsequent report, after mentioning the number of cases, he added the words, "*mostly cured*"

In a personal communication, M. Bertrand of Nyon, Switzerland, states that he does not believe in and will not recommend in his periodical (*Revue Internationale d' Apiculture*) the starvation method as used in America.

3. Treatment by Chemicals —In the treatment of bees by chemicals, we assume that such substances as are used are employed as antiseptics, and that their efficiency is due to the fact that they destroy the bacillus or prevent the germination of the spores, and thus bring about an internal disinfection; but we must remember that many of the substances used are more poisonous in their effects upon the cells of the bee than upon *B. alvei*. As is well known, quinine is frequently used as a specific for malaria; and in such cases the

cure is effected by the intervention of the body cell. The effectiveness of the remedy is due to the fact that it acts as a stimulus and exalts the natural forces of the body.

Whether the drugs used in the treatment of foul brood act antiseptically or by stimulating the cells of the bee and making them more active to ward off the disease, is a matter of doubt; but it must be admitted that certain drugs do seem to effect a cure, and some of them are regarded as specifics by practical beekeepers.

In taking up the different methods of chemical treatment, I shall as far as possible describe them in the chronological order.

(1) *Carbolic acid.* Carbolic acid was first proposed by Butlerow (52), who recommended one part of acid to 600 of syrup, this proportion being the limit in which one can give the remedy to bees. Cech (53) in a work published in 1877, also recommended carbolic acid

The Cheshire treatment (26) consists in using a treatment containing half a decilitre of carbolic acid in a litre of water, thoroughly shaking it up until the acid is entirely dissolved, and using half a decilitre of this in a litre of syrup. In this treatment it is also necessary to reduce the infected stock to the number of frames it can use, and if the queen is diseased to destroy her and substitute a healthy one. The syrup is given by pouring it into the empty cells of the brood nest.

This method of treatment has been frequently reported to be successful; but there have been many failures, perhaps partly owing to the fact that it is difficult to get the bees to take the medicated syrup

Experiments on the Antiseptic Value of Carbolic Acid According to McKenzie (28), two per cent. carbolic acid does not kill the spores in six days. One per five hundred of the acid prevented the germination of the spores, but when taken out of the solution and placed in ordinary beef broth it gave luxuriant growth. Hence McKenzie thinks that the explanation of the value of carbolated syrup in the treatment of foul brood consists in preventing the germination of the spores. The bee journals refer to numerous instances in which feeding carbolated syrup produced an improvement in diseased stock; but as soon as the treatment stopped, the disease broke out afresh.

Salycylic Acid. The salycylic acid treatment was first used by Hilbert in 1876. The following is the method of use:

Solution of Hilbert No 1—Pure salycylic acid, 12½ grams; alcohol, 100 grams.

Solution of Hilbert No. 2—200 drops of solution No. 1 (about five grams) in 200 grams of distilled water or rain water.

Fumigation—One or two grams of the pure acid for fumigation.

Syrup—From 200 to 240 drops of Solution No. 1 (or about 5 to 6 grams) in a litre of syrup, well mixed before the syrup cools.

As soon as the disease is noticed the hive is disinfected and the syrup fed; and this treatment is also used for other colonies as a preventive treatment. The fumigation is accomplished in a kind of tin lantern furnished with a small alcohol lamp, suspended over which is a small movable trough for placing the acid in. The flame of the lamp is regulated in such a manner that the acid is liquified and slowly evaporated without burning. Too great heat will decompose it and render it ineffective. The fumes of the acid spread through the hive in the form of a white vapour. Whilst the fumigation is in progress the entrance boards and all parts that can be disinfected are washed with No. 2 solution. Fumigation and washing are repeated every 4 or 5 days until a cure is effected. The diseased colonies receive,

every second evening, $\frac{1}{6}$ of a litre of acid syrup; and it is wise to give the same treatment to the neighbouring hives. A cure is usually effected in 3 or 4 weeks. If later, it is generally regarded as a sign that the queen is diseased, in which case it would be well to replace her. Occasionally the queens die during the treatment; but this is not frequent.

This treatment was very successful in diseased hives belonging to Bertrand (59). All the hives that were treated, were cured. Cowan (60), who has also used Hilbert's treatment with some slight modifications, has had the same success; and such is his confidence in the treatment that he does not fear to introduce into his apiary foul brood colonies for treatment. Some have found the treatment ineffective; but Bertrand thinks (59) that in all such cases there has been something lacking in the work, some precautions overlooked or neglected.

Experiments on the antiseptic value of salycylic acid. Salycylic acid agar was made containing 5 grams of $12\frac{1}{2}$ per cent. solution of salycylic acid in one litre of agar. Petrie plates were made from this and streaked on the surface with *B. alvei*. At the same time control cultures on ordinary agar were made. The results were abundant growth on the control plates and good growth, (but somewhat less than on the control plates) on the salycylic acid agar.

Salycylic acid Vapour. One gram of the acid was evaporated in our laboratory according to the directions given by Bertrand (59), in a box about the same size as a hive. Agar plates streaked with spores of *B. alvei* were left in different parts of the box during the fumigation for 10 minutes. The plates were then taken out, the covers put on and the plates incubated at 37°C. for 48 hours.

Results. Fumigated plates—no growth.
Control plates—abundant growth.

From these experiments it will be seen that the vapour of salycylic acid acts antiseptically, and that the feeding of the acid in the syrup, in the proportions specified, probably acts as a stimulant to the bees, enabling them to withstand or throw off the disease.

(3) *Camphor.* Ossipow (61) was the first to use camphor as a curative; and Bertrand (59) describes the use of it as follows: "There is," says he, "placed upon the bottom board of the hive, enveloped in a piece of muslin, "a piece of camphor about the size of a walnut, which is replaced when it has "evaporated. The presence of the camphor permits the bees to clean out the "cells containing dead larvæ and stop the development of the disease. So "long as a hive contains some of the substance foul brood will not develop, "at least according to our experience and to that of several other beekeepers. "The first thing to do then, when one doubts the state of health of a colony, "is to employ the Ossipow remedy before proceeding to more radical means. "One can administer camphor in food by dissolving it in its own weight of "alcohol."

Experiments on the antiseptic value of Camphor. Sloped agar in tubes was inoculated with one loopful of spores of *B. alvei*, and a crystal of camphor about the size of a large pea was dropped into the tube. The tubes were then capped with tin foil paper and kept at 22°C. and 37°C.; and control cultures were made at the same time. At 22°C., after two days, there was good growth in the camphor tube. At 37°C., after two days, compared with the control tube, the camphor tube showed slight restriction of growth, the extra heat having evaporated the camphor more quickly.

Another series was made by using agar Petri plates streaked with 2

loopfuls of spores. In each plate was placed a portion of camphor about the size of a large pea; and the plates were incubated at 37°C. In 24 hours there was good growth; but close to the lump of camphor, growth was slightly inhibited.

Thus, camphor in the quantity in which it might be kept in a hive has no antiseptic effect, the amount used in the experiments being far larger than would be used in a hive. This substance, therefore, if it has the effect mentioned by those who have used it, must act as a stimulant, strengthening the bees to overcome the disease.

(4) *Thyme.* Klempin (62) has used branches of dry thyme with success, burning them in the smoker for disinfecting his hives; but their effect, like that camphor, is not radical, and beekeepers are not all in accord as to their efficacy.

(5) In connection with thyme *thymol* may be mentioned. Zehetmayr (63) has recommended the use of thymol, and has made a little machine by which he steams the bees with this substance. If a little of it is placed in a hive it will prevent infection, because bees from uninfected hives will not come near it,—they object to the smell, until they become accustomed to it. Blow (63) thinks it very valuable, and Jones (65) remarks that, even in great dilution, it prevents the growth of the germ; but Cowan criticises its use, because it is disagreeable to bees, and if used in sufficient quantity, acts as a poison, and therefore cannot be good in food.

Experiments on the antiseptic value of Thymol. Crystals of thymol were placed in test tubes of sloped agar in our laboratory and inoculated with one loopful of spores of *B. alvei*. These were capped with tin foil paper and incubated at 22° and 37°C.

Result. Control tubes—abundant growth.
Thymol tube at 22°C.—slight growth.
" " 37°C.—very slight growth.

Agar plates, poured and streaked with two loopfuls of spores of *B. alvei*, were used in another experiment; and a piece of thymol the size of a large pea was placed in each plate. The plates were incubated at 37°, along with control plates, with the following results:

24 hours, control plate—abundant growth.
" thymol plates—good growth, but close to the lump, no growth.

Hence we conclude that this substance has a very slight antiseptic effect.

(6) *Carbolic Acid and Tar.* These substances were first used by Schreuter (66) and they are applied as follows:—"A piece of felt wool is placed in a small box, and soaked with a mixture of carbolic acid and Norwegan tar, in equal proportions The cover of the hive is slightly raised, in order to permit of the evaporation of the carbolic acid. The box is left upon the platform of the hive beneath the brood, and remains there permanently. The dose can be renewed as often as required. The addition of tar to the acid is for the purpose of making evaporation take place more slowly." This remedy has not been used to a very great extent. Borel (67) reports success with it; but others have not had the same results, and it is probable that it should be used only as a preventive.

Experiments on the antiseptic value of Carbolic Acid and Tar. Four drops of the mixture placed on blotting paper and inserted in a Petri dish containing agar streaked with spores, inhibited growth, from which we see that the mixture is antiseptic.

(7) *Creolin or Phenyle.* Creolin has been recommended by Cowan (68) and has been used with success by other apiculturists.

Recipes : Solution No 1—for sprinkling, disinfecting, etc.—half a teaspoonful of soluble creolin in a litre of water.

Solution No. 2 For washing hives, platforms, etc.—two teaspoonfuls of soluble creolin to a litre of water.

Solution No. 3—for feeding—a quarter of a teaspoonful of soluble creolin in a litre of syrup.

The water of the syrup ought always to be poured upon the top of the creolin and thoroughly mixed with it; and the mixture should be well shaken before using.

Use. Prepare a hive and a proper floor board, which has been washed with solution No. 2. Then, after having taken out the comb from the infected hive, shake off the bees, and sprinkle the comb with solution No. 1. Take out all superfluous comb and spray it with solution No. 2, and extract the honey from it. The honey can then be boiled, and if it is used for feeding the bees, it can be diluted and phenol added in the proportion of one quarter to a teaspoonful to a litre of the diluted honey. The combs are then put back and the bees fed with medicated syrup. If the bees take the syrup, the dose can be gradually increased; but we must be careful not to give more than one teaspoonful to a litre of syrup. If the bees refuse to touch it, which is not at all improbable, if they have access to other food, pour the medicated syrup upon the neighbouring combs, when the bees will quickly become habituated to it, and afterwards will take it in the ordinary manner. The vapour of creolin also acts as a disinfectant. A small phial of concentrated creolin may be placed in a corner of the hive, and lightly stopped with a cotton plug; and the lower part of the cotton being in contact with the liquid, capillarity will take place and draw up the creolin, and the heat of the hive will produce the necessary evaporation. A piece of blotting paper can be used by saturating it with creolin, and placing it upon the floor board or in a box covered with perforated zinc, so that the bees will not come into contact with the disinfectant.

Creolin is neither poisonous nor corrosive for man; but, in strong doses, it kills insects. Consequently it is necessary not to give greater strengths than those mentioned above. In the use of this remedy it is necessary to stimulate the production of brood by feeding liberally with medicated syrup; if the disease does not yield to this treatment, the queen should be removed.

Experiments on the antiseptic value of creolin. a. Sloped agar—each tube, inoculated with one loopful of spores, was plugged with cotton wool, saturated with creolin, and then capped with lead foil Tubes were kept at 22° C. and 37° C.

Result: After four days at 22° C.—No growth, except beneath the condensation water in the tubes.

After four days at 37° C.—No growth.

At the end of this time new cotton plugs were inserted into the tubes in the place of the creolin ones, and the cultures again incubated, when good growth ensued in 24 hours.

b. Agar plates were made and streaked with two loopfuls of spores. In each plate was placed a square inch of thick blotting paper, with four drops of creolin on it. The plates were kept in the incubator at 37° C., and removed in 48 hours, when very slight growth was manifest. On removal of the creolin and further incubation of the plates, good growth was obtained. Control plates gave copious growth. These experiments were repeated with only one drop of creolin.

Result, after 24 hours—abundant growth. With two drops of creolin,

the growth was restricted to the inoculation track after 48 hours at 37° C.

c. In addition to the above experiments, agar was made containing the same proportion of disinfectant as was used in feeding the bees of diseased hives; 15 c. c. of this agar was taken for making a plate culture, and several plates were streaked with two loopfuls of spores, and incubated at 37° C. Strength of agar,—2 c. c. creolin to 1 litre of water, i.e., about half a teaspoonful to a quart.

Results —Creolin agar, four tests—no growth.

Control agar, abundant growth.

This antiseptic in the strength used by Cowan for feeding purposes, would prevent the germination of the spores; and if there was a large amount evaporating in the hive, a slight antiseptic result would take place.

(8) *Eucalyptus* This substance was introduced by Beauverd (69). A small tin box, with a cover pierced with small holes, is placed upon the floor board of the diseased hive, and filled with essence of Eucalyptus. The colony receives every four or five days a litre of syrup containing a teaspoonful of tincture of eucalyptus (oil eucalyptus, 1; alcohol, 9). Then from time to time some drops of the same tincture are dropped into the hive. Auberson, who was the metayer of Bertrand's Apiary and was managing his own higher up the mountains, cured a number of colonies by means of this method. He finds that there is a great difference in the effect produced by the remedy. In some cases, the effect follows the remedy quickly; in others, the effect is slower. Sometimes more than a year passes without resulting in a complete cure. When the disease is of long standing, the remedy must be proportionate to the gravity of the evil. When there are only a few diseased cells, Auberson simply pours some drops of the essence along the back wall of the hive. He renews the dose every eight days; and in six weeks, sometimes sooner, the colony is cured. In cases where the hive is badly affected, he takes a clean hive and floor board and impregnates the interior, floor board, and division board with eucalyptus, and then transfers combs, brood, and bees to the new hive. He leaves the foul brood colonies their rotten combs, as this is the only handy means of disinfecting them. Three weeks later, during which he has twice poured eucalyptus on the floor board, he examines the new brood. If it exists in healthy patches he simply pours a few drops of the essence on the floor board until the cure is complete. If, however, the fresh brood still disclose some diseased spots, the queen is killed and replaced by another, and every fifteen days the essence is spread on the floor board until the cure is completed. If the colony is very weak, he strengthens it by the addition of bees and healthy brood. If he has to feed a diseased hive, he never fails to put the essence in the syrup.

Besides these well authenticated cases of cure by the essence of eucalyptus, there are a number of others, and the method has been extensively used in Europe. The great drawback to the use of this remedy is that it is liable to cause robbing.

Experiments on the antiseptic value of eucalyptus. (*a*) Eucalyptus oil. The cotton plug of a spore-inoculated sloped agar tube was saturated with the oil, and incubated at 37° C. In eighty-four hours there was no growth, but a fresh plug being inserted good growth occurred in twenty-four hours.

(*b*) Agar plates inoculated with spores and containing four drops of eucalyptus on a piece of blotting paper were incubated at 37° C. No growth formed, but when the eucalyptus was removed good growth immediately ensued. On plates containing two drops the growth was restricted to the inoculation track, but when the oil was removed abundant growth took

place. On plates containing one drop on blotting paper there was abundant growth in twenty-four hours.

(*c*) Eucalyptus agar was made by using a teaspoonful (4 c.c.) of tincture of eucalyptus to a litre of agar. Six plates were made with eucalyptus agar, each plate inoculated with spores, with the result that the growth on the medicated agar was only slightly less that that on the control agar. The medicated agar smelt slightly, but characteristically of eucalyptus oil.

A Queensland (Australia) correspondent of the *British Bee Journal* (71) is of the opinion that no foul brood exists among bees in that country. The reason of this is that the honey that goes into the combs is largely gathered from the eucalyptus, the medicinal qualities of which combat foulness in all forms. This statement, however, is not reliable, inasmuch as foul brood is known to be prevalent in Queensland.

(9) *Napthol Beta.* Napthol Beta was first used as a remedy by Lortet (72). The treatment is as follows :

The drug is administered in the food, in the proportion of one-third of a gram to a litre. This one third of a gram is at first dissolved in a little alcohol, as it is extremely insoluble in water. Afterwards it is mixed in a litre of water, and this liquid is used for making the syrup. In England the usage is to dissolve the napthol in the sugar, the proportion being about forty to fifty centigrammes to a kilo of sugar. It is, however, better to dissolve it in alcohol. Lortet thinks that external treatment by means of fumigation or spraying is helpful, as these methods contribute largely to the disinfection of hives, comb, etc ; but as he believes that it is always the digestive canal of the nurse bee which is infected and that it is by the act of feeding that the adult bee infects the digestive canal of the larvæ, therefore all efforts should be directed to the digestive canal of the worker bees, and the treatment ought to be internal and as energetic as possible. He states that when administered in the proportion of 0.33 gram per 1,000 of liquid it prevents all fermentation and decomposition and other changes caused by microbes. He further maintains that in addition to the use of this preparation first-rate hygienic conditions are necessary in order to give the bees vitality and recuperative power, which play an important part in enabling living organisms to resist the inroads of virulent microbes.

McKenzie found that (28) a beef broth containing one per thousand of B. Napthol prevented spores of *B. alvei* from germinating, and consequently had an equal value with one per five hundred of carbolic acid.

This remedy has been widely used and with considerable success.

Experiments on the antiseptic value of Napthol Beta. Napthol Beta agar was made in our laboratory the same strength as that recommended by Lortet for feeding, that is 0.33 gram B. Napthol to one litre of agar. Eight tests were made in Petri dishes, inoculated with spores of *B. alvei*, and in no case did growth result ; from which we learn that a dilution of one-third of the solution used by McKenzie completely inhibited growth. Napthol Beta agar containing 0.165 gram of the drug to a litre of agar was also tried, and the result of a number of tests was that some growth took place on the medicated plates and abundant growth on the control plates.

From these experiments, also those of Lortet and McKenzie, it will be seen that Napthol Beta has a strong antiseptic action.

(10) *Naphthaline.* This substance is regarded as a preventive rather than as a curative, although there are cases known in which it has effected a cure of diseased hives. A small quantity of the drug is placed on the floor board of the hive, a crystal about 2 c m. in diameter as far from the entrance

of the hive as possible. The evaporation is rapid and with very strong odour. Hence, if too much used, the brood will be deserted by the workers and death of the bees may take place. As soon as the dose has evaporated it is renewed.

As a preventive, naphthaline has been very favourably reported upon by a number of writers; and Cowan (73) states that he inspected very thoroughly a hive belonging to Merney which had been cured by this substance.

Experiments. In our laboratory, crystals of naphthaline about the size of a large pea were put into test tubes containing sloped agar, inoculated with one loopful of spores, capped with tin foil paper and kept at 22° and 37° C.

Results. After 48 hours—good growth in all tubes. Inoculated agar plates containing a crystal of naphthaline likewise gave good growth in 24 hours at 37° C, as did also the control tubes and plates. Hence, we conclude that naphthaline has no antiseptic power; and we are forced to look upon its use rather doubtfully. It may, perhaps, act as a stimulant.

(11) *Formic acid.* This substance was first suggested by Dennler in 1885 (74), but he did not ascertain the strength in which it could be used. Sproule (75) states, that since the year 1882 he had successfully treated foul brood with formic acid. He was the first apiculturist to use the remedy and give the treatment. The solution used is pure acid, 10 parts; water, 90 parts; and the treatment is as follows:—

A part of the comb is taken from the hive and as many bees as possible are shaken from the diseased comb; and then two or three empty combs are used, into one of the sides of which 100 grams of the solution are poured, while it is held inclined so as to allow the liquid to run into the cells and stay there. These combs are placed on each side of the brood, the side containing the solution next the brood. Eight or ten days after, an inspection is made; and if there is no cure, the dose is renewed and continued every week until the cure is complete, which is often after the first treatment. In fact the disease rarely resists the second or third application. To hasten the cure, this remedy can be given in the food of the bees—a teaspoonful to a litre of syrup.

Experiments Formic acid probably has an important rôle to play in the keeping properties of honey. As long ago as 1878, formic acid was found in honey; and Muhlenhoff (76) observed that when honey is not intended for immediate use, the bee deposits in each cell a drop of formic acid, secreted by the venom glands, and then seals the cell. Erlenmeyer (77) says that formic acid of the strength of 1.205 gr. to a thousand parts of water was antiseptic, Planta (78) refutes Muhlenhoff's idea that 100 grams of sealed honey contains .0186 grams of 22% formic acid. "100 grams is the capacity of 165 worker cells, but the smallest droplet of venom contains at least .0254 grams of formic acid, which would make for 165 cells, 4 1910 grams; that is to say, 200 times more than there is in reality." This opinion is, however, contrary to one expressed before by the same writer, in the year 1884 (79).

Formic acid seems to help bees to ward off the disease, especially when we supply it to them ready made; and that found in certain kinds of honey has probably an antiseptic effect. Two samples of clover honey and two samples of buckwheat honey were analyzed in our chemical laboratory with the following results:—

1	Buckwheat honey	0 15	grains of formic acid in 100 grains of honey.			
2	"	0.17	"	"	"	"
1	Clover honey	0 0579	"	"	"	"
2	"	0.057	"	"	"	"

Formic acid agar was then made containing the same proportion of formic acid as was found in the first sample of buckwheat honey, and weaker formic acid agar containing the same percentage of formic acid as was present in the first sample of clover honey; and spores placed upon the stronger formic agar did not germinate, while on the weaker formic agar the germination was only slightly retarded; and after the weaker agar was two days in the incubator, there was a large growth. Spores transferred from the strong formic agar (after being in contact with it for six days in the incubator) failed to grow on the weaker formic agar within two days; but after four days in the incubator they grew abundantly. The culture growing on the weaker formic agar was then transferred to the strong formic agar, to ascertain whether the germ could be accustomed to more unnatural food by previous cultivation on the weak formic agar. This transfer was, however, unsuccessful.

The germs used in these tests were isolated from samples of diseased comb from Ontario, Austria and Florida, U.S.A.

Formic acid bouillon was also made containing .15% of formic acid; and spores kept in this broth for eight months continued to germinate when transplanted to suitable material.

Formic acid agar was likewise made in the same proportion as suggested by Bertrand (59); that is, formic acid 10, water 90; and a tablespoonful of this mixture to a litre of syrup; but instead of syrup, agar was used. Fifteen c.c. of this acid agar was poured into each Petri plate, and the surface inoculated with spores.

Results: On 14 plates, no growth.
On 2 plates, very restricted growth, limited to one-eighth of an inch of the needle track (60 hours).
On control plates, abundant growth.

From these investigations, viz., the analysis of the honey, the experiments based thereon, and the tests with agar made in the proportion suggested by Bertrand, we would note three things: (1) That the amount of formic acid recommended by Bertrand for the cure of foul brood is almost identical with the amount found in buckwheat honey; (2) that formic acid is a good antiseptic; (3) that the formic acid in buckwheat honey may possibly tend more or less to ward off foul brood.

We may add that our analysis, showing a larger proportion of formic acid in buckwheat honey than in clover honey, is an interesting explanation of a fact well known among practical bee-keepers, viz., that the sting of bees when working on buckwheat is much more irritant than when working on clover.

In conclusion under this head, we may say that formic acid has given good results when used in the treatment of foul brood; and it is in a sense a natural remedy, being manufactured to some extent by the bees themselves.

(12) *Other substances used for treating this disease.* Among other substances that have been used for treating this disease are sulphuric acid, sulfaminol, various modifications of substances already mentioned, and some recommended in the McLean method (80), the Muth method, and others; but these have not had so wide application as those referred to in the preceding paragraphs.

Experiments on the Use of Drugs for Combatting the Disease.

I have already mentioned that, in one of my experiments, I endeavoured to find out if the virulence of the germ was attenuated by prolonged culture in artificial media, with the result that considerable attenuation occurred

after a large number of transfers; and in the following experiments I have endeavoured to meet any objections that might be made as to the virulence of my cultures, by isolating *B. alvei* from a badly diseased hive and then growing at once sufficient spores for the purposes of the experiment. Thus but three transfers from a diseased larva were made; and all the spores used in the following experiments were obtained in this manner:

Two small hives, each containing strong healthy swarms, were selected and placed side by side.

Hive A was given spores of *B. alvei* in syrup containing one-third of a gramme of napthol B. to a litre of syrup

Hive B was given spores of *B. alvei* in syrup containing from 1.6 to 1.8 c c. formic acid to a litre of syrup.

The spores given were scraped from the surface of an agar slope culture, put into 10 c c. of sterile water, and well shaken in order to obtain a good suspension of spores. The water and spores were poured into medicated syrup and the mixture thoroughly stirred. It was then given to the bees and was readily accepted. This procedure was continued four days a week for three weeks, and at the end of this time each hive had received the whole of the growth from twelve sloped agar tubes. During the feeding period the combs containing the brood were carefully examined, but none of the usual symptoms of the disease appeared, although cultures were obtained from different parts of the hives and from the digestive tract of the workers. At the end of three weeks the medicated syrup was discontinued for a week. Then ordinary syrup containing spores was given, and at the end of ten days typical symptoms began to be noticed, and after sixteen days the disease was well established. Both hives, so far as I was able to judge, were the same—no disease to be seen in either whilst medicated syrup was fed, but infection manifest in both soon after the formic acid and napthol B. were discontinued. This experiment goes to prove the benefit of feeding with syrup a substance which is antiseptic and which hinders the germination of the spores. It also confirms Lortet's opinion that the digestive canal of the nurse bee is alone infected. I have never been able to obtain Cheshire's results, viz, the isolation of the bacillus from the blood of the worker, but I have frequently found it in the digestive canal of bees from diseased colonies.

From the results of the above experiments I conclude that in certain cases the use of chemicals is beneficial, but I would not say that other measures, such as starvation and stamping out, should be abandoned as unnecessary or useless. Some of the drugs used are of very little, if any, value; but others, such as formic acid and napthol B, are undoubtedly very useful. In some cases, especially those in which the disease is very virulent, it may be advisable to resort to more drastic measures.

Toxins.

I endeavoured to find out whether or not the feeding of toxin (filtrate from a two weeks old culture of *B. alvei* in saccharose bouillon) mixed in syrup would enable healthy bees to withstand the disease. Small amounts of this filtrate were given in syrup to a healthy colony every other day for three weeks. The amount of filtrate fed was gradually increased, but as the amount got larger the bees refused to take it, so it had to be poured over the combs. At the end of three weeks spores of *B. alvei*, freshly isolated, were fed, and symptoms of the disease followed about fourteen days later. So the toxin had little or no effect, but further experiments are being made.

Legislation.

In the United States, six States have laws for the suppression of foul brood among bees. These are New York, Wisconsin, Michigan, Utah, Colorado and California. In Canada the Province of Ontario has enacted a foul-brood law. In Europe Mecklenburg also has a law.

These statutes differ a good deal from one another, and some of them are so drafted that evasion of the law is easy. The best are probably those of Wisconsin and Ontario, and the principal points in these acts are as follows:

1. The appointment of an inspector.
2. The inspection of all apiaries reported as diseased, and the duty of the inspector, if satisfied that the disease is present, to give full instructions as to treatment.
3. The enactment requiring the inspector, who is the sole judge, to make a second visit to all diseased apiaries, and, if need be, burn all colonies and combs that he may find uncured.
4. Various penalties (fines, and, in default, imprisonment) for—
   (*a*) Selling or giving away diseased colonies or infected appliances.
   (*b*) Selling bees after treatment, or exposing infected appliances.
   (*c*) Obstructing the inspector.
5. Persons who are aware of the disease either in their own apiary or elsewhere are to notfiy at once the proper authorities, and in default of so doing shall, on conviction, be liable to a fine and costs.
6. The inspector of apiaries to make an annual report, which shall include a statement of the number of colonies destroyed by his order, the localities where found, and the amount paid to him for his services.

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Ontario Department of Agriculture

FRUIT BRANCH.

BULLETIN 190

BEE DISEASES

IN

ONTARIO

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Ontario Department of Agriculture

FRUIT BRANCH

Bee Diseases in Ontario

Much dissatisfaction with beekeeping as a business is caused by so-called "bad luck," which is really due to a definite bee disease which any bee-keeper can learn to cure. Bees are quite as liable to disease as any other live-stock, and to be able to treat such disease intelligently is quite necessary to success.

Bee-moths are often blamed for the ravages due to disease; but moths never destroy a healthy normal colony, as they only feed on the deserted combs after the bees are nearly all gone. Heavy winter losses can often be attributed to disease. In fact, whenever a colony is not doing well the exact cause of its failure should be carefully sought to make sure there is no bacterial disease.

On the other hand, disease often makes its first appearance in the best colonies in the apiary, because infection is usually carried by robbing, and that is generally done by strong colonies. If not checked on the start it soon spreads through the whole apiary, and from it to other apiaries in the neighborhood.

The inspectors of apiaries can do a great deal for the health of bees in Ontario; but to be of real value their work must be supplemented by the earnest efforts of the individual beekeepers. Everyone should be his own inspector, carefully *examining every comb of every colony in the apiary at least once a year,* remembering that it is far better to detect it on the start in strong colonies than to wait until they are practically ruined and the disease has spread through the whole neighborhood. Only one cell of infectious disease makes it necessary to treat even the best colony in the apiary. And because one has kept bees for a number of years without seeing a case of disease is no reason why it should not make its appearance this year. Plenty of people have died of smallpox after having escaped it for fifty years.

When a case of infectious disease is suspected the beekeeper must first notify the Minister of Agricuture, Toronto, Ont., who will send word to the nearest inspector of apiaries; but if the case cannot have immediate attention the beekeeper should go ahead and treat the disease according to directions given in this bulletin.

Examining an Apiary for Disease.

The diseases which cause the most damage in Ontario attack the developing brood, causing much of it to die in the comb, and so reducing it that the colony soon dwindles from lack of young bees to replace the old.

When examining an apiary for disease the prime consideration is to avoid robbing. The best time is during a good honey flow as early as possible in the season.

It is necessary to have a good smoker, a hive tool for taking out combs, and a supply of wooden toothpicks for testing the brood.

In opening the hive just enough smoke should be used to keep the bees in subjection. Remove each comb in turn from the brood-chamber and examine the brood. It is best to sit on a box close to the hive with your back to the sun, and hold the comb so it will shine into the cells, and throw a strong light directly on the lower sides and bottoms of the cells. If there is no disease, the empty cells will be bright and clean, and the uncapped larvae will be plump in form and of a pearly white color. At first a number of cells of capped brood should be opened with the pick, until you are quite familiar with the outward appearance of healthy capped brood. Cappings which to any but the best-trained eye appear quite healthy often cover dead larvae. When diseased cells are present they are quite frequently found around the lower edge of the comb. If any of the brood cappings appear darker than the rest, or are flat, sunken, or perforated, they should be opened to see whether the brood they cover is dead. Healthy brood is sometimes found under flat, or perforated cappings; but there is a difference in appearance which experience soon teaches one to detect. Brood sometimes develops without ever being fully capped; this is not known to be an indication of infectious disease. When each hive is finished the pick used there should be left in the hive, and if any honey is daubed on hands or tools they must be washed thoroughly before opening the next hive.

There are three brood diseases prevalent in the apiaries of Ontario; American Foul Brood, European Foul Brood, and Starved or Pickled Brood. The first two are known to be infectious, the last is not so considered, although its cause is not well understood.

Distribution of Disease.

American Foul Brood is pretty evenly distributed over that portion of Ontario lying south and west of the Trent Valley. It has cost the Province of Ontario hundreds of thousands of dollars, not only in loss of bees and honey, but in its disheartening effect on the men engaged in the industry. Much, however, is being done, and more can and will be done, by the Department of Agriculture towards restoring a well grounded confidence in beekeeping as a business by various methods of instruction.

The greatest menace at present is European Foul Brood. This scourge is known to have practically wiped out the keeping of bees over a territory of perhaps three hundred square miles around Ottawa, and five hundred square miles in Northumberland, Hastings and Prince Edward; it has also gained a foothold at Fort Erie on the Niagara River. Much loss by this particular disease might have been saved if the beekeepers had kept Italian instead of common black bees, or if they had Italianized as soon as they were warned. As the situation now stands, it seems to be spreading from these districts like a blight, carrying all black bees before it. Only those who Italianize their bees can hope to save them, as no system of inspection can cure black bees of this particular disease. Men with the right strain of Italian bees are securing enormous yields of honey right in the centre of infected district. As the Irishman says, "It's an ill wind that blows nowheres," and there is not the slightest doubt that this wind of European Foul Brood, though ill enough, will blow money into the pockets of the men who will sit tight, get Italian bees, and weather the storm.

AMERICAN FOUL BROOD.

This disease is caused by bacteria known to scientists as Bacillus Larvae (not B. Alvei, as was formerly supposed). It reaches the healthy young larvae by means of infected food unsuspectingly fed to them by the nurse bees. In most cases the larvae dies when nearly ready to seal up, and most of the cells containing infected larvae are capped. The dead larva softens, settles to the lower side of the cell in a shapeless mass, at first white or yellow, changing to coffee-color and brown. At this stage it becomes glutinous, so that if it is picked with a toothpick the contents will rope out half an inch or so when the pick is slowly withdrawn. It adheres to the cell so it cannot be lifted out entire. It has the odor of a poor quality of glue. When the larva dries it forms a tightly adhesive scale, of very dark brown color, which cannot be removed without tearing the cell wall.

Where the infected larvae are capped the cappings turn a darker color and become flat or sunken, the workers perceiving that something is wrong usually start to tear off the capping, but, discovering the condition of the contents, they generally leave it with a small perforation in the centre until quite dry, then the capping is removed, and in time honey may be stored in the cells containing the scales of disease. The millions of disease spores then float out into the honey, which becomes a medium for carrying the disease to other healthy larvae by robbing, in the same or some other apiary. Some of the honey is also carried into the supers, to make room for alterations in the brood nest, and is marketed in the form of bottled or section honey. It goes into many homes, especially in

towns and cities. The wooden sides of the sections, and many of the empty bottles, or washings from them, are thrown out by housekeepers and cleaned up by bees of the neighborhood, and the disease is carried home to their healthy brood. This is why our inspectors find more disease in the apiaries around towns and cities than elsewhere.

The Treatment.

Now, to be cured of this disease a colony must be freed from all this infected brood, comb and honey. To do this we simply take it away. But in the operation some precautions are necessary. We must see that the colony will get healthy food as soon as the unhealthy food is taken away, and have means for building new comb at once. So the operation should be performed during a honey flow, and to make it perfectly sure it is a good plan to insert a division board feeder of sugar syrup. We must take precautions against starting robbing, or causing the treated colony to scatter to other hives or swarm out, be lost, and carry infection to other places. So the operation should be performed in the evening, when the bees are settling down for the night, and the entrance should be covered with queen-excluding metal to hold the queen in case of swarming out the next morning. A regular queen-excluder laid on the bottom board under the brood chamber will answer the latter purpose. They should also be given a clustering space to occupy, as in the case of a natural swarm. Whenever bees are disturbed in their hives they will fill their honey sacs with honey from the comb. As this will happen when the hive is being treated, and some of this diseased honey may be stored in the new combs, it is thought best to remove these after three or four days and require them to make a second start.

Method of Treatment.

When there is a good honey flow on, go to the colony in the evening, taking a set of frames with one-half inch starters of foundation in them. Take the combs out of the hives, shaking the bees from them, back into the hive. If any fresh nectar flies out, it will be necessary to brush the bees off instead of shaking them. Get these combs immediately under cover, and clean up very carefully any honey that may be about, so that robbers from healthy colonies cannot carry home disease. If the honey flow is at all uncertain, it is better to put in a feeder with thin sugar syrup.

On the third or fourth evening after the first operation, remove the hive from its stand and set in its place a clean disinfected hive containing frames with full sheets of foundation. Now brush the bees from what combs have been built on the starters into the new hive. Even greater care must be taken than at first to avoid leaving any honey or bits of comb about. Positively no comb must be used or left in the hive in either the first or second treatment.

You have now made an artificial swarm of this colony. It must be given the conditions a new swarm likes, or it will leave and carry its disease to parts unknown, or perhaps into some healthy hive in the apairy. A new swarm likes plenty of ventilation and shade, and also room to cluster for awhile without having to crawl in between sheets of foundation at once. To satisfy this natural desire, it is best to place an empty hive under the one containing the frames of foundation. If for any reason this cannot be done, two or three frames can be left out of the brood chamber for a couple of days. The bees will cluster in this at first, just as a swarm clusters on a tree; but they will soon go up and take possession of the foundation, then the empty can be taken away. This simple precaution will generally prevent the swarming out which so often happens in treating foul brood; but as an extra precaution it is best to use the excluder on the entrance as well.

All combs from the supers as well as from the brood chamber of the diseased colony, together with the first set of starters and whatever comb is built on them, must be either burned or melted, and boiled thoroughly before the wax is fit to use again. The honey that is removed is entirely unfit for bee feed, even after it has been boiled for a full half hour it is not safe. The only safe way to dispose of it is to burn it, or else dig a hole and bury it deep enough to be out of the reach of any bees.

If directions have been followed carefully and thoroughly, the treatment should be successful. To make sure, however, the brood must be examined again in about three weeks and again the following season. Please note in this connection Section 6 of the Act.

Saving Brood.

Brood from badly diseased colonies is of no value, and dangerous, and should be burned, buried or otherwise destroyed at once. Brood from colonies having only a few cells diseased may be placed over an average colony slightly diseased, and the queen caged. In ten days treat as given above.

Saving Combs.

It is never safe to use super-combs that have been on diseased colonies. Even though they may appear white and clean, germs of the disease are apt to lurk in them from year to year. To melt these down is no serious loss, as the wax will more than make foundation for new ones.

Disinfecting.

Hives which have formerly contained diseased colonies, or in which diseased combs have been stored or carried, should be burned over inside with a gasoline or oil torch.

EUROPEAN FOUL BROOD.

Until 1907 the only infectious brood disease known to exist in Ontario was the one already described. But another then made its appearance. It is called European Foul Brood (sometimes "black brood").

European Foul Brood has destroyed the apiaries in great areas of different States in the Republic to the south of us. It is now known to be rampant in at least three sections of Ontario. In one way it is much more to be dreaded than American Foul Brood, because it runs its course and destroys an apiary much more rapidly, and because the adult bees will carry out the disease scales and scatter them in the yard and farther, to find their way into healthy colonies.

In the part of Ontario where it was first discovered apiaries were wiped out at first something like this:

112 colonies reduced to 23 in two years.

180 reduced to 21 in one year.

60 colonies reduced to 44 in one year, and the balance all diseased the second year.

The following report in reference to European Foul Brood, received in the fall of 1910 from one of our apiary inspectors, will give an idea of the danger:

Inspector's Report for Peterboro, Northumberland, Hastings and Prince Edward.

Inspector W. Scott, Wooler.

"I travelled over the same ground as last year, and found that all the bees had been treated, except one apiary, but very little Italianizing had been done, and consequently the disease returned in every apiary and destroyed some of them completely. I found the disease spreading very rapidly; it has more than doubled since last year. The disease now covers fully 400 square miles; besides two outbreaks in Prince Edward County, also two in Hastings County, one in Roden Township, and one in Huntingdon Township. I think if the Department could encourage the beekeepers to Italianize ahead of the disease, it would prevent a great deal of loss, as the disease does not affect the Italians nearly so badly as the blacks. I think the disease could be prevented some if the law would forbid the moving of bees except by permission of the inspector. I found three cases in the past season, where the disease has broken out, caused by the moving of bees from a diseased territory to an undiseased one. Had this moving been prevented it would have taken it probably two years to travel of its own accord.

"I may say that $5,000 is not too large an estimate for the loss sustained by disease in my district last year, but these figures will be greatly

increased next year as European Foul Brood is spreading very rapidly. Last year (1909) it covered about 100 square miles. At the present date it covers 400 square miles, besides an outbreak in Prince Edward County, also in Hastings County near Ivanhoe."

Symptoms of European Foul Brood.

The symptoms are easily distinguished from those of American Foul Brood, as there is very little ropiness, and the odor is different. The larvae mostly die without uncoiling from their natural positions. The color in the earlier stage is lighter than in the American Foul Brood. The odor is very pronounced and offensive, like decayed fish; in fact, on a warm moist morning it is noticed on entering the apiary, and, when a diseased comb is held up for inspection, is almost sickening.

Use Same Treatment and Italianize.

The same treatment already described for American Foul Brood is effectual if applied to the whole apiary at once, even though only a few colonies show symptoms. Even then the cure is only permanent when pure-bred Italian queens are introduced to the affected colonies. It is quite impossible to cure an apiary of black bees of European Foul Brood without introducing pure Italian queens to all colonies.

We know of no reason why this plague should not sweep over Ontario as it has over most of the United States. If it does, all apiaries of black bees will be practically destroyed within the next few years. Its progress in the districts mentioned above has been appalling. No Government expenditure can touch the situation without the co-operation of the men themselves whose property is in danger. There is a remedy, however, right at hand. Pure-bred leather-colored Italian bees are almost immune to this disease, which works so much havoc among the common blacks.

It is very important, then, that all apiaries, especially in or near infected neighborhoods, should be Italianized at once, without waiting for a destructive outbreak of disease.

STARVED OR PICKLED BROOD.

A disease slightly resembling Foul Brood is called by some "Starved Brood," and by others "Pickled Brood." The most positive difference in the diagnosis of this disease is the absence of ropiness and of the glue-pot smell, which are always found in American Foul Brood. In Pickled Brood the larva decays from the inside, leaving the skin tough and in its natural shape; in European Foul Brood or American Foul Brood, the skin of the larva softens as the contents become glutinous, and all the natural wrinkles become smooth as the mass settles to the lower

side of the cell. In Pickled Brood the larva often dries up so as to become loose in the cell and fall out when the comb is inverted. In American Foul Brood it always cements fast to the lower cell wall, so it cannot be removed without tearing the cell. European Foul Brood attacks the larva generally at an earlier stage in its existence than Pickled Brood.

The cause of Pickled Brood is not definitely known. It is not considered to be infectious. McEvoy asserts that it is caused by an insufficient feeding of the larvae, due to a sudden check of the honey flow, or a constitutional weakness of the workers. The latter he charges to in-breeding of the queens. Re-queening with vigorous queens from other apiaries will often effect a cure, and it often disappears of its own accord.

AMERICAN FOUL BROOD.

From the reports of the inspectors of apiaries of recent years, we find that American Foul Brood is prevalent in the following counties and townships. This does not mean that townships not mentioned in this list are guaranteed to be free from this disease, because the apiaries of Ontario have not all been inspected as yet:

BRANT: Brantford, Dumfries South.

BRUCE: Arran, Brant, Bruce, Culross, Elderslie, Greenock, Kinloss, Saugeen.

CARLETON: Goulbourn, Osgoode.

DUFFERIN: Garafraxa East, Luther East, Mono.

DUNDAS: Winchester.

DURHAM: Darlington.

ELGIN: Dorchester South, Malahide, Yarmouth.

ESSEX: Gosfield North, Maidstone, Rochester, Sandwich East, Sandwich West.

FRONTENAC: Kingston Township.

GREY: Artemesia, Collingwood, Euphrasia, Glenelg, Keppel, Osprey, Proton, St. Vincent, Sarawak, Sydenham.

HALDIMAND: Cayuga, Walpole.

HALTON: Esquesing, Nelson, Trafalgar.

HURON: Grey, Morris, Turnberry, Wawanosh West.

KENT: Harwich, Romney, Tilbury East.

LAMBTON: Bosanquet, Moore, Warwick.

LEEDS: Bastard, Elizabethtown, Kitley, Yonge.

LINCOLN: Louth.

MIDDLESEX: Adelaide, Biddulph, Delaware, Lobo, London, McGillvray, Metcalfe, Westminster, Williams East, Williams West.

MUSKOKA: Draper, Macaulay, Muskoka.

NORFOLK: Charlotteville, Townsend, Walsingham, Wyndham, Woodhouse.

ONTARIO: Brock, Pickering, Reach, Scott, Thorah, Uxbridge, Whitby East.

OXFORD: Blandford, Blenheim, Dereham, Norwich North, Norwich South, Oxford East, Zorra West.

PEEL: Albion, Caledon, Chinguacousy, Toronto.

PERTH: Blanshard, Downie, Easthope North, Easthope South, Ellice, Elma, Fullarton, Hibbert, Mornington, Wallace.

SIMCOE: Adjala, Essa, Gwillimbury West, Innisfil, Medonte, Nottawasaga, Orillia, Tay, Tecumseth, Tiny, Vespra.

VICTORIA: Bexley, Eldon, Mariposa.

WATERLOO: Dumfries North, Waterloo, Wellesley, Wilmot.

WELLINGTON: Garafraxa West, Guelph, Luther West, Nichol, Puslinch.

WENTWORTH: Ancaster, Barton, Beverly, Binbrook, Glanford.

YORK: Etobicoke, Gwillimbury East, King, Markham, Scarborough, Vaughan, Whitchurch, York.

EUROPEAN FOUL BROOD.

From the reports of the inspectors of apiaries we find that European Foul Brood is prevalent in the following counties and townships. As this disease is spreading rapidly, it is very likely to appear in the townships adjoining these during the season of 1911. All beekeepers should be very much on the alert and examine their bees carefully for the symptoms of this disease:

CARLETON: Fitzroy, Gloucester, Nepean.

HASTINGS: Huntingdon, Rawdon, Sidney.

LEEDS: Bastard.

NORTHUMBERLAND: Brighton, Cramahe, Murray, Percy, Seymour.

PRINCE EDWARD: Ameliasburg, Hillier.

RENFREW: MacNab.

WELLAND COUNTY: Bertie.

The names of cities and towns located in these townships are omitted for brevity, but as a matter of fact, bees in cities and towns are more often diseased than in the country.

AN ACT FOR THE SUPPRESSION OF FOUL BROOD AMONG BEES.

His Majesty, by and with the advice and consent of the Legislative Assembly of the Province of Ontario, enacts as follows:

1. This Act may be known as "The Foul Brood Act."

2. The Lieutenant-Governor in Council, upon the recommendation of the Minister of Agriculture, may from time to time appoint one or more Inspectors of Apiaries to enforce this Act, and the Inspector shall, if so required, produce the certificate of his appointment on entering upon any premises in the discharge of his duties. And the Minister shall instruct and control each Inspector in the carrying out of the provisions of this Act. The remuneration to be paid to any Inspector under this Act shall be determined by order of the Lieutenant-Governor in Council.

3. The Inspector shall, whenever so directed by the Minister of Agriculture, visit without unnecessary delay any locality in the Province of Ontario and there examine any apiary or apiaries to which the said Minister may direct him, and ascertain whether or not the disease known as "foul brood" exists in such apiary or apiaries, and wherever the said Inspector is satisfied of the existence of foul brood in its virulent or malignant type, it shall be the duty of the Inspector to order all colonies so affected, together with the hives occupied by them, and the contents of such hives, and all tainted appurtenances that cannot be disinfected, to be immediately destroyed by fire under the personal direction and superintendence of the said inspector; but where the inspector, who shall be the sole judge thereof, is satisfied that the disease exists, but only in milder types and in its incipient stages, and is being or may be treated successfully, and the inspector has reason to believe that it may be entirely cured, then the inspector may, in his discretion, omit to destroy or order the destruction of the colonies and hives in which the disease exists.

4. The inspector shall have full power in his discretion, to order the owner or possessor of any bees dwelling in box or immovable frame hives, to transfer them to movable frame hives within a specified time, and in default the inspector may destroy, or order the destruction of such hives and the bees dwelling therein.

5. Any owner or possessor of diseased colonies of bees, or of any infected appliances for beekeeping, who knowingly sells or barters or gives away such diseased colonies or infected appliances, shall, on conviction thereof, before any Justice of the Peace, be liable to fine of not less than $50 or more than $100, or to imprisonment for any term not exceeding two months.

6. Any person whose bees have been destroyed or treated for foul brood, who sells or offers for sale any bees, hives or appurtenances of any kind, after such destruction or treatment, and before being authorized by the inspector so to do, or who exposes in his bee-yard, or elsewhere, any infected comb, honey, or other infected thing, or conceals the fact that said disease exists among his bees, shall, on conviction before a Justice of the Peace, be liable to a fine of not less than $20 and not more than $50, or to imprisonment for a term not exceeding two months, and not less than one month.

7. Any owner or possessor of bees who refuses to allow the Inspector to freely examine said bees, or the premises in which they are kept, or who refuses to destroy the infected bees and appurtenances, or to permit them to be destroyed when so directed by the inspector, may, on the complaint of the inspector, be summoned before a Justice of the Peace, and, on conviction, shall be liable to a fine of not less than $25, and not more than $50 for the first offence, and not less than $50 and not more than $100 for the second and any subsequent offence, and the said Justice of the Peace shall make an order directing the said owner and possessor forthwith to carry out the directions of the inspector.

8. Where an owner or possessor of bees disobeys the directions of the said inspector, or offers resistance to, or obstructs the said inspector, a Justice of the Peace may, upon the complaint of the said inspector, cause a sufficient number of special constables to be sworn in, and such special constables shall, under the directions of the inspector, proceed to the premises of such owner or possessor and assist the inspector to seize all the diseased colonies and infected appurtenances and burn them forthwith, and if necessary the said inspector or constables may arrest the said owner or possessor and bring him before a Justice of the Peace to be dealt with according to the provisions of the preceding section of this Act.

9. Before proceeding against any person before a Justice of the Peace, the said inspector shall read over to such person the provisions of this Act or shall cause a copy thereof to be delivered to such persons.

10. Every beekeeper or other person who is aware of the existence of foul brood, either in his own apiary or elsewhere, shall immediately notify the Minister of the existence of such disease, and in default of so doing shall, on summary conviction before a Justice of the Peace, be liable to a fine of $5 and costs.

11. Each inspector shall report to the Minister as to the inspection of any apiary in such form and manner as the Minister may direct, and all reports shall be filed in the Department of Agriculture, and shall be made public as the Minister may direct or upon order of the Legislative Assembly.

12. Chapter 283 of the Revised Statutes of Ontario, 1897, intituled "An Act for the Suppression of Foul Brood Among Bees," is repealed.

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July, 1926 Extension Service Bulletin No. 138

Control of Bee Diseases and Pests

(Courtesy of Frank C. Pellett, Am. Bee Journal)

Putting combs in sacks.

IOWA STATE COLLEGE OF AGRICULTURE
AND MECHANIC ARTS

EXTENSION SERVICE

R. K. Bliss, Director

AMES, IOWA

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Control of Bee Diseases and Pests

Bee diseases constitute one of the chief causes of the present day inefficient production of honey. This indicates that beekeepers need a better knowledge of the diseases and the means of reducing the loss from them. There are two general classes of apiary diseases, those of the adult bees, and those of the young or brood. At present the brood diseases cause by far the greater portion of the loss in the apiary so more attention must be given to them. Their diagnosis and control have been carefully worked out and the practical application is merely a matter of knowledge.

BROOD DISEASES

AMERICAN FOULBROOD

Origin and History: American foulbrood as a distinctive disease of bees has been known for about 20 years. Prior to that time it was confused with what is now known as European foulbrood, both being included in the term foulbrood. It became evident that there were at least two distinctive diseases since beekeepers were getting different reactions to identical treatments under apparently the same conditions.

The term "American" foulbrood does not mean that this disease originated in America, nor that its distribution is restricted to America. It is merely a term to indicate a definite disease and distinguish it from the European foulbrood. Foreign records indicate that this disease has been prevalent thruout most beekeeping sections much longer than it has in the United States.

Distribution: American foulbrood is now known to occur quite generally in every state in the Union. Its spread has been restricted in some sections, but no extensive beekeeping region enjoys immunity to this brood disease. In Iowa, it has been felt that in certain restricted localities American foulbrood did not occur, but careful and extensive inspections indicate that the areas where foulbrood does not exist are very small indeed. Some think that the disease is spreading and increasing its distribution, but the fact that it has not been recorded from a definite locality does not signify that it is not present. It probably has not been discovered, but may be found at any time.

Common Names: While American foulbrood is, in a way, a common name, beekeepers often refer to this disease in a general way as "black brood". This name certainly indicates the condition of the dead larvae. In other areas it is referred to as "ropy brood," which is another term describing a stage of the disease. With the gradual disappearance of European foulbrood it has come to be a practice, among beekeepers, to refer to American foulbrood simply by the term "foul". Closely associated with this is the common term, "diseased brood"

Transmission: American foulbrood is a germ disease, caused by a specific organism known as **Bacillus larvae.** The disease is highly infectious, so that the germs will readily produce the disease when introduced into a healthy host. American foulbrood germs are exceedingly minute and they travel unobserved by the human eye. The bacteria grow and develop in the bee larvae, but whenever they are placed in honey, they immediately develop into the spore or seed stage. This is important because it is considered that the sale of honey may be one means of spreading the disease.

The bacteria of American foulbrood are hardy compared with the average forms of germs. If they were not so hardy they would not be able to continue the disease in spite of all adverse conditions to which they are subjected in attempted control measures. This point is important because of its bearing upon the apparent disappearance of the disease in the treated colonies and apriary and the unexplained appearance of the disease at a later time.

The spores remain capable of producing bacteria for 30 or 40 years, so that when the material is once contaminated it is exceedingly difficult to say that all possible sources of re-contamination have been eliminated. One must know how the disease works and appreciate the dangers in handling infected material, in order to conduct a proper fight against the spread of the disease. It is also necessary to appreciate the ravages of the disease in order that one may be careful and use the necessary precautions in the treatment of the disease and the disposal of infected material.

Diagnostic Characters: The larvae which are infected with the bacteria causing American foulbrood usually reach maturity and the cells are capped. Death occurs, however, except in rare cases, before the larvae have an opportunity to change to the pupal stage. The bacteria seem to require seven days to cause the death of the larvae. Apparntly the bees are inquisitive to learn why certain sealed cells are not giving forth adult bees. Consequently a tiny hole is gnawed in the capping, which is all that is needed for the bees to tell the condition inside.

In the early stage of the disease the character which will attract the attention of the beekeeper in the colony is sunken, discolored and perforated caps on a few scattered cells thruout the brood area. If these cells are examined early in the development of the disease, it will be found that the larvae are discolored and appear a shade of chocolate. If the examination is made in this stage the larvae can be tested with a toothpick or pointed match and it will be found that the contents will "string" or "rope" as the match is withdrawn from the larvae. This condition is very typical of the disease. The odor which may be detected from the dead larvae is supposed to be characteristic and is described as a "gluepot odor," but for one unfamiliar with the disease this character cannot be used exclusively.

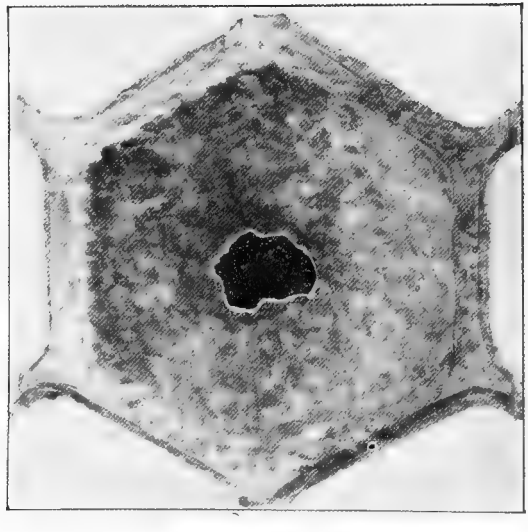

Fig. 1. Perforated cap of American foulbrood.

If the disease has progressed

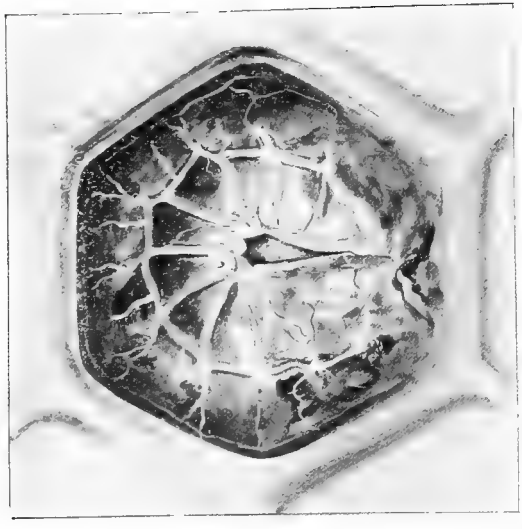

Fig. 2. Dead larva melting away from trachael system.

for some time in the colony, or if the combs are examined after the colony is dead, it will not be possible to get the characteristic ropiness of the larvae. If the larvae continue to dry, they eventually form a scale along the lower side of the cell and cover most of the bottom of the cell. This scale is black in color and adheres tightly to the cell wall; in fact, it is necessary to tear down the cell in order to get the scale out of it. These scales can be seen readily by standing so that the light comes over the shoulder and by tipping the comb with the bottom bar away from the observer so as to throw the light on the lower side of the cells. The black scales ban se seen readily if the light strikes them at the proper angle.

Seasonal History: American foulbrood works slowly in causing the extinction of the colony. A colony may become infected during the early spring, but the disease may increase slowly thruout the summer and the colony in the fall may still be apparently strong enough to go thru the winter. Beekeepers often remark that such a colony appeared a little below average in strength, but apparently had plenty of stores so that it seemed it should survive the winter. Under these conditions the colony invariably fails to withstand the cold weather and dies during the winter. The stores in such a hive are unprotected during early spring and are subject to the ravages of robbers, which carry the honey to their hives. This robbing occurs very early in the spring and is usually unnoticed by the beekeeper.

It is evident, then, that a colony which dies from disease may serve as a feeding ground for robber bees from several other colonies, which may then become infected from this contaminated honey, brought from the dead hive. These newly infected colonies may show the disease very soon or not until late in the season, or even perhaps not until the next season, or two seasons later.

The appearance of the disease in a colony depends upon the time when the diseased honey is used for rearing brood. Small amounts of the diseased honey may be stored in the bottom of the cells and later covered up with nectar or sugar syrup, and the cells may not be emptied for a season or two. Usually disease shows up the first season if robbing has occurred, for the contaminated honey is stored where it will soon be used in rearing brood. Since colonies are robbed out early in the season, early examination should be made to deter-

mine if a colony is alive or dead. It is not safe to start a diseased colony into the winter, for it is sure to be abnormally low on young bees, and the chances are decidedly against it surviving.

Treatment: The method of treatment will depend entirely upon the proportion of infestation in the apiary. Fundamentally, the treatment of American foulbrood consists of separating the adult bees from the contaminated material. The adult bees when separated are put on a starvation basis for four days in order to rid them of all contaminated material which is carried in their honey sacs. This process is known as the "shaking treatment," or is sometimes referred to as the McAvoy treatment.

If the disease is occurring for the first time in an apiary and but one colony is diseased, the safest and surest method is to destroy the entire hive and its contents. To do this, dig a deep pit and start a fire in it. The entire hive in which the bees have been killed during the previous night, is placed on top of the fire and after the material has been burned thoroughly the remainder of the contents of the hive are covered with the dirt. The pit must be deep enough so that the remains of the hive are far below any ordinary disturbance of the soil such as spading, plowing and ordinary grading. This method is open to objections and the real difficulty lies in not sufficiently covering the remains of the colony. The honey is not disinfected in the least for it runs out of the combs when they melt at about 135 degrees, whereas, to disenfect honey, it must be heated to 212 degrees and held there for 30 minutes.

An actual experience will illustrate the difficulty. A diseased colony was killed, a pit was dug and the colony put on the fire. The fire did not completely burn the material, but the pit was refilled. In time an odor came thru the soil which attracted dogs which dug down and uncovered the unburned remains of the colony. After reaching the decaying mass they left in disgust. The bees in the apiary found this hole and had acsess to the buried honey. The slight heating had not destroyed the bacetria in the honey, the disease, instead of being eliminated from the apiary, spread to six or eight additional colonies. It is of utmost importance to make a thoro disposal of the contaminated material in a colony in order to prevent the spread of American fourbrood.

A diseased colony should be taken from the yard where the healthy colonies are located if treatment is to be given. It should be moved in the evening after all of the field bees are in the hive. On the new location, a confusion board should be put in front of the entrance so that the field bees will not tend to return to their old location. It is impossible to treat a diseased colony satisfactorily in the same yard with healthy colonies, tho where it is difficult to move a colony very far, where one has but a few colonies in a back yard, the diseased colonies should be separated as far as possible from the healthy ones before the shaking treatment.

The shaking process should be given to the diseased colony between sunset and dark, and all preparations should be made in advance so that it can be accomplished quickly. The new equipment should be made ready, including three or four sheets of foundation, since the remainder of the frames need not be put in the hive until later. When the new or clean equipment is ready, the diseased colony is removed from its stand and placed with its entrance elevated 45 degrees and but a foot from the original entrance. The new equipment is placed on the old stand and a newspaper or old cloth is spread in front of the entrance. The bees should be smoked as little as possible in order to keep down the confusion among them. One by one the

frames are lifted from the diseased colony and the bees brushed onto the paper or cloth.

The bees from the first frame will be badly confused and a little attention may be needed to get them started toward the entrance to the new hive. If the entrance has been located, the bees from the other frames will start immediately to the entrance. The process is facilitated if the frame containing the queen can be among the first ones brushed. It does not pay, however, to spend any particular time looking for the queen. The entire brushing must be done with reasonable haste, but so as to cause as little confusion as possible. After a comb is freed from bees it should be placed in a sack. After all of the combs have been brushed the bees which are still in the hive body and on the bottom board, can be dumped in front of the entrance to the new hive.

The combs may then be replaced in the hive body or left in the sack, depending on what disposal is made of this contaminated material. However, the old hive and its contents must be promptly removed from the yard. As soon as the bees going into the clean hive are off the paper or cloth on to which they have been brushed, this material should be picked up and burned immediately, not in the yard, but in a place far removed from access by bees. It is not safe to burn any material in a furnace because the honey simply runs down on the ashes without any of the diseased spores which it contains being rendered inactive. Then when the ashes later are thrown out the bees will seek this infected honey.

The proper disposal of the contaminated material demands some sacrifice on the part of the beekeeper. It is undoubtedly cheapest in the long run to burn the frames with their contents. For this purpose, the destruction by the pit method as described previously is probably the best. In this way the chief source of contamination is eliminated in such manner that it is not likely to reappear. The contaminated material must be disposed of immediately after the bees have been shaken. The practice of taking hive bodies and their contents to a bee-tight room has proven wholly ineffective in controlling the disease. The reason for this is that the normal bee-tight room is sooner or later made accessible for bees which hastily rob the contaminated honey and take it to their hives.

If several colonies are to be treated at one time, it may be desirable to shake a portion of the colonies and stack the brood chambers on two colonies in order to save the brood which will continue to emerge. These two hospital colonies must be carefully attended because their forces will be spread out in an effort to care for the immense increase in brood. Under these conditions the colony is subject to the attacks of robbers so that it is probably advisable to reduce the entrance to a space which will permit only one or two bees to enter at a time. These nursery colonies are then given the shake treatment in 21 days. The use of nursery colonies is suggested only for the beekeeper who is thoroly familiar with disease treatment, for they require very struct attention and if neglected in any manner will spread disease faster than the beekeeper can check it. When shaking the nursery colony it will be necessary to place in the clean brood chamber all of the frames with foundation, as the population will be so great that a few frames will not serve to accommodate all the bees.

Bees shaken onto foundation must immediately build comb in which the queen can lay eggs, and in which honey can be stored. The honey which they have brought over from the old hive is soon digested and converted into comb-building material. It is this process which apparently destroys the disease germ. If the shaking

(Courtesy of Frank C. Pellett, Am. Bee Journal)

Fig. 3. Wreck of a neglected apiary.

has been carefully done the chances are that the bees will use up all of their contaminated honey in making comb for the new hive or at least before any of the eggs hatch and the larvae need to be fed. Then it should not be necessary to shake again.

Occasionally disease will reappear and a "double shake" will be necessary. After the colonies have recovered and have a supply of open brood in the combs, they must be provided with stores as they may not be able to get sufficient stores in the field. In case the "shaking" was made on four frames of foundation, additional frames can be added as they are needed by the colony. In a normal colony two additional frames of foundation can be added each week, but the beekeeper must decide if more are needed. Careful inspection should be made before additional foundation is made to determine if the disease is re-occurring or not.

It is not advisable to attempt to salvage old brood combs containing honey. Some beekeepers feel that inasmuch as wax is worth considerable money, it may be best to render up such combs and sell the wax. This practice is to be discouraged because during the time when combs are held before treatment, they are apt to be robbed; during the treatment there is a tendency for the bees to get at the contaminated honey, and after the treatment there is still the problem of disposing of the contaminated honey The salvage process is expensive and the amount of wax recovered will not pay for the labor involved. It does not pay to attempt to save the honey even tho this honey is not in any way injured for human consumption. Such disease laden honey may so easily become attractive to robber bees and the disease spread in this way.

Recently beekeepers have been able to buy disinfecting materials which render combs fit for re-use in the bee-hive. The first question for the beekeeper to decide is whether the combs are worth treating. It is a growing practice among modern beekeepers to replace

their brood combs every three to five years. This eliminates the amount of drone brood and also pollen-clogged combs from the brood chamber. Therefore any brood combs which are old are apt to be so imperfect that it is not advisable to spend much time and money in fitting them for re-use in the beehive. Consequently, only combs which are really worth using again in the brood chamber should be treated. These combs must be free from honey and all of the cells must be uncapped for perfect results.

The process of treating combs consists of immersing them in a tank containing the disinfecting material for a period of 48 hours. After this time the combs are extracted in order to free them from the disinfecting material. Then these combs are aired for a short period after which they may be used again in the hives. The real service of the disinfecting materials comes in treating super or extracting combs. These combs are usually very good and are well worth saving. Extracting combs may be treated and after short airing used again for the storage of surplus honey.

Having disposed of the frames and their contents, attention should be given next to disinfecting the bottom board, hive body, inner cover and outer cover. A highly satisfactory method is to immerse this equipment in a tank of boiling lye water. This not only kills the disease spores but it cleans the equipment of wax and propalis. The process requires but a relatively short time. After the material is taken out of the lye solution, it should be rinsed quickly with cold water and then stacked so as to air slowly. The lye solution is made at the rate of one can of lye to ten gallons of water. The hives can be painted again and are practically as good as new equipment in the yard. Other methods of disinfecting this equipmenet are not advised.

EUROPEAN FOULBROOD

Origin and History: European foulbrood has been known to beekeepers for a long time. In the early development of beekeeping there was apparently only one foulbrood disease and consequently when the disease was introduced in this country it was known as European foulbrood. Only in the last 20 years has European foulbrood been differentiated from American foulbrood. There has been considerable confusion in regard to European foulbrood for under certain conditions it may slightly resemble American foulbrood.

Distribution: European foulbrood was very serious in the east at the time American foulbrood was determined to be a distinct disease. Since that time European foulbrood has been very devastating in California apiaries. There probably is no definite territory in the United States which is free from disease. In Iowa, it occurs in practically every section, but is decidedly more pronounced in some areas than in others. It is worst where the black bee prevails and it frequently occurs in those areas where there is usually a deficiency of spring honeyflow. There may be out-breaks in unusual territory in times of extremely adverse spring weather.

Common Name: This disease is usually referred to as foulbrood and the designating term "European" is not often used. It is sometimes called "melting" brood. These common names are not entirely sufficient to distinguish it from American foulbrood.

Transmission: European foulbrood is an infectious disease of the bee larvae, caused by a germ called **Bacillus pluton,** and is transmissable from one colony to another. The chief mode of transmission seems to be thru robbing out a diseased colony. There is

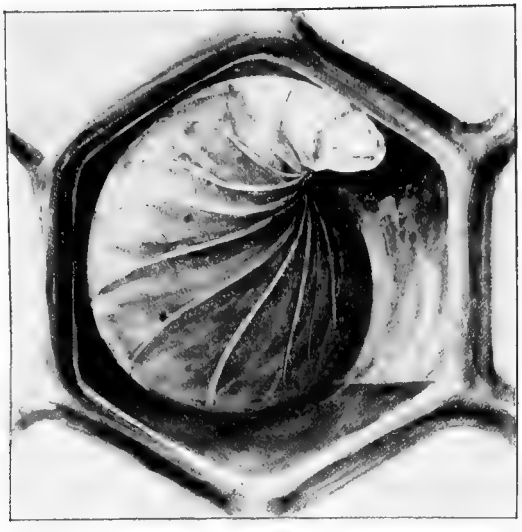

Fig. 4. Movement prior to death in European foulbrood.

not so much likelihood that it is carried by the beekeeper on his hands and clothing as is the case with American foulbrood, nor does empty equipment seem to spread the disease as readily as is the case with American foulbrood.

Diagnostic Characters: Any weak colony in the spring may be suspected and should be carefully examined for this disease. Its presence or absence cannot be determined by the appearance of the adult bees. The infection of the larva probably takes place during the second day of its life, when the infected material is given to the larva with its feed by the nurse bees. The action of the disease is very rapid and the larva dies in about two days after the germ has been introduced into its body. Thus most of the larva die before they have completed the fourth day of growth, or before they have left the bottom of the cell, which means that they are still curled. The appearance of a dead larva is very characteristic. Its general color is that of putty; the larva appears to have melted down, and it has lost its usual shining, glistening and pearly white appearance. After the tissue melts down the tracheal or breathing system of the larva seems to stand out in relief and usually is observed by the beekeepers for the first time. As the larva dries down it develops into a scale, which never has any suggestion of chocolate color as is true in American foulbrood. The scale is loose in the cell so that it can be shaken out by tipping up the comb. The odor of the larva is not particularly characteristic, but is described as "sour". The body contents of the larva are simply "watery" and not "ropy".

Some toher organisms, but principally **Bucillus alvei,** may be found at most any time along with European foulbrbood. These are called secondary invaders and are germs which live upon decaying tissue, after the larvae have died from European foulbrood. Their presence may cause an unusually dark color and there may be a tendency to "ropiness". These secondary germs make a condition midway between those typical of European and of American foulbrood. In such cases it is necessary to use extreme care in making the diagnosis.

Seasonal History: This disease seems to be most prevalent in the later spring, particularly when the weather is exceedingly unfavorable, with cold, damp, raw days prevailing. The disease is not commonly observed after weather conditions become settled in the late spring. Occasionally cases are evident thruout the early summer, but these

are exceptions to the rule. The disease works very rapidly and a colony may become extinct in ten days. Very often a colony dies between the inspections made by the beekeeper in his regular apiary management. If the disease is not severe it may seriously reduce a colony and sometimes there is a tendency to diagnose the condition as physiological rather than pathological. If the disease is not serious, the colony sometimes survives and may build up slowly with the coming of favorable weather in the late spring. European foulbrood is especially severe among the black and hybrid bees. Formerly the Italian race of bees was found to be more resistant and certain strains were evidently more resistant than others. At the present time apparently all strains of Italian bees are really resistant. Weak colonies need special attention, especially the entrance should be reduced so that it will be difficult for robbing to get started.

Treatment: Inasmuch as this disease seems to be more prevalent with the black and hybrid bees, the first step in eliminating European foulbrood is to Italianize all colonies by introducing a queen of a good strain. Wherever colonies are found to be struggling in an effort to rid themselves of the disease, it is advisable to requeen. If the requeening is done in the spring it is well to give a thin feed to further stimulate brood rearing by the new queen. Colonies which have been requeened need special attention and it may be advisable to add a frame of sealed brood from a healthy colony. Since there is not the likelihood of transmitting the disease by the interchange of equipment, it is much easier to handle this disease than American foulbrood. Furthermore, the bees will readily clean out the scales, and cells are apparently made clean enough by the bees so that succeeding larva will not contract the disease. European foulbrood is no longer feared by beekeepers and its chief control measure is "better beekeeping". This term includes the items of introducing better stock and of giving better management in the form of protection and ample stores. Frequent examinations are also a part of better management and enable the beekeeper to detect the presence of disease before it gets a start.

Fig. 5. Tongue adhering to roof of cell.

SACBROOD

Early History: Sacbrood has been known for a good many years, but this name has been applied to the disease in only recent years. It was confused with a condition called "pickled" brood and was not easily differentiated from what is now known as "starved" brood, and "chilled" brood.

Distribution: Sacbrood has occurred quite generally in all localities of the United States and Canada. Its effects have never been serious and consequently it has not received the attention that it really deserves. It occurs frequently in Iowa, with no partciular regard to locality. It is more commonly associated with European foulbrood than with American, but is not so restricted to black bees.

Common Name: The name is quite distinctive of the condition in which the larvae are found after death. The appearance of the dead larvae has been likened to a sack of wheat tightly tied at the top end.

Diagnosis: Sacbrood is infectious, altho the specific organism has not been isolated. It is transmitted by the nurse bees in feeding the young larvae. Sick larvae when used experimentally will produce the disease again in healthy larvae. The larvae die usually after the cell is sealed, but very often the bees have entirely taken away the capping, so it appears that the larvae die before they are capped. The larvae swell until there is no trace of the segments or divisions of the body. Their color might be termed gray, altho in some instances there is a tendency to a yellowish tint, but hardly enough to be called brown. The tightly tied sack appearance is very characteristic. Another characteristic of this disease is that the skin of the larva becomes almost parchment like in texture. Therefore, it is very easy to remove the larva from its cell without puncturing the body wall; in fact, if the comb is tipped up and shaken, the larva will drop out. The contents of the larva are very watery in consistency. These symptoms are very characteristic and are sufficiently different from either American or European foulbrood so that it should be possible to make a diagnosis.

Seasonal History: Sacbrood seems to be more common during the spring and especially when unfavorable weather conditions prevail, as is the case with European foulbrood. It is more common with the black and hybrid bees than with the Italian bees, altho the correlation is not as positive as is the case with European foulbrood. It works much more slowly in the colony than European foulbrood and more often a colony will overcome the disease and perhaps regain in strength by the end of the honeyflow. It is seldom found during the summer honeyflow.

Treatment: Better management seems to lend itself exceedingly well to control of sacbrood. The first consideration is to re-queen the colony which is affected. This can be accompanied with a stimulative feed and it may be advisable to add a frame or two of sealed brood from some strong colony. Under these conditions a colony will recover very rapidly and the disease will probably not appear again in that colony.

MISCELLANEOUS DISEASES

Closely associated with American and European fulbrood and Sacbrood are minor disease and physical conditions which may be confused in making a diagnosis for a brood disease. Before sacbrood was definitely named, beekeepers frequently referred to a so-called disease, "pickled" brood. With the coming of better methods of beekeeping this so-called disease has practically disappeared from the apiary. Occasionally brood will decay in a manner which is not typical of any other brood diseases which have been described. This may be the result of a specific organism, but more often it is a condition rather than a disease. In the majority of cases the term "pickled" brood has been applied to sacbrood and as a specific disease is not of importance.

In Iowa, where spring conditions are especially erratic, the beekeeper may find a condition in the brood nest which is a result of chilling or desertion. A colony may be ready to build up and when a cold spell occurs it is necessary to draw in the cluster so that some of the brood is left to exposure. This brood dies and the beekeeper may be led to feel that a disease is present in the hive and under those conditions the situation will appear more serious than it really is. It is necessary for the beekeeper to keep clearly in mind the typical characteristics of American, European and Sacbrood.

ADULT DISEASES

The diseases of the adult bee have been considered as unimportant to the beekeeper. There are times, however, when conditions develop within the colony which are confusing and the cause of some little loss. Now there is more need for the beekeeper to understand thoroly adult bee diseases than was true formerly.

DYSENTERY

Dysentery is not a disease, but more properly a disorder, of adult bees. It has been frequently referred to in beekeeping literature and in a good many instances it has been confused with true diseases of the adult bee. It is the result of an accumulation of undigested matter which accumulates when the bee is not able to fly. Therefore, this condition is most often observed in bees which have been confined in the cellar for the winter, or in cellar wintered bees which have been placed out of doors before a rather cold wave in the spring. It is evident by the so-called spotting of the hives. Colonies seldom die from dysentery, but inasmuch as the adult bees which have suffered from this condition soon die, the adult population becomes materially reduced in a short time. Inasmuch as this is most prevalent in the spring, a reduction of the adult population is a serious handicap to the colony. The relief is a flight for the bees and in some instances it may be well to requeen in order that new vigor may be introduced into the hive. Usually it is well to give a stimulative feed in order to

TABLE OF DISEASE CHARACTERISTICS

	American	European	Sac
Cause	Bacillus larvae	Bacillus pluton	Filterable virus
Age	After capping	3-4 days	Fully grown
Color	Brown	Gray-yellow	Yellow-brown
Position	Lengthwise	Curled on bottom	Lengthwise
Consistency	Ropy	Slimy, moist	Watery
Odor	Glue pot	Sour	None
Trachae	Not apparent	Outstanding	Not apparent
Skin	Disintegrates	Tough	Parchment
Cappings	Sunken, perforated, discolored	None	Open
Pupa	Tongue adhering to roof of cell	None	None
Scales	Adheres, dark	Thin gray, loose	Brown-free
Casts	Worker, mostly	All	Worker
Season	Summer and fall	Spring	Spring-summer
Spread	Moderate	Very rapid	Moderate
Treatment	Shaking	Requeen	Requeen

encourage brood rearing to the fullest extent. Colonies which are given this treatment recover very rapidly, and often make fine colonies for honey production.

PARALYSIS

Occasionally a beekeeper will observe adult bees on the alighting board that are trembling. If this condition is in an advanced stage the bees are rather shiny in appearance and the body is quite swollen. The bees seem to be making an effort to fly, but are unable to do so. Then they crawl around on the ground in front of the hive in an effort to get on some high point to make another effort to take wing. Being unable to fly, these bees soon develop dysentery and then the disorder above is outstanding. It seems that paralysis is more prevalent among black bees than among Italian. The relief measure is to requeen with a good strain of Italian bees and give a stimulative feed. If this condition has seriously depopulated the colony it is advisable to give a frame of hatching brood from a healthy colony.

NOSEMA

This disease has been confused with the conditions just described and in a good many instances it is quite likely that nosema was the cause of the trouble. It is caused by a specific organism, which is infectious and probably is carried by bees in collecting water for the supply inside the hive. Robbing of diseased colonies is apparently a source in the spread of nosema. It works slowly within the hive and it is quite likely that a good many colonies recover from the disease before it is observed by the beekeeper. The organism which causes nosema attacks the digestive tract of the adult bee, causing a breaking down of tissues and resultant inability to digest food. In a colony which is badly attacked the most evident condition is that of dysentery. Probably the most satisfactory program of relief is to requeen the colony and give additional brood. This will enable the colony population to develop more rapidly than the disease will attack and the chances are that a cure can be effected.

ISLE-OF-WIGHT

This disease does not occur in the United States, as far as the records show at present. However, it may appear and the beekeeper should be alert to detect its presence. This disease has been very severe in Europe and the British Isles. The cause of this disease is a small mite or "chigger", which gets inside of the bee at the breathing pores and there attaches itself and reproduces very rapidly. In time the bee is unable to supply itself with the needed oxygen and inasmuch as it is not able to fly, the dysentery condition readily develops. Affected bees in the last stages are inhabited by countless numbers of the mites. Any suspected material should be examined very carefully.

PESTS

Bee Louse. The bee louse pest has recently become of economic importance in a few eastern sections of the United States. There are records of its occurrence in the west, but apparently it has not established itself very extensively. The beekeeper should be on the lookout for it and should be familiar with the precautions and treatment It has been quite a serious pest in Europe, where it is necessary for the beekeeper to constantly fight to save colonies from its ravages.

The lice attach themselves to the backs of the bees, usually one louse to each bee, and takes its nourishment from the body of the bee. A large population of the worker bees of a colony may be infested with the lice thruout the season. The louse is more apparent in weak colonies and seemingly more abundant in poor seasons. This must be considered as relative, since a strong colony will survive a more severe attack of the pest than a weak colony, and of course all colonies are handicapped in gaining strength during unfavorable weather conditions. The lice work sometimes on the drones, but not nearly as extensively as on the workers and the queen. Frequent examination of a colony should enable the beekeeper to determine the presence of this pest. At the present time, the control measures are rather indirect and not entirely satisfactory. When the infestation is slight, it seems advisable to destroy the adults of the colony. The medical treatments which have been proposed do not seem to be wholly satisfactory.

Bee Moths. The moths have been a pest of bees since the earliest beekeeping times. They are found wherever bees are kept. It is interesting that a pest which is so old as the bee moth should persist for centuries without any apparent natural factors of control. There are two kinds of moths, but the greater bee moth is by far the more common than the lesser. Their habits vary and of course their size should enable the beekeeper to determine which species is prevalent.

Greater Bee Moth: This is now considered mostly as a pest of poorly kept apiaries. The bee moth is not the cause of any condition in the apiary, for it appears after other facts or mismanagement have occurred. Weak colonies are subject to the attack of this pest and black bees seem to be more susceptible than Italian bees. The pest is considered as a secondary invader of the beehives and consequently the first factor in the control is to have all colonies with Italian bees and as strong as possible.

The moth flies entirely at night, generally during the evening twilight. At this time it is possible for the moth to gain entrance to the hive, especially in a weak colony which is poorly guarded. The eggs are preferably laid on the comb and when the young larvae hatch they burrow thru the cell wall to the mid-rib of the comb. Here they are quite secure from the attacks of the bees and their work can progress with little disturbance. Their feeding areas are well protected with a very tough silken thread, so that when they are once established in a comb it is very difficult for the bees to reach them to fight and destroy them. The larvae, when their growth is completed, may leave the comb and spin their cocoons between the end bar and the hive body, or perhaps on the underside of the inner cover. These cocoons are parchment like and serve as a most excellent protection. When the combs become badly infested the bees apparently lose all hope of the fight and in some cases leave the combs to the ravages of the worms. In well managed apiaries the bee moth is able to maintain itself only thru its work on stored combs. It seems to preserve its numbers so that whenever a colony is weakened it is ready prey for the moth.

The control measures are first to keep all colonies in strong condition and in numbers and then direct the energy toward protecting the stored combs. The combs which are not on the beehive should be examined to detect the early work of this pest. The combs can be fumigated and for this purpose carbon bisulphide has been quite satisfactory. This material is effective against all stages of the moth, but it must be remembered that this material is inflammable and every possible precaution is necessary to keep it away from all possible sources of fire. One should be more careful even than in handling

gasoline. The fumigation should not take place in any residence or outbuildings which carry insurance, for fire which may result from the use of carbon bisulphide will nullify the insurance policy. Carbon bisulphide is not effective in average doses at temperatures below 70 degrees, and loses most all of its effectiveness below 50 degrees. Carbon bisulphide is a liquid which evaporates very rapidly and the resulting gas being heavier than air goes to the bottom of the container. All material which is to be fumigated should be stacked in bodies or supers and the bisulphide should be poured on a towel or cloth placed on the top bars of the top body of the stack. A safe rule is to use one-half ounce of carbon bisulphide for each 10 frame Langstroth hive body. Of course the pile should not be over eight bodies high and as many of the cracks should be eliminated as possible.

The **lesser bee moth** is primarily a pest of stored combs and especially of honey in storage. This moth works primarily on the cappings. All stages of this pest can be controlled by fumigation with carbon bisulphide in the manner just discussed.

Ants. There are at least three general classes of ants common to the bee yard. A large black ant is sometimes found between the inner and the outer cover of a colony. They apparently do not molest the bees in any way, but seek these locations in order to take advantage of the escaping heat from the bee hive to assist in developing their young. A small black ant and a small brown ant at times enter the hives in search of food, which is usually honey. These ravages are not severe, but it is well to destroy the ants if they are numerous or likely to prove a nuisance.

The best method of destruction is to trace the ants to their nest. The entire population can be destroyed by fumigating with carbon bisulphide. Fumigation should be done late in the evening, when most of the ants are normally in the nest. A sharp stick can be thrust into the center of the nest and the carbon bisulphide poured into this hole. The amount will depend upon the size of the nest, but two or three ounces will be sufficient for the average sized nest. To make the fumigation more effective it is well to place a damp sack over the nest to further direct all of the fumes down into the nest. In handling carbon bisulphide, use the necessary precautions to prevent fire.

A relief measure is to construct legs for the hive stand and place these legs in a small tin cup which contains a heavy oil. This prevents the ants from gaining entrance to the hive, but of course it does not reduce the supply of ants in the apiary.

Mice. Many beekeepers do not seem to appreciate the loss which is caused by mice getting into the hives. The mice cause the greater portion of their damage by getting into the colonies during the fall and spending the winter in the hive for whatever protection may be afforded. When the mice get inside of the hive they make very careful preparations to construct an efficient nest for winter protection, usually in one of the lower back corners of the hive. At this time of the year the bees are not very vigorous in their efforts to repel the invaders. The damage caused by the mice is in comb destruction, probably to secure feed.

If supers are stored out-of-doors the mice may establish themselves in these combs during the summer. Precaution should be taken to prevent the mice from getting into the hives during the early fall. For this purpose it is well to insert in the entrance of the colony a piece of hardware cloth, which can be lightly tacked in place during the early fall period. This will prevent the mice from getting into the hive, but will provide ample facilities for the bees to fly.

BEE-YARD SANITATION

Every practice about the apiary at all times should be such as to prevent robbing. At no time should frames of honey be taken from a colony which may be diseased and given to another colony for food. If there is any possibility of robbing, one should never open a diseased colony. It is best to remove diseased colonies from the apiary as soon as discovered. Carelessness is the cause of most of the spread of these diseases. There are some who feel that if a frame does not contain diseased larvae it is safe for use in another colony. Never use combs which have come from a region where foulbrood is known to exist.

No beekeeper can expect to eradicate American foulbrood from his apiary who is careless and neglects at any time to remove all possible sources of infected honey or combs.

Failure to eradicate may be attributed to:

1. Indifferent manipulation during the treatment.
2. Improper attention to hospital colonies.
3. Failure to remove all infected honey from equipment.
4. Exposure of diseased combs of honey to robber bees.

The beekeeper can save much trouble and expense by using every precaution to prevent disease from gaining a foothold in the apiary. Honey should never be fed if there is any question as to whether it came from a disease-free colony. Bees should never be purchased without an inspection certificate. A weak or dead colony should not be robbed out at any time. It may contain disease, but even if it doesn't, robbing is a serious handicap to get started in the yard. The use of old combs and second hand equipment is a dangerous practice. Second hand hives and equipment should be treated in hot lye water before they are used for disease-free colonies.

FOULBROOD LAW

The law which was passed by the 37th General Assembly of Iowa was amended by the 41st General Assembly. It permits the state apiarist to make inspection of bees and buildings which may harbor equipment suspected of containing disease material. The law provides that directions shall be left for the treatment or destruction of disease material. If such treatment is not completed within ten days the same can be made by the state apiarist and the cost charged and collected as taxes. The law is sufficiently clear that disease can be fought in a territory with an expectation that the disease can be eliminated. Copies of the law may be secured upon request. Those interested in inspection should communicate with the State Apiarist, Ames, Iowa.

(MARCH, 1937

Ontario Department of Agriculture

ONTARIO AGRICULTURAL COLLEGE

BEE DISEASES

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VIEW OF APIARY
ONTARIO AGRICULTURAL COLLEGE

CONTENTS

BEE DISEASES

INTRODUCTION

Diseases of bees are divided into two main classes, namely, brood diseases and those which attack the adult bee. Although adult bee diseases have caused great damage in sections of Europe, so far they have not been serious in any parts of North America.

The three important brood diseases of Ontario are American Foulbrood, European Foulrood and Sacbrood. In general these diseases have quite similar characteristics. Whereas European Foulbrood and Sacbrood are easily controlled, there is no cure for American Foulbrood. To avoid serious losses it is essential that beekeepers become sufficiently informed to correctly diagnose all three diseases.

Through the destruction of infected colonies American Foulbrood causes an annual loss of about four thousand colonies in Ontario. Besides the loss in bees, honey and equipment, many beekeepers become discouraged and sell their bees or, which is worse, neglect their colonies, leaving them as a source of infection to other people's bees. Although some parts of the Province have suffered from disease more than others, no area is outside their range.

Due to the inspection system in Ontario, disease has been held in check and beekeeping on a commercial scale is made possible. Complete eradication, however, can never be attained until every beekeeper becomes his own inspector. It is the aim of this bulletin to place before the beekeeper in a clear and simple form the essential facts regarding identification and control of bee diseases. Do not let disease destroy your colonies while waiting for the inspector. Learn symptoms and keep the colonies disease-free at all times. If not sure of your diagnosis, send a smear to the Apiculture Department, as explained later.

EXAMINATION OF COLONIES

Control of disease is best effected by giving the colonies two thorough inspections each year. The first examination should be made in the spring at the beginning of the fruit bloom and dandelion flow before the first super is added. If disease is controlled at this time the loss of one or more supers is avoided. In addition to this, the disease does not get a chance to spread to other colonies and cause trouble later on in the season when there is surplus honey in the hives. Any infection picked up by bees robbing diseased colonies during the fall and early winter is usually evident and may be brought under control before it spreads too far.

A second thorough examination is advisable during the summer just prior to the removal of the light honey crop, particularly where disease is suspected in the supers. When the bee escapes are being placed on the hives, very little additional effort is required to examine the brood combs. Finding disease at this time allows the beekeeper to keep diseased supers away from clean equipment. Colonies having disease during the fall will likely die before spring and act as a potent source of infection to other colonies.

Detection of disease is much easier on a bright day. When examining a frame shake most of the adhering bees into the hive. By standing in such a manner that the sun shines into the base of each cell, little difficulty will be experienced in detecting abnormalities of the brood. A thorough

examination in the above manner should always be given before interchanging frames of brood or honey with another colony.

Prevention of robbing from dead and weak colonies in the early spring is one of the most important duties of a beekeeper in the control of disease. All dead colonies should be examined on the first visit to the yard, and diseased colonies destroyed. If this is not possible, dead colonies should be made absolutely bee-tight, or, more preferably, removed from the yard. It is believed that much disease is spread by robbing from dead colonies in the spring.

It is impossible to control disease if colonies are kept in box hives Infection cannot be detected unless combs are removable. Box hives are therefore a menace to the industry. The Foulbrood Act demands that all bees in such hives be transferred to movable frame hives.

NORMAL DEVELOPMENT OF HEALTHY BROOD

There are two types of insect development. In the case of the grasshopper for example, the egg hatches into a young insect or nymph very similar in appearance to the adult. This is called incomplete metamorphosis. The second type of development, under which the honey-bee is classed, is called complete metamorphosis. The honey-bee egg develops into a small white grub or larva. This later spins a fine cocoon and goes into the pupal or transition stage from which it emerges a fully developed bee.

In order to detect unnatural conditions of the brood the beekeeper must know the life history and the appearance of healthy brood at every stage of its development. A study of the life history chart in conjunction with Figure 1 will give the story of this development.

AVERAGE LIFE CYCLE OF THE HONEY BEE

Stage	Queen	Worker	Drone
Egg	3 days	3 days	3 days
Larva	5½ days	6 days	6½ days
Pupa	7½ days	12 days	14½ days
Total	16 days	21 days	24 days

The egg of the bee is a small, white cylindrical object about 1|10 of an inch long, somewhat larger at one end (future head end) and slightly curved. It is deposited on the base of the cell by the queen and is fastened in place by a secretion.

The larva, at first very small, grows rapidly, and in a few days occupies the whole of the base of the cell. The healthy larva before being sealed lies curled up in the base of the cell and is a glistening pearly white colour, with the segmentation of the body clearly shown. Three or four days before the larva actually transforms to the pupal stage, the cell is sealed by the nurse bees. The curled larva straightens out in the cell and spins a thin cocoon preparatory to transformation to a pupa. Figures "a" and "b" show the egg and larval stages before capping, "c" and "d" the larval and pupal stages after being sealed.

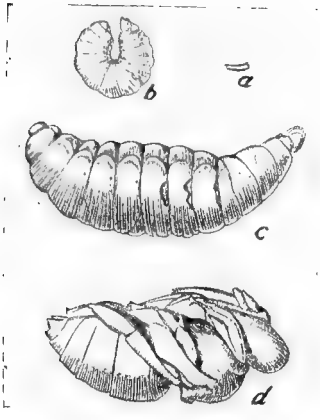


Fig. 1.

The honey bee: a, Egg; b, young larva; c, old larva; d, pupa. Three times natural size. (U.S. Dept. of Agr. Farm Bulletin 447.)

AMERICAN FOULBROOD

The term "American" is used simply to differentiate this disease from other brood diseases. It does not imply either origin or location of the disease.

(a) Symptoms

Appearance of diseased comb. If disease has been present in a colony for some time the comb will assume a mottled or pepper-box appearance. This is caused by open cells containing dead larvae or scales being interspersed with normal brood. This mottled effect is also characteristic of other diseases.

Condition of cappings. Normal healthy cappings are light brown in colour, becoming slightly darker with age. They are somewhat rounded or convex in shape. If a diseased larva or pupa is present in the cell the capping frequently becomes sunken and dark brown in colour. Many cappings are perforated with one or two small holes by the bees as though they were investigating the tardy emergence of the brood.

Time of death. Death occurs from American Foulbrood almost invariably two days before or two days after the transition to the pupal stage at which time the cells are capped.

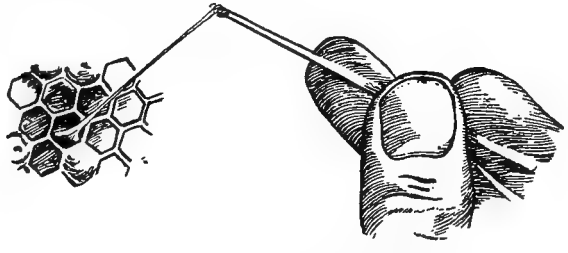
Position in the cell. Larvae lie curled in the cell at the time capping takes place. After the cell is sealed the larva straightens out, lying flat along the bottom of the cell. Three to four days after capping the change to a pupa takes place. It is within two days either before or after this transformation that death occurs. Due to the uniform position in the cell at this time the decaying brood settles in a regular uniform mass, to the bottom of the cell. This characteristic is contrasted to European Foulbrood where the decaying brood is usually twisted in the cell.

Colour changes. Healthy larvae are pearly white in colour. Decay-

ing larvae dead of American Foulbrood are first a light yellow brown. As they settle down in the cell the colour changes gradually to a dark, coffee brown, which is of uniform shade over the entire body surface.

Appearance of affected pupae. Pupae dead of the disease go through the same process of decay and settling and similar colour changes as the larvae. The tongue of the pupa is nearly always extended towards the top wall of the cell. This is a definite sign of American Foulbrood.

Ropiness of American Foulbrood. American Foulbrood destroys the larval or pupal tissue. The body wall soon becomes soft and easily ruptured. Dead brood passes through several stages of decay, each stage varying in colour, shape and consistency. After about three weeks of decay, until the scale is formed, a characteristic ropiness is exhibited. During this period a toothpick inserted into the mass will draw out a fine gluey thread of decaying matter. (Fig. 2.)



The ropiness of American Foulbrood
(U.S. Dept. Ag., Far. Bul. 442)

Although this ropiness is a definite characteristic of American Foulbrood, it should be remembered that it only occurs at a certain stage of putrefaction. When a suspicious cell is found during inspection, all other symptoms should be carefully observed before inserting a toothpick and destroying the larva or pupa for further inspection. Too many beekeepers have the habit of inserting a toothpick immediately they see a suspicious cell.

This destroys other important symptoms.

American Foulbrood odour. In the early stages of American Foulbrood no odour is evident. In advanced stages, however, where much brood is dying and the disease has been present for weeks, the odour is quite distinct. The smell is characteristic, but is probably best described as resembling that of heated glue.

The value of odour is overlooked by many experienced beekeepers. Even when a small amount of infection is observed a diseased larva may be removed on a match or toothpick and an effort made to detect the characteristic odour. Some people have a very highly developed sense of smell and with such this point is a great help in diagnosis.

Formation of a Scale. After four to five weeks the decaying brood dries down to a hard dark scale. American Foulbrood scales are characteristically uniform in shape, covering the greater portion of the lower cell wall and extending part way up the back wall. The scale adheres tightly to the cell and cannot usually be removed without tearing the cell wall.

(b) Cause of American Foulbrood

In 1902 Dr. G. F. White, United States Department of Agriculture, first demonstrated that the disease was caused by a bacteria which he named Bacillus larvae. Certain bacteria have the ability to form a protective covering around themselves. In this stage, called spores, they are capable of existing for long periods away from their host. Bacillus larvae is a spore forming bacteria. It will live for years in honey and will often withstand boiling in water for twenty minutes. This spore forming characteristic makes B. larvae very difficult to exterminate.

(c) Method of Infection

When honey is stored in cells containing scale the spores of American Foulbrood become dispersed through the honey. Larvae fed with this diseased honey become infected and death occurs a few days later. Thus the cycle is maintained.

(d) Methods of Spread

American Foulbrood is spread chiefly by robbing infected honey from diseased colonies, either dead or alive. Great care should be taken to see that dead or weak colonies are not robbed out in the spring. The entrances of these colonies should be closed or the hives removed to a bee-tight dwelling about the first of April. This precaution is extremely important. It is possible for one diseased colony to infect a whole apiary. Infection may also be carried from one hive to another by drifting of the nurse bees, especially during manipulation of the colony. Great care should be taken in interchanging combs from one hive to another. Stray swarms should be hived on foundation rather than on drawn comb. Then if diseased honey is present in the crops of the swarming bees it is used up in drawing out the foundation, rather than being stored and later fed to the larvae.

Bee trees are often given too much credit for the spread of disease. Although colonies in trees may spread American Foulbrood, such swarms which become weakened and die from disease do not long remain a menace. They are soon cleaned up by wax moths, ants and other insects or animals.

(e) Immunity to American Foulbrood

Although workers are most commonly infected, drones and queens are also susceptible to the disease. From the results of experimental inoculations and beekeepers' experience it appears that no race of bees possess any marked immunity to the disease.

(f) Eradication of American Foulbrood

The old shaking treatment, whereby the bees of a diseased colony are shaken on to foundation and thus saved can no longer be advocated. In fact, it has been found that the treatment in general tends to spread disease rather than eradicate it. When combs are shaken there is a tendency for young nurse bees, not having marked their location, to fly into nearby hives carrying diseased honey with them. Another criticism of the treating system aside from general spread of disease at the time of treatment is that diseased combs are often stored for some time before

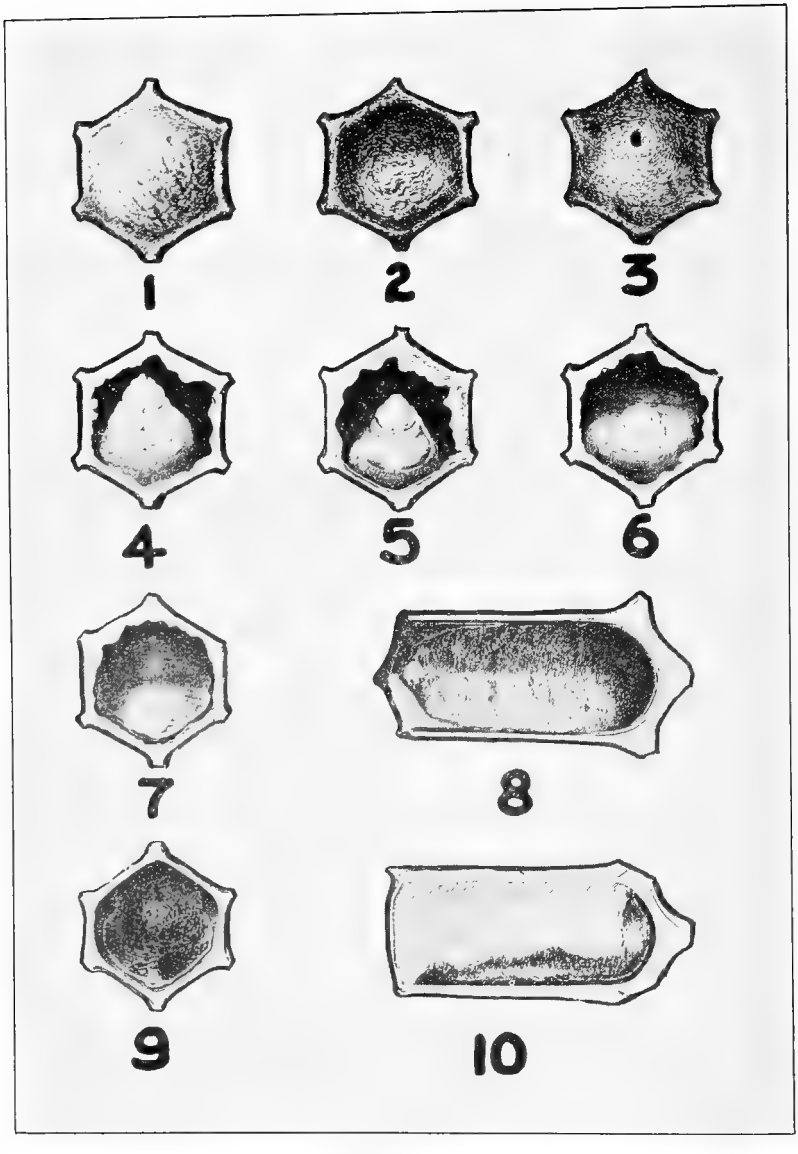


Plate 1.

American Foulbrood death occurring in the larval stage: 1. Healthy capped cell; 2, 3. Capped cells containing dead larvae; 4, Healthy larva; 5, 6, 7, 8. Progressive decay; 9, 10. dry scales.—(Reproduced from U.S.D.A. Bulletin 809.)

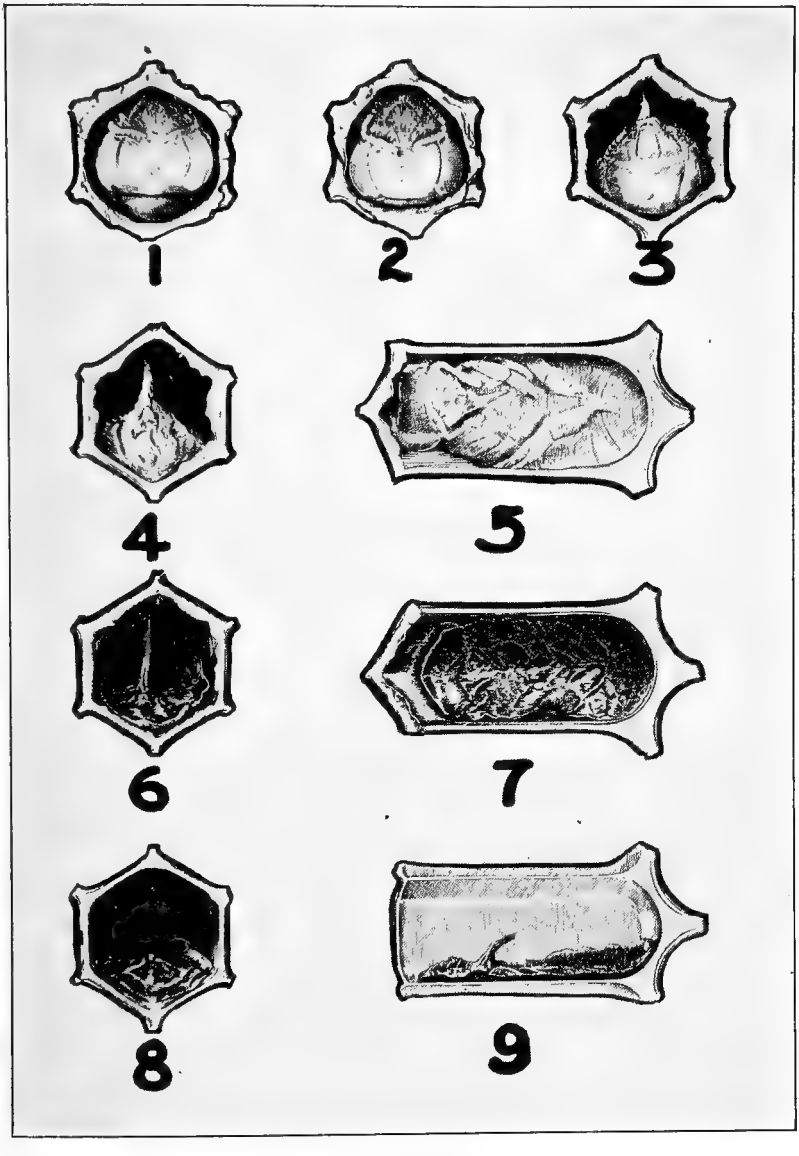


Plate 2.

American Foulbrood, death occurring in the pupal stage: 1. Healthy pupa; 2, 3, 4, 5, 6, 7. Progressive decay of dead pupae; 8, 9. Dry scales.—Reproduced from U.S.D.A. Bulletin 809.)

being rendered. This is very dangerous as there is always a grave danger of infection by bees robbing stored combs. The treating plan proved to be false economy. It is cheaper to kill diseased bees and make increase from healthy colonies.

Burning diseased colonies. The only known method at present by which American Foulbrood may be completely stamped out is by burning the bees and combs and sterilizing other parts of the hive by fire. Disease has been entirely cleaned up in restricted areas by this plan and there is every reason to believe it could be completely eradicated from the Province. Immediately the disease is found it should be destroyed. Delay is generally costly.

Considerable work has been done in an effort to find some drug or chemical that would effectively cure a colony of American Foulbrood. So far there has been no success along this line.

Steps in Eradicating American Foulbrood

1. Inspect the colonies giving each frame a thorough examination. If disease is found, mark the colonies carefully so there will be no possible danger of overlooking them later.

2. Dig a hole in the ground large enough to accommodate all the combs. Unless the hole is large diseased honey will be spilled around the surface of the soil and spread disease. Do not dig the hole too near a tree or the fire will wilt the leaves and injure the tree. Allow for the wind and be sure the fire is situated where there is no danger of spreading. Place some rough chunks of wood in the hole to ensure a good draft and sufficient fuel to thoroughly consume all diseased material.

3. Kill the bees. In the past gasoline was used for this purpose but it is not at satisfactory as cyanogas, which is a commercial preparation of cyanide in powder form. When gasoline is used the bees fly from the hive unless the entrance is closed. Cyanogas kills the bees without their realizing any danger. Field bees will also fly into the hive. When using cyanogas sprinkle a dessert-spoonful on top of the frames and a little in the entrance to catch the incoming bees. Five minutes is long enough to leave the colony after adding the cyanogas, otherwise many of the bees recover as the powder becomes spent and the gas drifts from the hive. Great care should be taken when using this poison as a few good breaths might easily prove fatal. There is no necessity to wait until evening to kill the colonies.

4. Light the fire. It is advisable to use coal oil and get a good strong flame so the diseased material may be disposed of as quickly as possible.

5. Carry the hives complete to the fire and throw on the combs. Be sure no dead bees drop from the entrance as most of them exude a drop of honey which sticks to their tongues and may be diseased. If the combs are heavy with honey keep the fire well bolstered up with chunks of wood.

6. Scrape thoroughly and disinfect by scorching all boxes, bottom boards and covers. Covers and bottom boards may be sterilized by

holding them over the fire for a few seconds, on a fork or long stick. Boxes may also be treated in this manner or else piled one on top of the other sprinkled inside with coal oil or gasoline and ignited. The flame may be extinguished by placing a cover on the top box, thus excluding the air.

7. All dead bees and other debris that might carry infection should be scraped into the fire. When everything has burned down to an ash, fill the hole in thoroughly.

8. Hive tools should be disinfected with fire and the hands washed with soap and water before examining other colonies.

Disease in Super Combs

Chemical treatments such as the use of formalin, chlorine, etc., which where once recommended for diseased super combs have been definitely proven unsatisfactory. If a beekeeper is suspicious of his super combs the best practice is to render them into wax and have it made into foundation. The actual cost of this procedure is not great and it is considered sound economy by many successful beekeepers.

EUROPEAN FOULBROOD

Less than forty years ago this disease caused the beekeeping industry considerable anxiety. It spread with remarkable rapidity, wiping out whole apiaries. Today beekeepers have learned that it can be easily controlled by keeping the colonies strong and using resistant Italian stock. It is no longer a menace to the industry.

(a) Symptoms

The appearance of larvae dead of European Foulbrood varies considerably and the symptoms are more variable than those of American Foulbrood. Always keep in mind the appearance of healthy brood when inspecting for indications of disease.

Appearance of comb. The presence of many uncapped cells, containing disease larvae in the early stage of development, gives the comb a spotted irregular appearance. Should the majority of larvae die at a later stage of development after the cells are sealed, the cappings are sunken, perforated and decidedly greasy in appearance.

Time of death. All larvae die before the transformation to the pupal period. About ninety percent die one or two days before the cell is capped. Probably ten percent die the first or second day after capping, i.e. when the larva is beginning to straighten out in the cell preparatory to its transformation to the pupa. Occasionally an outbreak of European Foulbrood takes place where colony after colony contains larvae most of which are attacked at this later stage. This type is often confused with American Foulbrood. The variability displayed in time of death makes diagnosis more difficult. In typical European Foulbrood, however, the cell is uncapped.

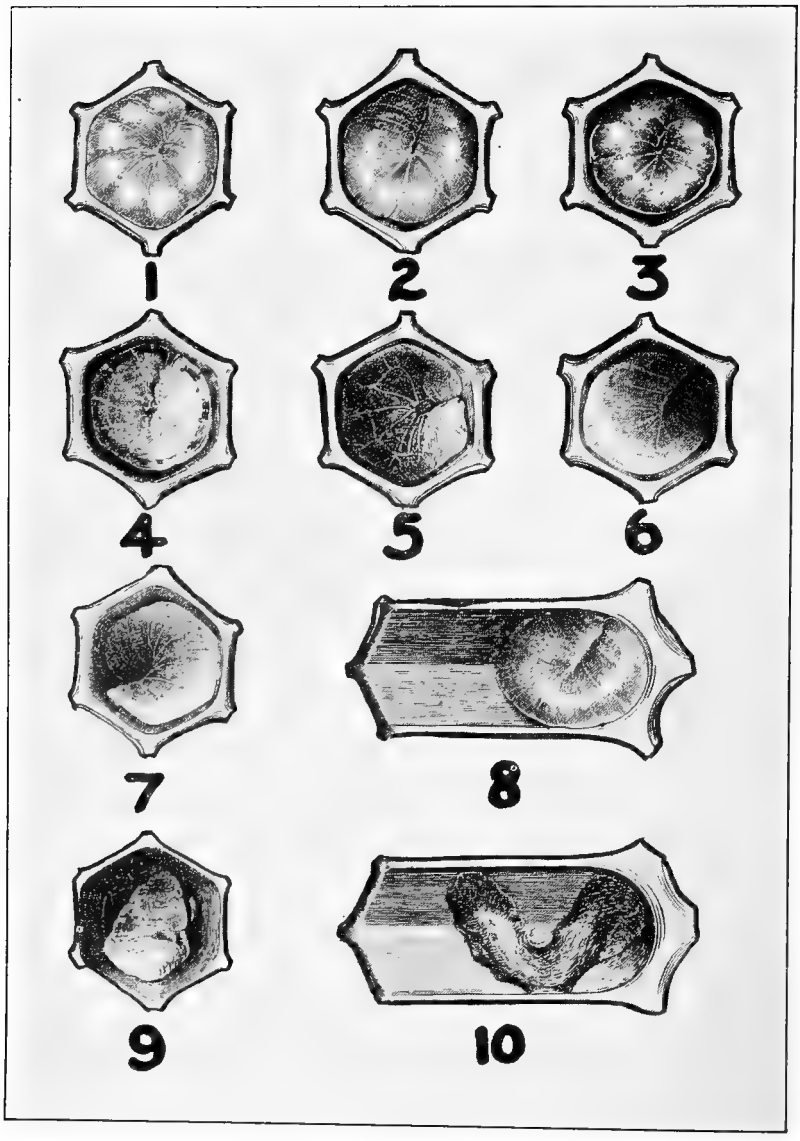


Plate 3.

Characteristic European Foulbrood, death occuring in the early larval stage: 1. Healthy curled larva; 2 to 10. Various stages of decay; 4, 5. Shows tracheae; 6, 7. Larva twisted in cell; 9, 10. Dried out scale.

—(Reproduced from U.S.D.A. Bulletin 210.)

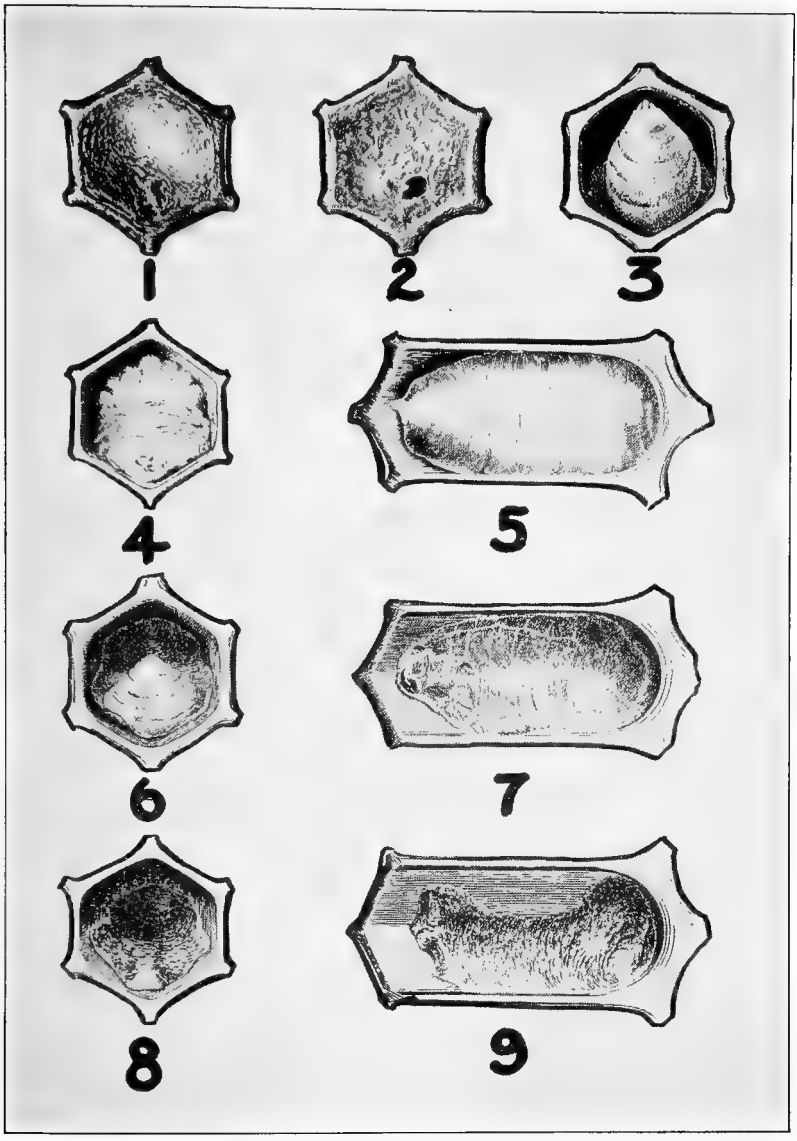


Plate 4.

European Foulbrood, death occurring in the late larval stage: 1, 2. Capped cells containing larvae dead of European Foulbrood; 4. Dead larvae showing part removed by the bees; 3, 5, 6, 7. Various stages of decay; 8, 9. Dried out scales.—(Reproduced from U.S.D.A. Bulletin 810.)

Position in the cell. Larvae exhibit marked variation in their position according to the age at which they die. Young larvae dying at the characteristic stage while still curled generally squirm around as though in pain assuming unnatural positions in the cell. Those larvae that die after capping are larger and lie more uniformly extended in the cell.

Colour changes. The pearly white colour of healthy larvae changes to greyish yellow. As putrefaction continues the colour deepens to a dark greyish brown mass.

Consistency of dead larva. After approximately three weeks of decay the larva becomes a sticky, somewhat granular mass. The granular appearance at this stage is contrasted to the smooth glue-like appearance of American Foulbrood.

Tracheae visible. The tracheae are glistening, silvery air tubes situated below the skin. In many larvae dead of the disease these tracheae may be plainly seen and remain visible during the complete process of putrefaction. The presence of tracheae is an important symptom to differentiate infection in the advanced larval stage from American Foulbrood.

European Foulbrood odour. In advanced cases, especially where many larvae die in the capped over stage, there is a very offensive odour. It is described as resembling that of rotten fish.

Scale formation. The dried larval remains are less brittle and more rubber-like than American Foulbrood scales. They are shrivelled, brown in colour and can easily be removed from the cells. The fact that brood dead of European Foulbrood can be removed by the bees makes it possible for strong colonies to clean up the disease.

(b) Cause of European Foulbrood

Bacillus pluton, a non spore former, is considered to be the organism which actually causes the death of the larva. Invariably, however, there are secondary organisms present, the chief of which is Bacillus alvei. When a laboratory diagnosis is made the presence of Bacillus alvei indicates European Foulbrood.

(c) Methods of Spread

The organism causing European Foulbrood does not form spores so is unable to live over winter in honey. It is carried over in pieces of dead larvae. From this small beginning in the spring it can spread very rapidly under favourable conditions. If nectar is stored in contaminated cells and fed to larvae they contract the disease. Further spread may be caused by robbing, interchanging combs or drifting nurse bees.

(d) Immunity to European Foulbrood

Workers, drones and queens are all susceptible to European Foulbrood. Italian bees, due to their vigorous house cleaning habits, stop the spread of disease and eventually eradicate it. Some strains are far more efficient in cleaning out dead larvae than others. Black bees are very susceptible to the disease.

(e) Eradication

European Foulbrood has not caused very serious trouble since beekeepers have learned the present preventive measures. Most definite progress in its control has been made through Italianizing all colonies and breeding queens from resistant stock.

It must be remembered that European Foulbrood is a disease of weak colonies. The introduction of an Italian queen to a very weak colony is useless if European Foulbrood is present. Weak colonies should be united to stronger ones before requeening. If resistant Italian queens are used and the best methods of beekeeping, which ensure strong colonies, are followed there will be little trouble with European Foulbrood. A colony at the beginning of the honey flow should be strong enough to have eight full combs of Langstroth size filled with brood.

Proper wintering of bees is a matter of highest importance in regions where European Foulbrood is found. As very little infection is carried over the winter the first brood of the year usually escapes with little loss. For this reason it is essential to have as much brood as possible get away to a good start early in the spring. The emerging bees are then able to ward off the disease throughout the season.

Where the disease has become well established, it is sometimes difficult for the bees to make headway in cleaning it up. Removal of the queen for a few days causes a period in which no brood is being fed. This gives the bees a chance to make more rapid progress. As soon as the dead larvae are removed, the queen is returned or better still the colony is given a young Italian queen.

How the disease spreads is not thoroughly understood. Honey has a definite devitalizing effect upon the organism so is not a serious carrier. It is never necessary to destroy or disinfect combs, brood, or honey from European Foulbrood colonies. Normally requeening and strengthening the colony will satisfactorily control the disease.

SACBROOD

Sacbrood is an infectious disease of the brood of bees. Although it is not particularly malignant and rarely, if ever, causes the death of a colony, it is responsible for the loss of much brood. Where the disease is advanced the death of many worker larvae results in the weakening of the colony. Brood rearing space may also be considerably reduced by the presence of dead larvae in the cells.

The disease has never proven very serious in Ontario but it is essential that beekeepers be able to differentiate between it and other brood diseases, especially American Foulbrood.

(a) Symptoms of Sacbrood

Character of comb and cappings. As in other brood diseases the presence of affected brood, interspersed with healthy brood, gives an irregular appearance to the comb. Larvae die after the cells are capped.

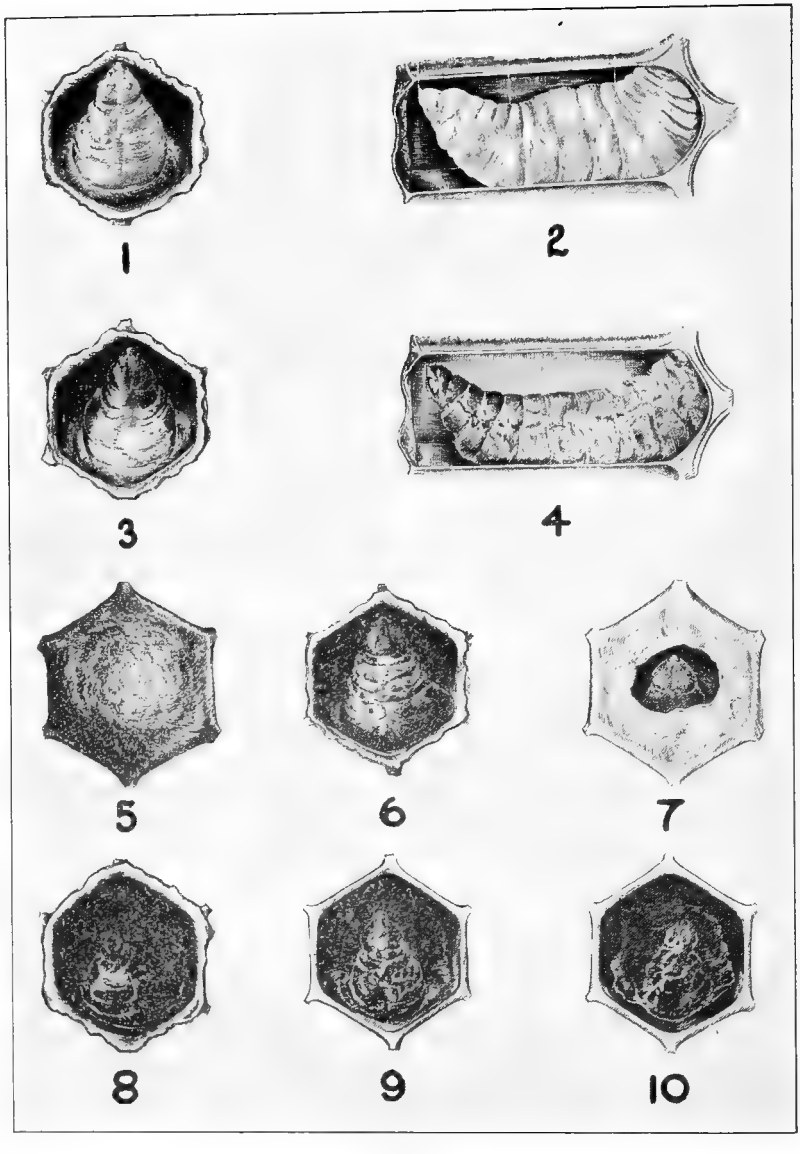


Plate 5.

Sacbrood: 1, 2. Healthy brood at the age at which it dies of Sacbrood; 3, 4. Brood recently dead of disease; 5, 6, 7, 8, 9, 10. Brood in various stages of decay.

—(Reproduced from U.S.D.A. Bulletin 431.)

COMPARISON OF BROOD DISEASES

	A. F. B.	E. F. B.	Sacbrood
Cappings	Many sunken, dark and perforated.	Usually none. When larvae in advanced stages of development, capping, sunken, perforated and greasy.	Many uncapped; some perforated.
Time of Death	During prepupal stage or within two days after transformation to pupa.	Before change to pupa. Generally quite early and before capping. Advanced larval stages often capped.	Before change to pupa. After capping.
Position in the Cell	Straightened out, regular and uniform. Occupies most of lower cell wall. Posterior, or hind end, extending up back wall of cell.	90% of cases curled in the cell and in unnatural positions. 10% in advanced larval stages fairly regular and extended in the cells.	Uniform shape and position in cell with head end curled up.
Colour Changes	From light yellow-brown to dark coffee-brown. Uniform throughout.	From greyish-yellow to dark greyish brown. Not uniform.	From a light yellowish grey to dark brown. Head end darker.
Appearance of Pupa	Same process of decay as larva, tongue extended upwards.	None die.	None die.
Consistency	Soft mass. Exhibits ropiness at certain stage.	Somewhat sticky and granular.	Body wall tough, contents watery.
Tracheae	Not visible.	Often plainly visible.	Not visible.
Odour	Odour of heated glue in advanced stages.	Fishy odour in advanced stages.	No odour.
Scale	Hard, dark, uniform scale. Adheres tightly to cell.	Rubber-like, irregular, shrivelled, dark brown scale. Easily removed.	Black, roughened curled up at anterior or head end.

A considerable proportion of the cappings are uncapped by the bees. As a rule the proportion of brood affected is not large.

Time of death. Affected larvae die after the cells are capped but before the change to the pupal stage is completed. The majority die during the two days prepupal period, i.e. within the two days preceding the transformation. Many uncapped cells are generally observed on diseased combs but they are uncapped by the bees after the death of the larvae.

Position in the cell. Dead larvae are extended lengthwise along the floor of the cell. The position is similar to that of larvae affected with American Foulbrood. Sacbrood, however, can be determined by the appearance of the head which is dark, somewhat shrivelled and turns up towards the roof of the cell.

Colour changes. Soon after death the larval remains are slightly

greyish white in colour. This greyish colour turns to a greyish brown tint which deepens as the process of decay continues. Throughout this period of decay the head end appears somewhat shrunken and much darker than the more posterior, or hind, portion of the body.

Consistency of dead larva. The body wall of a larva dead of Sacbrood becomes toughened and may be easily removed intact from the cell. When removed from the cell the larva is sac-like in appearance. The contents of the sac are watery, containing many fine brown granules.

Odour. Sacbrood has no distinctive odour.

Scale formation. Scales formed from sacbrood are greyish black, roughened and generally curl up at the anterior or head ends. Scales are not common as the bees generally remove the dead larvae before the scale is formed. When present they are loose in the cell.

(b) Cause of Sacbrood

No organism has been found present in dead larvae which can be demonstrated to cause the disease. It has therefore been concluded that the disease is caused by a virus which will pass through the finest of filters. A colony may be inoculated with the disease by feeding syrup or honey containing the virus from dead larvae.

(c) Spread of Sacbrood

Sacbrood virus is readily destroyed. Larvae dead of Sacbrood cease to be infectious after one month. How the disease winters over is not known. Colonies infected in the spring generally recover during the honey flow. The weakening effect of the disease during the spring stays with the colony throughout the honey flow.

(d) Control Measures

Vigorous colonies rarely suffer to any extent from Sacbrood. Requeening and strengthening will generally clean up any infection that may occur. It is never necessary to destroy any part of a hive infected with Sacbrood.

FUNGOUS DISEASES

Fungous diseases, although never particularly serious, cause considerable damage in the aggregate loss of bees. There are many types of fungi affecting bees both as brood and adults. Conditions such as the so-called stone brood and chalk brood are typical of the work of fungi. The dead remains become dry and mummified. Molded combs and equipment and mouldy fruits, etc., are believed to cause the infection.

DISEASES OF ADULT BEES

To date there has been no serious trouble from adult bee diseases in any part of Canada or the United States. In England and other parts of Europe they have caused great loss. It is necessary that we know something of the symptoms of these diseases and be ready to stamp out any infestation that might occur.

(a) Isle of Wight Disease

This disease has been a very serious source of loss to British beekeepers. It is caused by a minute mite which crawls into the bee's spiracles choking off its supply of air and possibly secreting a toxine which paralyzes the wing or flight muscles. Most adult bee diseases have quite similar symptoms, making diagnosis from external characteristics difficult.

The following is taken from leaflet No. 253, issued by the Board of Agriculture and Fisheries, 4 Whitehall Place, London, England:

Symptoms:

1. The first symptom noticed is a disinclination to work. They fly about aimlessly and do not gather stores.

2. Later they lose their powers of flight and are unable to travel more than a few yards without alighting.

3. As the disease progresses, the bees are unable to fly more than a few feet, when they drop and crawl. They may be seen crawling up grass stems or other upright objects, but they soon fall down and die. Towards night some may be seen gathered in groups, but they usually die before morning.

4. The abdomen is often swollen.

5. The wings often appear disconnected. Sometimes the legs seem affected, and the bees stagger in their attempt to walk.

6. Finally the whole colony of workers is found clustered in front of the hive, except a few which are found crowded around the queen.

7. The queen and the brood are not attacked, though "chilled brood" often appears subsequently, owing to there being insufficient bees to keep the hive warm.

(b) Nosema Disease

This disease although fairly widespread is mild in character. It is caused by a protozoan or one celled parasite, Nosema apis, which infests the alimentary canal of adult bees. Bees can be infested with Nosema spores without showing any marked symptoms of disease, and apparently with little effect on their ability to carry on. Death may result from infection and in the case of excessive infection bring about a weakening effect on the colony through the shortening of the life of the individuals. Bees are often observed crawling around outside the hive or climbing up blades of grass. Requeening and strengthening by giving additional capped brood usually brings relief. Cases of this disease have been known in Canada.

(c) Dysentery

Dysentery is more properly a disorder than a disease of adult bees. Bees are only able to void faeces while in flight. During the winter waste matter accumulates in the lower intestine. If the food is of good quality and the bees are not confined to the hives for too long a period, they will be healthy in the spring. If the food contains considerable indigestible material and the bees are unable to fly they often die in great

numbers. In cases of advanced dysentery faeces are voided within the hive and the disorder becomes evident by the resultant spotting.

Material reduction in the adult population is a serious handicap during the spring building-up period. Dysentery can be prevented to a great extent by proper precautionary measures. Bees wintered on good honey or sugar syrup are rarely affected. Poor food, such as honey-dew or honey of high water content, nearly always produces the disorder. Proper winter protection and freedom from disturbances during winter are also factors in the control of dysentery.

(d) Paralysis

Very little is known about bee paralysis. It is possible that there are several conditions which result in the so-called paralysis. At any rate beekeepers frequently report that they have observed worker bees crawling in front of the hive with their abdomens trembling. They keep crawling up the side of the hive and up blades of grass and tumbling to the ground. Occasionally individual colonies become rapidly depleted in bees. There is a possibility that the disease may be caused by certain foods.

(e) Spring Dwindling

Spring dwindling is the term used to describe the condition when adult bees in a colony die off more rapidly than they are replaced by emerging brood. The condition is brought about by poor wintering and by the colony going into winter with too large a percentage of old, worn-out bees. To prevent this colonies should be supplied with vigorous queens that will continue brood rearing as late as possible in the fall.

SENDING SAMPLES FOR DIAGNOSIS

Brood is frequently found dead in a colony from causes other than infectious disease. We find chilled brood, starved brood and overheated brood. Generally the appearances are characteristic but at times the symptoms may be quite similar to one of the brood diseases. If in doubt a sample of comb, or more preferably a smear consisting of the diseased larva or pupa, folded in a piece of waxed paper, should be sent to the Apiculture Department, Ontario Agricultural College, for examination. Also, if a beekeeper notices adult bees showing symptoms of disease he should send a good number to the Department for examination. Bees that have been dead for for some time are not satisfactory for examination. If pieces of comb are sent no honey should be present and the comb should not be crushed.

PESTS OF THE APIARY

(a) The Greater or Common Wax Moth

This is a pest of weak colonies and of stored combs. It is most prevalent in warm climates. The adult moths fly almost entirely at night. The eggs may be laid in masses between the supers or in corners and cracks within the hive. The tiny larvae emerge within a few days and being extremely active soon disperse throughout the hive. In brood combs they burrow along the midrib, out of reach of the bees, spinning a

tough cocoon as they travel. These larvae feed chiefly on pollen, pupal cases and other impurities in the comb. It is thought that very little, if any, wax is digested. When confined to wax and honey as in comb honey infestations, the larvae are unable to develop into the adult stage. Adult moths do no damage to comb.

Combs left exposed act as breeding places for this troublesome pest, and the beekeepers should be careful to keep all combs out of reach of the moth.

When weak colonies are attacked the beekeeper should remove as many of the bee moth larvae as possible as well as the webbing and cocoons. The colony should then be united with a strong colony. Italian bees are more resistant than black bees. Strong colonies are rarely infested. Weak or dead colonies in box hives form excellent breeding grounds for moths.

Various disinfectants can be recommended for the control of wax moth in stored combs:

Sulphur. The amount recommended is 3 pounds per 1000 cu. ft. of storage space, burned for 24 hours in a room made as air-tight as possible. As the sulphur dioxide fumes given off do not destroy the eggs it is necessary to fumigate again two to three weeks later.

Its use incurs considerable fire hazard. Fire Insurance under the Ontario Beekeepers' Association is not available to those who disinfect with sulphur. It is most conveniently used in the form of flowers of sulphur. To use, obtain a large flat pan and place some water in it. Stand the pan in which the sulphur is to burn inside this on two bricks. Place live coals in this pan and put the flowers of sulphur on top. Supers are criss-crossed in piles.

Carbon bisulphide. Is a yellowish oily liquid which gives off a very disagreeable odour. It may be used quite effectively, although the gas does not kill the eggs, and as is the case with sulphur it is necessary to give a second treatment two to three weeks later. The method used is to place two tablespoonfuls or one ounce of the liquid in a small dish and place this *on top* of a stack of five supers. For best results, the cracks between the supers should be closed by means of gum paper, or they may be effectively sealed by moistening strips of newspaper and sticking them around the supers. The stack should remain sealed at least 12 hours. At low temperatures this evaporation is very slow, so it is necessary to have the room fairly warm.

Every precaution should be taken when using this disinfectant, as the gas given off is highly inflammable. When a dish of carbon bisulphide is used on the top super it is necessary to close off the pile by means of an empty super and lid.

Cyanogas. Is a commercial cyanide preparation which is extremely poisonous to humans and animals. Great care should be taken when using it. The gas given off is almost the same weight as air. Cracks in the pile of supers should be closed up and about one tablespoonful of the powder may be placed on a piece of paper somewhere in a pile of five

supers. Be careful not to inhale any of the fumes. It is advisable to do the fumigation outside or in a well ventilated room. Do not tear the supers down for twelve hours. *Do not use cyanogas in a dwelling house—it is extremely poisonous.*

Paradichlorobenzene (P.D.B.) Is one of the most satisfactory insecticides that can be recommended for wax moth control. It is a white crystalline material, with a not unpleasant odour. It is non-injurious to humans at ordinary concentration. It is non-inflammable and very easy to handle.

The gas given off is heavier than air, so a handful of crystals is added on a piece of paper at the top of a stack of five supers. More crystals can be added occasionally throughout the storage period. It is most effective at around 70 degrees F.

Storing Combs. Where much trouble is experienced from mice and wax moth, it is advisable to store the supers of comb in stacks of five with a metal lid or queen excluder at the top and bottom of each pile. One of the various disinfectants should be used and cracks sealed up as far as possible. The most suitable disinfectant recommended for general use is Paradichlorobenzene (P.D.B.). When disinfecting supers, best results are obtained if the stacks are not made more than five supers high. Where the danger of wax moth is not particularly great beekeepers stack the supers up with a sheet of newspaper between each super. Printers' ink apparently acts as a repellant to the wax moth.

Comb Honey. Wax moth larvae can very soon spoil a season's crop of comb honey. Moths will lay their eggs in the cracks between the supers; on hatching the young worms crawl to the comb honey and destroy the wax capping. If trouble with wax moth is expected it is advisable to remove the honey from the colonies as soon as possible. Place 8 to 10 shallow frame supers of honey one on top of the other and use similar fumigation methods as used for stored combs. P.D.B. is particularly recommended for use with comb honey. Some beekeepers use sulphur for this purpose, as the fumes tend to bleach wax, thus making the cappings whiter.

(b) Other Moths

The larvae of several moths destroy comb. The Mediterranean Flour Moth and Indian Meal Moth larvae sometimes damage comb when burrowing for pollen. The larvae spin silken threads similar to those of the Common Wax Worm, but do not cause as much damage to the comb.

The Lesser Wax Worm does damage similar to the Common Wax Worm, except that the webs are finer and more generally on the surface of the comb. This is not an important pest in Canada, but causes considerable damage in warm climates.

These moths can be controlled by fumigation in the same manner as recommended for the Common Wax Worm.

(c) Skunks

Skunks are troublesome in most sections of Ontario. They will scratch in front of a hive to annoy the bees. When the bees rush out they become entangled in the skunk's fur and are rapidly eaten. Skunks also

do much damage in winter by destroying tar paper and exposing hives packed with this material. The control recommended for skunks is to make a small hole in an egg at the air cell end. Then stir one grain of strychnine well into the egg with a tooth pick or small piece of wire. A grain of strychnine is about as big as a pin head. The egg is then half buried near the hive where the skunk can get at it. Every precaution must be taken when using strychnine. Where there is danger of poisoning domestic animals, the egg should be placed under the hive or some place where larger animals cannot get at it. *Persons placing out strychnine in this manner are responsible for any damage that may result to livestock.*

(d) Mice

Bee yards in all parts of the Province are troubled by mice. These pests will destroy comb and build nests alongside the winter cluster in good colonies. The disturbance will generally result in the loss of the colony. They will also build nests in stored super combs during the winter. In some sections chipmunks will do similar damage. Winter entrances should be constructed small enough that mice cannot enter, and piles of supers in storage should be protected both above and below with queen excluders. Poison grains are effective as a means of control. A commercial poison bait known as "Mouse Seed" may be obtained from the Bee Supply Companies, or a mixture of strychnine and grain may be prepared at home. This is done by dissolving 25 grains of strychnine in about 1½ pints of water and placing this in a large container. Completely soak some grain with this liquid. The grain must be dried before using, otherwise it will mold. A small amount of strychnine may also be combined with icing sugar and then mixed with flour or crumbs, or spread on bread.

(e) Other Pests

Pests of lesser importance in Ontario include bears, ants and kingbirds. A stencil sheet of Apiary Pests and their control may be obtained free on request from the Apiculture Department, Ontario Agricultural College, Guelph.

PERMITS TO MOVE OR SELL BEES

All beekeepers are asked to co-operate to the fullest extent with the Apiculture Department in the matter of obtaining permits to move or sell bees.

In the past great losses have been experienced in many sections of the Province, and disease has been introduced into many disease-free areas through the criminal carelessness of certain beekeepers in promiscuously moving diseased colonies or equipment.

The slight inconvenience and possible delay of a sale that sometimes has to occur before a permit can be granted is dwarfed to insignificance by the fact that co-operation in this matter is absolutely essential if disease is ever to be brought under control in the Province.

Persons guilty of moving or selling bees without a permit are subject to a fine of not less than $50.00 nor more than $100.00 under the Foulbrood Act. Apiary Inspectors are of the opinion that this section of the Act must be strictly enforced if we are going to hold what we have gained through efficient inspection.

If intending to move or sell bees or equipment write to the Apiculture Department for one or more application forms. These applications to move or sell should be filled out in detail. When the application is received at the Department a permit is sent to your local Inspector. The Inspector in turn issues the permit when he is convinced that the colonies or equipment are free of disease.

When bees or equipment are being sold and moved to a new location only one permit is required to cover sale and removal. This should be procured by the seller.

PRECAUTIONS

(1) Never buy colonies of bees unless they have been examined by an authorized Inspector and a permit granted by the Department of Apiculture.

(2) If drawn combs, irrespective of age, are purchased with used apiary supplies, they should be rendered into beeswax, unless positive no American Foulbrood existed in the apiary from which they came.

(3) Be sure to prevent spring robbing by closing the entrances of all dead colonies early in April, thereby avoiding the spread of disease. The entrances to weak colonies should also be reduced.

(4) Combs should not be interchanged from one colony to another where there is danger of foulbrood being present.

(5) Destroy all colonies having American Foulbrood immediately they are found. Make sure your diagnosis is correct before proceeding with the destruction.

If in doubt remove the contents of one or more cells and fold in a piece of waxed or hard finished paper. Place this, with your name and address, in an envelope and forward to the Apiculture Department, Ontario Agricultural College, Guelph, for diagnosis.

(6) Since honey is one means of transmitting American Foulbrood, it should not be fed to bees unless it is known to be free from Foulbrood spores. When in doubt feed sugar syrup.

(7) When locating an apiary don't crowd any other beekeeper. Never keep bees in box hives. The control of American Foulbrood is the beekeeper's most important problem. In order to remain in the business and ensure success, constant vigilance must be maintained.

THE BEE DISEASE ACT

Bees are of greater economic importance as pollinators of fruit trees, clovers and other crops than they are as honey gatherers. Because of the very important place of bees in agriculture the Foulbrood Act has been drawn up to safeguard the beekeeping industry. Its purpose is to combat disease and protect the careful beekeeper.

An Act for the Prevention of Diseases Among Bees

(INCLUDING AMENDMENTS WHICH CAME INTO FORCE ON MARCH 31, 1931)

1. This Act may be known as **The Bees Act, 1931.**

2.—(1) The Lieutenant-Governor in Council, upon the recommendation of the Minister of Agriculture, may from time to time appoint one or more Inspectors of Apiaries to enforce this Act.

(2) The Inspector shall, if so required, produce the certificate of his appointment on entering upon any premises in the discharge of his duties.

(3) The remuneration to be paid to an Inspector under this Act shall be determined by order of the Lieutenant-Governor in Council, and shall be payable out of any sum appropriated by the Legislature for the enforcement of this Act.

2a.—(1) Every person keeping bees in the Province of Ontario shall on or before the 30th day of April in every year apply to the Minister of Agriculture in writing, signed by the applicant, for a certificate of registration.

(2) The application shall be in such form as may be prescribed by the regulations and shall be accompanied by the prescribed fee for registration.

(3) Every application shall be addressed to the Provincial Apiarist, Ontario Agricultural College, Guelph.

(4) Where a person commences keeping bees after the 30th day of April in any year, he shall apply for a certificate of registration as hereinbefore provided within ten days after coming into possession of the bees.

(5) Every person keeping bees who neglects or refuses to comply with the provisions of this section shall incur a penalty of not less than $5 nor more than $10 and costs.

3.—(1) The Inspector shall, whenever so directed by the Minister, visit any locality in Ontario and examine any apiary to which the Minister directs him, for the purpose of ascertaining if any infectious or contagious disease of bees exists in such apiary.

(2) If the Inspector finds that foul brood exists in a virulent or malignant type he may immediately destroy by fire all colonies of bees so affected, together with the hives occupied by them and the contents of such hives and all tainted appurtenances that cannot be disinfected.

(3) Where the Inspector, who shall be the sole judge thereof, finds that an infectious or contagious disease, not being foul brood of a virulent or malignant type, exists among the bees he shall give notice in writing to the beekeeper, instructing him as to the treatment of such disease and stating the time within which such treatment shall be given, and if at the expiration of such time the diseased colonies have not been treated by the beekeeper in accordance with the notice, the same may be treated by the Inspector and the beekeeper shall be liable to the Inspector for all expenses incurred in such treatment.

4. The Inspector may order the owner or possessor of any bees dwelling in box or immovable frame hives to transfer them to movable frame hives within a specified time, and in default the Inspector may destroy or order the destruction of such hives and the bees dwelling therein.

4a. For the better prevention of foul brood, the Lieutenant-Governor in Council may, on the recommendation of the Minister, declare a quarantine of bees at any point within the Province and may fix the duration of such quarantine and all other conditions in connection therewith, and any Inspector appointed under this Act shall have full authority to inspect bees in such quarantine when directed so to do by the Minister.

5.—(1) The owner or possessor of an apiary shall not sell, barter, give away or

remove from the premises any bees or used apiary appliances or apparatus until he has secured a permit from the Provincial Apiarist that such bees, used apiary appliances or apparatus have been properly disinfected and are free from disease.

(2) Bees or used apiary appliances or apparatus shall not be imported into Ontario from any other Province in Canada or from any State in the United States of America unless accompanied by a certificate from a provincial or state officer certifying that such bees, used apiary appliances or apparatus are free from any infectious or contagious disease, but this shall not apply to the importation into Ontario of bees apart from combs.

(3) Every person who contravenes the provisions of subsection 1 or of subsection 2 of this section shall be guilty of an offence and shall incur a penalty of not less than $50 nor more than $100.

6. Any person whose bees have been destroyed or treated for foul brood who sells or offers for sale any bees, hives or appurtenances of any kind after such destruction or treatment and before receiving a permit from the Provincial Apiarist so to do, or who exposes in his bee-yard, or elsewhere, any infected comb honey or other infected thing, or conceals the fact that such disease exists among his bees, shall incur a penalty of not less than $20 or more than $50, or he may be imprisoned for a term not exceeding two months.

7. Any owner or possessor of bees who refuses to allow the Inspector to freely examine bees, or the premises on which they are kept, or who refuses to destroy the infected bees and appurtenances, or to permit them to be destroyed when so directed by the Inspector, shall, in the complaint of the Inspector, incur a penalty of not less than $25 and not more than $50 for the first offence, and not less than $50 and not more than $100 for the second and any subsequent offence, and the convicting justice shall, by the conviction, order the said owner or possessor forthwith to carry out the directions of the Inspector.

8. Where such owner or possessor of bees offers resistance to or obstructs the Inspector, a justice of the peace may, upon the complaint of the Inspector, cause a sufficient number of special constables to be sworn in who shall, under the directions of the Inspector, proceed to the premises of such owner or possessor and assist the Inspector to seize all the diseased colonies and infected appurtenances and burn them forthwith, and if necessary the Inspector or constables may arrest the owner or possessor and bring him before a Justice of the Peace to be dealt with according to the provisions of the next preceding section.

9. Before proceeding against any person before a Justice of the Peace, the Inspector shall read over to such person the provisions of this Act or shall cause a copy thereof to be delivered to him.

10. Every owner or possessor of bees, and any other person who is aware of the existence of foul brood either in his own apiary or elsewhere, shall immediately notify the Minister of the existence of such disease, and in default of so doing shall incur a penalty of $5.

11. Each Inspector shall report to the Minister as to the inspection of any apiary in such form and manner as the Minister may direct, and all reports shall be filed in the Department of Agriculture, and shall be made public as the Minister may direct or upon order of the Assembly.

11a. The Minister of Agriculture, with the approval of the Lieutenant-Governor in Council may make regulations,—

(a) prescribing the form of application for registration;

(b) for fixing the fees to be paid for registration and upon a certificate of registration;

(c) for the registration of beekeepers and prescribing the form of the register and the particulars to be entered therein;

(d) for requiring beekeepers to make such returns and to furnish such information to the Department as may be deemed necessary or desirable;

(e) generally for the better carrying out of the provisions of this Act.

12. **The Ontario Summary Convictions Act** shall apply to all prosecutions for offences against this Act.

REPORT

OF THE

Maryland State Beekeepers' Association

TWENTY-SEVENTH ANNUAL MEETING

Baltimore, January 15,

1936

Reprinted from the 1935 Annual Report of the Maryland Agricultural Society—The Maryland Farm Bureau Federation.

REPORT

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TWENTY-SEVENTH ANNUAL MEETING

OF THE

Maryland State Beekeepers' Association

January 15, 1936

Baltimore, Maryland

The Twenty-Seventh Annual Meeting of the Maryland State Beekeepers' Association was held on Wednesday, January 15, 1936, in conjunction with the Maryland Agricultural Society-Maryland Farm Bureau Federation meetings in the Lord Baltimore Hotel, in Baltimore.

The meeting was called to order by President S. R. Damon at 7.00 P. M. He gave the usual address by the President.

Following this Dr. E. N. Cory, Secretary-Treasurer, made a report.

Mr. George J. Abrams, Specialist in Apiculture, University of Maryland Extension Service, gave the following address:

REPORT OF EXTENSION ACTIVITIES IN APICULTURE FOR 1935

By George J. Abrams

Specialist in Apiculture

The work of the Extension Specialist in Apiculture was unfortunately curtailed during the summer of 1935 because of a serious bone infection of the right hand. It was unfortunate that this disability occurred at the busiest time of the year, necessitating a suspension of all work for the month of July and rendering inspection work after July 1 impossible.

The inspection work suffered most from this incapacitating of the extension worker. The program of work as submitted at the beginning of the year was not followed as outlined, but was altered considerably to meet the exigency.

Educational Work

The greatest need of the Maryland beekeeper is instruction in bee be-

havior and the fundamentals of colony manipulations and apiary management. With this need in mind, more time was allotted to this part of the yearly program than to the other phases of the work. Beekeepers in all parts of the State, many of whom are just learning of the existence of a State worker in apiculture, indicated an increased desire for instruction. They want this instruction in the bee yard where personal instruction can be given, where they can ask questions and can be actually shown how things should be done. The extension worker has expressed the belief in previous annual reports that there is no adequate substitute for this method of teaching bee culture and that whatever else is done can be only of an auxiliary nature.

General Work in the Apiary

In working with the beekeeper in the apiary, it was the policy not only to help him with the things for which he requested help, but to outline a yearly plan of colony manipulation and apiary management. It is believed that the most valuable instruction and information given to the beekeepers was dispensed in this way. First the apiary site is discussed and recommendations made for altering it if advisable. Then the yearly cycle of work is discussed, starting with the preparation of bees for winter, continuing with spring management, swarm prevention, control practices, methods of supering, removal of the honey crop. This brings to mind many questions which the beekeeper often would not have remembered to ask at the time. Next, the problem of equipment is gone over thoroughly and recommendations made. The preparation of honey for the market is discussed and recommendations regarding pack and suggestions as to outlets are made. Grading, according to the U. S. Standards and Grades for honey, is always urged. Finally all beekeepers are urged to use the cost account sheets prepared by the extension worker. Great difficulty has been experienced in getting the beekeepers to use these record sheets or even record sheets of their own devising. A few men are using them and it is hoped that each year the number can be increased.

Requeening

One of the greatest specific needs of Maryland beekeeping at the present time is better stock. A large majority of the colonies in the State need requeening with new queens of good blood. There can be no question but that a general requeening in the State would greatly increase the State average of colony honey production, cut down winter losses and bolster up the whole tone of Maryland beekeeping. At every opportunity, in the apiary, at beekeepers meetings, and through letters and publications, this thought was stressed, as it is felt that this is a fundamental need of prime importance.

It had been the plan of the extension worker to actually do as much of

this requeening as possible in an effort to get the work started, the plan being that the beekeeper should buy queens for a small percentage of his colonies, and the extension worker would do the actual work of introducing them. With this initial help and instruction it was felt that the beekeepers would then be able to finish the job of requeening the remainder of the apiary without further help. This plan was just started when it was necessary to abandon it because of injury. This work will be resumed during 1936. Already a number of beekeepers are awaiting notice, from the extension worker, of when to order queens from the south.

ELIMINATION OF BOX HIVES

Through direct extension work and its collateral effect, more and more box hives are being transferred over into modern movable frame hives each year. This phase of the work will of necessity progress slowly because of cost of new equipment, but with the extension worker and county leaders constantly demonstrating the increased profits to be derived from modern hives, it can be expected that gradually the box hive will be eliminated through education and not make oft proposed legislation necessary.

TWO-STORY BROOD CHAMBER

In conjunction with this quiet steady drive to eliminate box hives, the value of the two-story brood chamber is always advocated. Much success has been made in getting beekeepers to use two brood chambers in place of one. The method of attack has been to urge, almost on a sporting basis, that the beekeeper try out several colonies with the two brood chambers, and run them in competition with one brood chamber hives. Instruction in colony manipulation under the two chamber method is given and the success from the extension and production standpoint has been gratifying. The greatest drawback to this change in colony management is the lack of enough equipment.

EDUCATIONAL WORK ON DISEASE AND PESTS

Work done in the control of the bee diseases and pests is reported fully elsewhere in this report. However, much educational work is accomplished each year in teaching beekeepers to recognize the various bee diseases and pests and how to combat them. Talks were given at beekeepers meetings, instructions were given in the apiary and literature has been sent out, all in an effort to familiarize the beekeepers with the diseases and pests before they encounter them, so that should the apiary become infested or infected they will be able to recognize the trouble immediately and before the condition has a chance to spread.

THE POLLEN BASKET

Even since extension work in apiculture was started in Maryland, a need has been felt for some sort of extension paper through which the extension worker could reach every beekeeper on the mailing list period-

ically, with timely information, notices of meetings, market reports, and other information of general interest. Such a paper was started during 1935 as a monthly, the first issue being sent out in February. It has been continued each month since then.

This paper is called "The Pollen Basket." It is mimeographed, and during 1935 varied in size from two to four pages. An effort was made to prepare a paper that not only carried specific information and timely suggestions, but which would be newsy and readable.

One of the commonest complaints registered against the large established trade journals and government bulletins and bee books is that they are written to be read by beekeepers throughout the country, and often do not apply to the specific conditions of a locality. This of course is unavoidable, as literature with nation-wide circulation must contain general information. "The Pollen Basket" is, therefore, slanted especially for the Maryland beekeepers. It takes the general fact or practice and reshapes it specifically for the Maryland beekeepers.

In all modesty, the extension worker wishes to report that this paper has been almost enthusiastically received. Numerous unsolicited letters attest this fact.

"The Pollen Basket," aside from its educational value in giving definite information relative to Maryland bee culture, has assumed the place of official publication for the State beekeepers and has done much to bring the State group together.

Orchard Pollination

Work with the Maryland fruit growers was of necessity limited only to those orchardists requesting help. With the limited travel funds for apiculture being exhausted each year before all necessary work with the beekeepers can be accomplished, it was decided unwise to attempt any sort of campaign with the fruit growers calling for added expense. The second factor determining this policy is the present shortage of colonies in the State available for orchard rental. There is no point in working up a market that cannot be supplied.

A number of the larger fruit growers are already using bees for pollenizers. Those requesting help were given assistance and several others becoming interested in this phase of orchard management were visited and advised regarding number of colonies needed and methods of securing them.

One radio talk was given in the spring regarding the use of bees as pollenizers in orchards, and an educational display pertaining to bee culture was set up at the annual meeting of the State Horticultural Society.

Honey Marketing and Publicity

In general, it can be safely said that the Maryland beekeeper is not seriously troubled in marketing his crop. The market is as a rule slow, but steady, and it is the usual case that the great majority of the producers sell out their crop before the new crop is produced. There are numerous exceptions to this rule, especially where beekeepers live in isolated parts of the State. This steady demand for honey should be expected in an Eastern State near the large retail markets, and especially in a State embracing a city the size of Baltimore, and in such close proximity to Washington, Philadelphia and New York.

The price of honey has remained good with considerable variation between retail city markets and retail small town markets. Under either condition, however, honey can be sold at a profit.

It is believed that the prevailing fair prices and steady market demand will be reflected in greater State production of honey. This increase can easily be consumed locally.

NATIONAL HONEY WEEK

Some really worthwhile State-wide advertising of honey was done in Maryland in conjunction with the national observation of Honey Week. The extension worker directed this work in Maryland, and took active part in placing exhibits and radio broadcasting.

First, publicity about Honey Week was given through "The Pollen Basket," and beekeepers all over the State were urged to set up local displays of bee products in stores and other suitable display places. A number of beekeepers responded with attractive displays so that the smaller towns and Baltimore city were well covered.

The State worker contacted two large bakeries; one in Cumberland and one in Hagerstown, both users of large quantities of honey in their bread mixtures, and obtained their cooperation in securing advantageous display space in stores selling their bread. Local beekeepers were then contacted and in each town cooperators were secured to supply enough comb and extracted honey to set up an attractive display. An observation hive was supplied for each display by the extension worker and the live bees were furnished by local beekeepers. The extension worker set up these exhibits at the beginning of Honey Week. They included comb and extracted honey, an observation hive, beekeeping equipment and bread made with honey.

The bakeries were also asked to allow the extension worker to use their time on the local broadcasting stations. They consented and three talks about bees and honey were given on Monday of Honey Week, in Hagerstown, and one talk was given the next day in Cumberland.

In addition to these radio talks, two talks about honey were given at the regular weekly University of Maryland Extension Service's period over Station WBAL, Baltimore, on the two Fridays immediately preceding Honey Week.

The Home Demonstration Agents, through their leader, were asked to observe Honey Week by publishing recipes, using honey, in the local papers and by demonstrating the uses of honey in their work of that week.

The students in apiculture at the University arranged a very attractive display of honey (supplied by a local beekeeper) in the salesroom of the Dairy Husbandry Building at the University.

BAKERIES

Three of the largest bakeries in the State, outside of Baltimore City, using honey were contacted in an effort to gain an outlet for the darker grades of Maryland honey. One bakery, using roughly 75,000 pounds of honey per year, and formerly buying from New York brokers has started buying Maryland honey. Another is now also buying locally as far as they can be supplied. The third bakery is also buying some local honey but being located in a comb-honey area the local extracted honey supply is limited.

FAIRS AND DISPLAYS

Two fair exhibits were set up during 1935 by the extension worker; one at the Timonium Fair, the other at the Harford County Fair. The honey was supplied for both exhibits by the beekeepers and was for competition. The large U. S. D. A. display telling the story of honey was obtained and used at the Timonium Fair Exhibit.

Through the Washington and Frederick County Beekeeper groups, the fair officials of the Hagerstown and Frederick Fair Associations are being contacted as an effort to open premium lists for bee products and it is hoped to add these two large fairs to the list of fair exhibits next year.

Inspection, Disease Control and Pests

BROOD DISEASES

Inspections for brood diseases were limited to those apiaries in which infection was found the previous year. These apiaries were given an inspection during the spring, seven yards, including two hundred and eleven colonies being examined.,

It had been planned to conduct an area clean-up campaign in seven counties, an over ambitious plan rendered impossible not only by the physical incapacitation of the extension worker through illness, but because of lack of funds.

However, much educational work on bee diseases and pests was accom-

plished through personal talks to beekeepers. This is a subject of vital importance to beekeepers in general, and one in which all are interested whether they have had experience with disease or not. As a consequence, considerable time was usually spent in describing the disease, its manner of spread and control measures, to beekeepers on personal visits. Two talks were given at meetings on American foulbrood at which time diseased material was exhibited.

PESTS

An increased number of inquiries were received this year in regard to the Greater Wax Moth, a pest inhabiting bee hives. Help was given when requested and two talks at beekeepers meetings were given explaining the life history and control of the moth.

Another serious pest of honeybees, the bee louse, *Braula coeca*, was discovered and investigated in Montgomery County, and was also located in Washington County. This pest was found to be doing considerable damage to comb honey, rendering sections unfit for sale. Thirty-nine (39) visits. were made to Montgomery County apiaries in an attempt to determine the extent of the infestation. The infested area appears to lie between the Frederick pike and the Potomac river in that area north of Rockville as far as Sugarloaf Mountain. Six widely scattered apiaries in this region were found to be definitely infested, and others are suspected.

Work With the Maryland State Beekeepers' Association

Further progress was made during 1935 in organizing new county groups of beekeepers into chapters of the Maryland State Beekeepers' Association. Particular success was experienced in Washington, Frederick and Montgomery counties. In these three counties are located a large percentage of the Maryland beekeepers.

In Washington County, two meetings were held and the group formally organized themselves into a chapter of the State Association. This group plans to meet once a month with the extension worker. As a group, they cooperated on the Hagerstown Honey Week exhibit as their initial group venture. They also plan an exhibit at the local Food Show and have made the necessary overtures to the county fair officials to obtain a premium list for bee products.

This group is interested in having the extension worker hold regular bee culture classes in Hagerstown, which will be done in 1936.

The Frederick and Montgomery county groups have not formally organized and elected officers; but will do so at their first 1936 meeting. The beekeepers in both of these counties have nearly all been contacted by the extension worker in personal visits and through meetings, and are ready to organize and are desirous of obtaining instructions through bee culture classes.

The Eastern Shore Chapter of the Association, organized last year, met once during July, and the Baltimore group originally comprising the whole of the State Association, held five meetings; one the annual meeting in conjunction with the Maryland Agricultural Society-Maryland Farm Bureau meetings, one summer field meeting and three indoor winter meetings.

These Baltimore meetings were well attended by both beginners and experienced beekeepers. Having two classes of beekeepers present at the same meeting has made it difficult to arrange programs suitable and interesting for both, as a beginners program proves unprofitable and uninteresting to the experienced man and the advanced program goes over the heads of the beginner.

It is hoped to alleviate this situation during 1936 by holding a beginners class in Baltimore, primarily for the novice in addition to the regular association meetings.

PARTIAL-DRONE-LAYING QUEENS

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Before taking up the discussion of partial-drone-laying queen bees, it seems desirable to review briefly for comparison the work of normal fertilized queens and of unfertilized queens. Queens that have mated normally lay only fertilized eggs in worker and queen cells and unfertilized eggs in drone cells and cells that are larger than the average worker cell. In rare cases young queens may lay a few unfertilized eggs in worker cells before beginning to lay fertilized ones regularly. Old or exhausted queens may lay both worker and drone eggs in worker cells for a few days before they are superseded. The brood of a normal queen with the exceptions mentioned consists almost entirely of worker brood in worker cells, drone brood in drone cells and queen brood in queen cells. Drone and queen brood may be entirely absent even when much worker brood is present. Queens that fail to mate can, of course, lay only unfertilized eggs and their brood consists entirely of drones. These queens are known as drone layers. They usually delay longer than mated queens before beginning to lay but may lay just as prolifically. The eggs are deposited more or less regularly and until the brood is capped it can readily be mistaken for the brood of a fertile queen. Drone-laying queens do not seem inclined, as do prolific fertile queens, previous to the swarming season, to leave the worker comb in order to lay in drone cells.

In rare cases queens become partial-drone-layers at a time when they should be at their best. They lay both drone and worker eggs in worker cells and their brood consists principally of drones and workers irregularly scattered in worker comb. There may also be present drone brood in drone comb and queen brood in queen cells.

This abnormal condition exists in some of the queens inseminated artificially by the method devised by Watson[4] but was more common at first than since the method has been improved. It may be present in any degree when artifically inseminated queens first begin to lay or may develop at any time thereafter.

Partial drone-laying queens are not uncommon among queens that have been shipped in mailing cages. Although the percentage of queens that are affected is usually small, considerable loss occurs at times among queens reared in the South and sent to northern States in mailing cages. The affected queens may be partial drone-layers as soon as they are introduced but more often the disorder does not show up for a few weeks or until the following season. Even the worst affected queens may be prolific layers and have the appearance of normal young queens. Such queens are of little value even though there are but a few drones in worker cells. The percentage of drones usually increase more or less rapidly and the colonies do not prosper. The best remedy is to kill the queen and introduce a normal one or unite the bees and combs with another colony.

When colonies are seriously poisoned from working California buckeye the queens frequently become drone layers. Drone laying is supposed to come on gradually, although this point has not been fully investigated. In case of buckeye poisoning, however, drone-laying queens have returned to normal egg laying when introduced to normal colonies of bees outside the buckeye territory.

When queens remain to head the colonies in which they are reared and are not subjected to injury, they rarely become partial drone layers while they are still young and vigorous. There are, however, a few references to this condition in beekeeping literature. Root[3] states that "any queen is liable any day of her life to begin laying drone eggs altogether or in part." Langstroth and Dadant[1] state that "some very prolific queens occasionally lay drone-eggs in worker cells. It may be due to fatigue." Langstroth and Dadant[2] also report as follows regarding an experiment by M. Barthelemy of France. "A queen may be temporarily rendered a drone layer by exposure to cold, as was proven in an experiment conducted by Mr. M. Barthelemy of Marseilles, France in 1918. He tried the experiment of introducing a young queen by dipping her in cold honey. The refrigeration caused her to lay drone eggs for several weeks after the operation when she again resumed her laying of fertilized eggs." There are other reports of queens laying only drone eggs after having been exposed to low temperatures.

In April, 1932 the writer found two partial-drone-laying queens that were reared during the summer of 1931 in the colonies that they headed at Somerset, Maryland. It seems probable that the condition may have been brought on by some injury to the queens but this was not determined. Since there are but few reports of observations on the work of such queens

it was decided to make observations on the two colonies at Somerset and other colonies with partial-drone-laying queens as opportunity availed. The observations reported below were made on the two colonies mentioned, supplemented by observations on colonies with partial-drone-laying queens that had been shipped in mailing cages and samples from such colonies sent in by beekeepers for diagnosis.

The two colonies were of Italian stock but some of the worker bees in one colony were darker colored than is usual for pure Italians. Both queens appeared to be young and vigorous. In their general appearance, actions, and the position and regularity of their eggs in the cells, they resembled normal fertile queens. They were fairly prolific layers and during most of the season layed in the combs of two standard hive bodies. When the queens were first found their brood showed a typical concentric arrangement. Irregularity of the brood gradually increased but appeared to be caused largely by the difference in the time of development of the workers and drones and by the death of part of the drone brood. At first less than 10 per cent. of the brood consisted of drones but the proportion of drones gradually increased. In September about 50 per cent of the brood in one colony and about 75 per cent. in the other consisted of drones.

The workers seemed to accept both queens as normal young queens. Supersedure either was not attempted or was unsuccessful, since the queens remained in the colonies and no queen cells were observed during the season. Colonies with partial-drone-laying queens that had been shipped in mailing cages have also been observed in which supersedure was not being attempted.

In April both worker and drone brood appeared healthy and drones and workers were observed emerging from worker cells. In May a few drone larvae died before they were capped. Thereafter the death rate among the drone larvae gradually increased and dead drone pupae were also found A few dead larvae were found in cells with nearly flat cappings but owing to the advanced state of decay their sex could not be determined. It seems probable that they were drones. The dead brood resembled that of laying workers and drone-laying queens and showed the same wide variation in symptoms. Some of the dead larvae resembled larvae killed by the brood diseases. American foulbrood could be eliminated in the apiary by the consistency and odor when the appearance of the dead brood resembled that of American foulbrood. Microscopical examination was necessary to distinguish some of the dead larvae from larvae killed by European foulbrood.

By the first week of August the two colonies under observation were noticeably weakened. The bees still occupied the combs of two standard hive bodies but were less crowded on the combs than in case of other colonies in the apiary with normal queens that had been of about equal

strength earlier in the season. The proportion of old bees also appeared to be greater than in colonies with good queens. Both colonies died out during the winter of 1933-34.

The reason why some queens while still young and apparently vigorous lay both worker and drone eggs in worker cells has never been definitely determined. It seems natural to suspect insufficient insemination and this explanation has been advanced as a possible cause. The explanation is purely theoretical, however, as proof to support it has never been obtained. The fact that mated queens are more likely to become partial drone-layers after they have been shipped in mailing cages would seem to be adverse to this theory. Also W. J. Nolan in unpublished work found, by microscopical examination, that an artificially inseminated queen that layed only worker eggs in worker cells appeared to have received a comparatively small amount of semen. Likewise the cause of the death of the drone brood of partial-drone-laying queens as well as of laying workers and drone-laying queens has never been determined. In case of laying workers and drone-laying queens, improper nutrition, perhaps owing to the absence of young nurse bees, has been suggested as a possible cause. In case of partial-drone-laying queens, however, the drone brood dies even when young bees are present. More work on these questions seems to be desirable.

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THE BEE LOUSE

By ERNEST N. CORY
State Entomologist

For many years, particularly in the southern European section, the bee louse has been recognized as a pest of the hive bee, but relatively little accurate knowledge of its habits has been developed in the native habitat of this insect. It has been introduced into this country presumably with imported queen bees on a number of occasions, and was found in what seems to be in serious proportions in Maryland in 1920, and apparently the first record occurred in Minnesota in 1911. Mr. Nusbaum, in whose apiary it was found in 1920, reported that he had observed the insect at least five years previous to that date without knowing at that time its identity. It was found in Cumberland County, near Harrisburg, Pennsylvania in 1918 and again in 1923. In 1925 Mr. Argo found the insect either in the adult, or in the form of larval tunnels in honey from Allegany County, two points in Washington County, Prince George's County, Baltimore County, and many places in Carroll County. In the latter part of that year he found it in honey produced near Rochester, New York. During

the past summer Mr. Abrams has found the work of this insect in honey produced in Montgomery and Washington Counties.

Braula coeca, the technical name of the bee louse, is a wingless, reddish-brown insect about one-sixteenth of an inch long, with the body covered with numerous spine-like hairs. The legs are quite long in comparison with the body, and the last joint of each leg has a comb-like structure with 15 or 16 teeth on each side of the middle line of the tarsus. The insect clings to the hairs of the bee by means of these comb-like structures. The abdomen is considerably compressed and the whole insect is nearly round in outline, including the outstretched legs. The newly emerged adults are light tan in color.

In heavily infested colonies the lice may be found throughout the year in varying numbers. In early summer the lice appear to be more numerous on worker bees than on any other caste, but in late summer, or early fall, they may be found more numerously on queens than on workers.

The eggs may be found under the newly formed cappings on honey in the brood nests or in the supers. Argo reports that by tracing back tunnels to their beginning he found egg shells in each case glued lightly to the undersurface of the cappings slightly to the center as though the egg had been deposited there just prior to the completion of the cap. In addition to the empty egg shell, cast larval skins and pupal skins have been found throughout the tunnels. These tunnels have the same appearance as the very early larval tunnels of the wax moth. The presence of these tunnels, of course, throws any comb honey out of first grade. Whether the insect lives over winter in one of the immature stages, emerging early in the spring, or whether it lives over winter as an adult on the honey bees has not been definitely determined, but it would seem, from investigations of Argo, that the latter supposition is the more tenable since he failed to find any living material in the tunnels when the hives were first opened in the spring. Apparently gravid females were collected as early as May 16, but no larvae were found until June 12. This would seem to indicate that there was only one brood per year.

The feeding habits of the bee louse have been observed by several workers. The lice attach themselves to the hairs of the head near the upper lip and seems to tickle the labrum or upper lip of the host with the legs that are used in holding on to the hairs. In a short time the bee extends the tongue and the louse quickly moves forward so that it is able to place its mouth parts at the base of the bee's tongue just beyond the upper lip, and apparently sucks up such liquid as may be emitted by the bee. As soon as the bee louse has obtained a sufficient amount of the material excreted by the bee it returns to its position on the thorax.

The bee louse, therefore, is what is known as a commensal, rather than a parasite, as was supposed in the early days. Beekeepers and observers

generally are undecided as to whether the lice cause any economic loss in the death of bees or the queen. Some observers feel that the presence of large numbers of bee lice on the queens may lead to supersedure, but it may well be that a strong colony would be able to divest itself of these intruders and that a weak colony would suffer greater loss from the pest than the strong colony. It would seem reasonable at least that the presence of the bee louse might be a contributing factor in the loss of weak colonies, through death or supersedure of the queen.

There is no doubt that a considerable economic loss occurs in the production of comb honey where the pest is numerous, as the tunnels of the cappings disfigures the comb, throws it out of grade, and allows for leakage. Several methods of control have been suggested using chemicals on the bottom board, but it is very doubtful whether such materials could be used safely in sufficient concentration to cause the lice to release their hold on the bee without either killing some of the bees or causing them to leave the hive. In the event that an apiary becomes thoroughly infested, the wise procedure for the comb honey producer would be to extract all honey and render out the wax in the fall of the year, thoroughly scrape and sterilize the hives and start anew the following spring with package bees, secured from some source where the bee louse is known to be absent. There seems to be a tendency, in the north at least, towards this procedure as an annual practice to obviate the cost of packing, cellar wintering, and the consumption of at least forty pounds of honey necessary to carry the colony throughout the winter. The extraction of an extra forty pounds of honey in the fall would go a long way towards paying for the cost of new bees in the following spring. The disadvantage lies in the fact that all wax in the colonies would have to be rendered and the package bees would have to be started on new foundations and unfinished sections could not be used with safety in the brood chamber.

It is extremely doubtful whether a producer of extracted honey need concern himself very greatly about the presence of the bee louse, unless conditions get much more serious than they have ever been to our knowledge, in which event the same procedure advocated for the comb honey producer could be followed.

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THE WAX MOTH AND ITS CONTROL

By WARREN WHITCOMB, Jr., *associate apiculturist, Division of Bee Culture, Bureau of Entomology and Plant Quarantine*

CONTENTS

The wax moth (*Galleria mellonella* L.) is known under many names in different sections of the United States. Beekeepers know the insect as the "wax moth", "bee moth", "bee miller", "wax worm", "web worm", and "wax miller." It is better known in the larval or worm stage than as the adult or moth and is therefore generally referred to as the "wax worm" or "web worm."

ECONOMIC IMPORTANCE AND SOURCES OF LOSS

No careful estimate has ever been made of the damage caused by the wax moth. The losses in Texas were at one time estimated at 5 percent, and in 1911 reports from 136 Texas beekeepers placed colony losses at from 5 to 95 percent, according to Texas Agricultural Experiment Station Bulletin 231. Losses in the Southern States are considerably higher than in the North because of the longer season of bee and moth activity. Moreover, apiary practices in the South, especially that of keeping empty combs on the colonies during long, slow honey flows, increase the opportunity for wax moth damage. The complete destruction of colonies, however, does not represent the total of wax moth damage, since combs in supers may be ruined even when the colony is of fair strength. This is particularly true when two or more hive bodies are placed on the colonies during slow flows, or late in the fall for storage.

Probably the most noticeable loss from wax moth injury is in combs in storage, especially if these combs are in a warm, protected place, and consists in the destruction of the combs by the larvae, which leave them a mass of webs and debris (fig. 1). In the North such losses are more common than the destruction of entire colonies.

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The larvae of the wax moth cause a considerable amount of damage each year to comb honey. The eggs of the wax moth are probably laid on the comb or section boxes before the comb-honey supers are removed from the hives, but the damage usually occurs some time after the honey has been placed in storage. The damage consists of small, rather inconspicuous tunnels and borings through the thin wax cap of the honey cells. These small holes through the cappings cause the honey to leak out, which makes the affected section unmarketable. This type of damage is sometimes termed "weeping."

A rather indirect loss that might be charged to the wax moth is the winter loss of colonies in the Southern States. Owing to the necessity of preventing wax moth damage to stored combs after

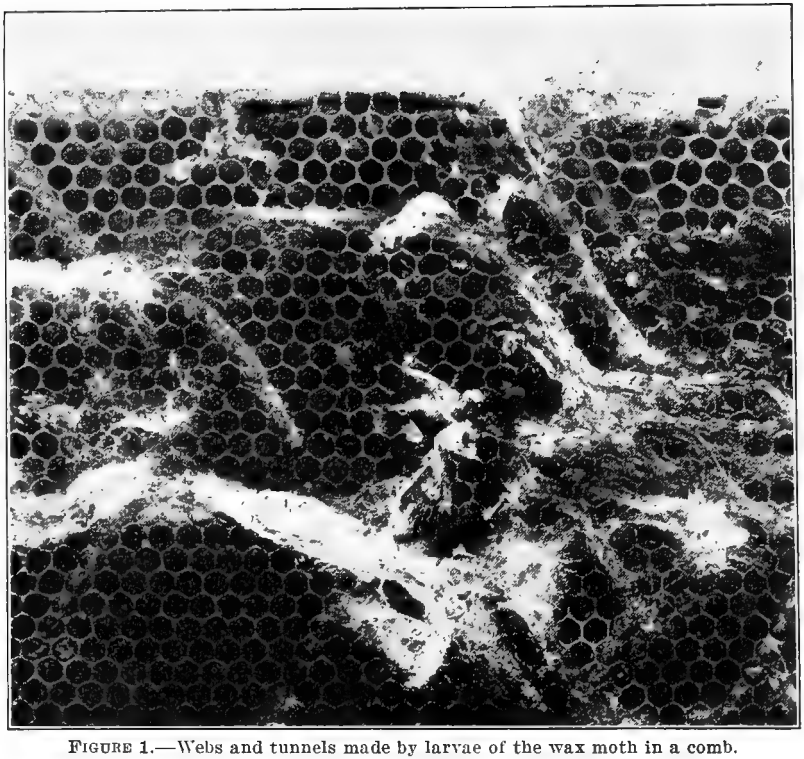

FIGURE 1.—Webs and tunnels made by larvae of the wax moth in a comb.

extracting, and the expense of storage room and treatment for such combs, many beekeepers store supers of these empty combs on the colonies during the winter. This gives added room for the bees to occupy during a warm spell, and a sudden change in temperature may chill or even kill them before they can return to the cluster. It also gives an opportunity for the dissipation of colony heat and thereby increases the quantity of food consumed by the bees, and, during long periods of cold or inclement weather, weak colonies or colonies short of stores may starve. In many such cases of starvation the wax moth destroys the combs before the beekeeper becomes aware of the death of the colony.

In any study of the economic importance of an insect, not only the loss but the benefit from the insect must be weighed. The wax moth is not an unmitigated evil. In the first place, the destruction of combs by the wax moth has not only tended to prevent the keeping of bees in box hives but has also tended to improve general beekeeping practices. The wax moth has also been an ally of the beekeeper by helping to destroy combs in bee trees or other inaccessible places, which might harbor germs of some of the brood diseases.

Since bees in box hives cannot be examined, requeened, or otherwise controlled, the colonies are likely to become weak, and under such conditions an invasion of the wax moth destroys the colony and the combs. Many States have laws to prevent the keeping of bees in box hives, and the wax moth has furthered the aim of this legislation by destroying such colonies in its spread. So thorough is the destruction of colony and combs in most box hives that, unless there are large stores of honey in the hives, bees are no longer attracted to them.

Particularly in the Southern States, where the honey flow is slow and extends over a long period, it has become a practice to give more super room at the beginning of the flow than the bees actually need. This is not the best practice if a large crop of honey is desired, and the destruction of such unprotected combs by the wax moth has been of direct benefit to beekeepers in forcing a change of method.

The destruction by wax moth larvae of combs in bee trees is probably a great aid in preventing the spread of bee disease through the robbing of honey by other bees and, in those areas where queen breeders and package shippers are located, the destruction of stray colonies has also been of real value. Since the germs of American foulbrood have been found in the excrement of wax moth larvae there is a theoretical possibility that the disease might be spread by this means, but actually there is no evidence to warrant pinning additional guilt on this pest.

The benefits of the wax moth are small, however, when compared with the losses of entire colonies and of stored combs, or the extra care and manipulation necessary to combat the insect.

HISTORY AND DISTRIBUTION

The earliest works on beekeeping contain references to the wax moth. Aristotle (384–322 B. C.), Virgil (70–19 B. C.), and Columella (middle of the first century, A. D.), all mention the wax moth as an enemy of the honeybee.

The range of foods that can be eaten by the larvae of the wax moth would suggest that it might at one time have had other foods than those obtainable in the hive, but at present wax comb in some form is practically its only food.

F. B. Paddock, who has made a study of the present-day distribution of the wax moth, was unable to determine the date of its introduction into the United States. From his distribution records some interesting inferences may be drawn: (1) The wax moth has been spread by man more than by the natural activity of the moth. The introduction of the moth into Sweden with beehives from Germany prior to 1750 and its introduction into Australia, New Zealand, and other island regions all point to the conclusion that the wax moth

must have been aided in its distribution by man and by poor beekeeping methods. (2) The insect finds its most favorable conditions in the Temperate Zone. According to Paddock, the wax moth is present in Ontario, Canada, but has been unable to establish itself in Manitoba and British Columbia. The high altitudes of the Rocky Mountains are also free, but the wax moth can be found almost anywhere else in the United States where there are bees.

In the Southern States the wax moth does damage practically throughout the year, with the possible exception of December, January, and February; and during mild winters wax moths may appear even in January. It is probable that colonies are infested, at least with eggs, throughout the whole season of bee activity and that only in active colonies is wholesale damage prevented. In supers and hive bodies brought from the apiary and stored, larvae of all stages will be found, ordinarily within a week, unless the combs are treated. Under storage conditions, the lengths of the egg, larval, and pupal stages vary considerably, and the number of broods per year is largely determined by temperature and humidity. Distribution, under such conditions, is rapid because of the movement of combs and bee equipment, even without the active flight and dispersion of the adult moths.

LIFE HISTORY

THE EGG

The egg of the wax moth is small, white, somewhat elliptical, and rather inconspicuous (fig. 2). It measures about one fifty-fourth of an inch in greatest length and about one-sixtieth of an inch in greatest width. The size and shape vary somewhat, depending on the number of eggs laid in one spot and the character of the site in which they are laid.

At 75° to 80° F. the eggs hatch in from 5 to 8 days, but with low temperatures (50° to 60°) the period may extend to 35 days. Under apiary conditions the incubation period is probably almost entirely dependent on temperature.

The eggs of the wax moth are probably laid most frequently in the cracks between hive parts; that is, between supers, between hive body and bottom board, or between the super and cover. Egg masses have been found in cracks between the inner cover and top super of the hive, where they had been deposited by the female, apparently from the outside of the hive. Eggs are also laid inside the hive in more or less unprotected places. Under controlled conditions, when females were allowed access to combs, the eggs were found on the comb (fig. 2) along the edges of the frames and almost always in the portions of the hive farthest from the light. Egg masses in the hive are difficult to see and may often be overlooked.

THE LARVA

The young larvae, upon hatching, are very active and do not look like the familiar wax worms. Beekeepers have called them wood lice and have not connected the appearance of these forms with the damage from the worms, which they noticed later. They are often seen upon the inner covers of hives and in the cracks between supers and

hive parts. They are less often observed within the hive, especially those with strong colonies, partly because they are very small and very active, and partly because they resemble the wax in color.

The young larvae attempt to burrow into the wax almost immediately after emergence from the egg. The first burrows are often incomplete and may be mere roughenings of the surface of the wax. After the first day, however, they make small tunnels between the cells and toward the midrib of the comb, in which the typical silken strands of the web may be found.

The growth of the larvae depends upon several factors, of which the quantity and quality of food and the temperature are most

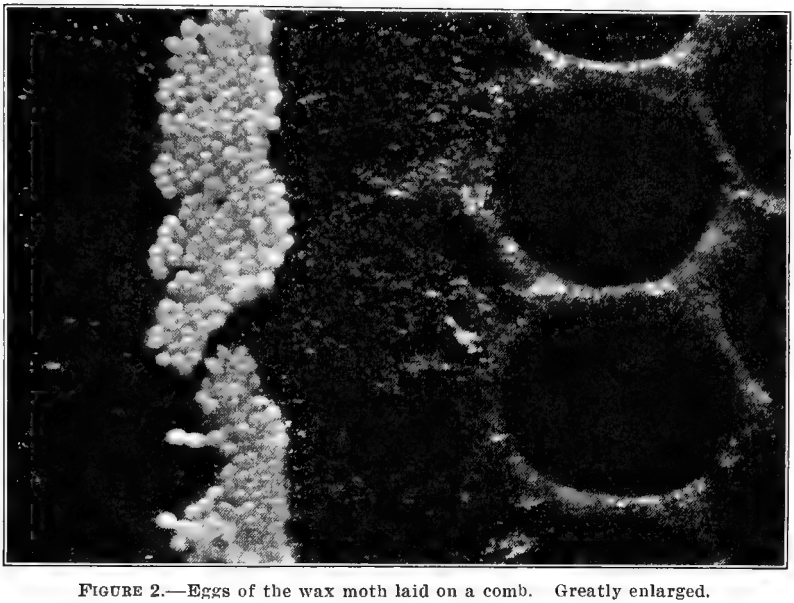

FIGURE 2.—Eggs of the wax moth laid on a comb. Greatly enlarged.

important. The length of the larval period, from the time of the hatching of the egg until pupation, has been found to range from 28 days to 4 months or even as long as 140 days or nearly 5 months. During this period the large larvae have grown from about one twenty-fifth of an inch to seven-eighths of an inch in length.

The food of the larvae is not confined to beeswax, and it is even probable that little pure wax is digested but rather that the larvae derive most of their nourishment from the impurities in the wax. Foundation, especially in frames, is seldom attacked and then usually only by the small larvae. In some cases newly emerged larvae have been seen chewing, or attempting to chew, other larvae which had been injured.

The larvae prefer the darker brood combs to the white extracting combs. In the brood combs the larvae confine their work mostly to the midrib and bases of the cells, and combs are often found with perhaps the outer one-fourth of the length of the cells untouched and the central portion, including the midrib, completely destroyed and replaced with a mass of web and refuse. Under such conditions

the cells containing pollen are mostly avoided, although cells containing honey may be riddled. It is known, however, that larvae will eat pollen and develop on it. Wax moth larvae sometimes chew off the cappings of the cells containing sealed brood, and, while the bees may repair some of the damage, many cells will be left only partially closed.

Although larvae can develop on foundation, the mortality of such larvae is high, and the developmental period of those which survive is much longer than that of normally fed larvae, and the resulting adults are small and almost white. It is almost certain that damage reported by beekeepers in Louisiana as caused by the lesser wax moth (*Achroia grisella* Fab.) is caused by such poorly fed larvae of *Galleria mellonella*, since no specimens of the true lesser wax moth were observed during the author's studies. When the larvae are forced to exist on the lighter comb and the outer portions of the cells which have been left untouched by the previous broods, the damage done by them, such as the webbing and external feeding, and their later appearance greatly resemble the work and appearance of the lesser wax moth.

The optimum temperature for the development of the larvae is between 85° and 95° F., about that normally found in a beehive during the active season. At lower temperatures development is slower, but, unless the temperature falls below 60°, no other influence on the larva has been noted. At temperatures of 40° to 45° the larvae seem to become dormant, and no feeding or growth takes place.

THE PREPUPA

Before pupation the full-grown larvae spin a dense, tough, silken cocoon. Usually this cocoon is firmly attached to the side of the hive, to the frame, or other solid support, but in some cases the cocoons are found in the mass of tunnels and refuse of the wax of the frames or on the bottom of the hive (fig. 3). In many cases a hollow is chewed out of the wood of the hive or frame, and the cocoon is placed in this for added protection. Frames may be found in which holes have been bored completely through the end or top bars, and the cocoon and pupal case will be found inside these holes. This habit of the wax worm is responsible for a considerable part of the damage caused by the insect, since in heavily infested colonies not only the wax but also the frames are destroyed. In such cases particles of the wood borings are incorporated in the cocoon, which is then well disguised. The fully grown larvae migrate to considerable distances before the cocoons are spun, and pupal cases may be found beneath the hive and even on the more protected parts of the hive stand.

THE PUPA

Within the cocoon the larva changes to the pupa. The duration of the pupal stage within the cocoon ranges from 8 to 62 days, depending on temperature. As with many other insects, the pupal period allows the wax worm to pass through the fall and winter protected against climatic influence to a large extent. In the South, especially during warm winters, the adults may emerge at any time during the winter.

THE ADULT

The adult wax moths are about three-fourths of an inch in length and have a wing spread of about 1 to 1¼ inches in well-developed specimens. They are commonly seen in the resting position with their grayish-brown wings folded, rooflike, closely about them (fig. 4, *A* and *B*). The moths are not easily disturbed, but when molested they run rapidly before they take wing. The males are slightly smaller than the females and may be distinguished from them by the shape of the outer margin of the fore wing, which is smooth in the female but roundly notched in the male. The sexes

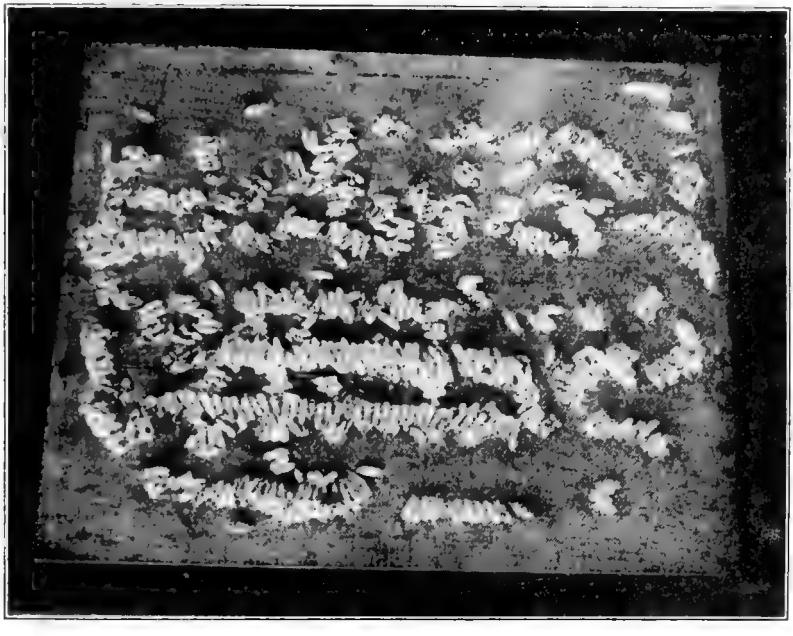

FIGURE 3.—Pupal cases, or cocoons, of the wax moth.

may also be distinguished easily by the palpi of the mouth parts, which are prominent in the female but absent in the male.

The moths vary widely in size and color, according to the type of food consumed by the larvae and to the length of time of development. Small, poorly nourished larvae, or those which, because of low temperatures or other factors, develop slowly, transform into small adults, sometimes less than half the normal size. Such small adults might easily be confused with the lesser wax moth. Larvae fed on dark brood combs transform into adults which may be dark gray to almost black, while larvae which survive on pure wax, or on foundation, transform into moths that are silvery white and smaller than those reared on brood comb.

The female starts depositing eggs from 4 to 10 days after emergence and continues depositing them as long as her bodily vitality lasts. Egg laying may be rapid at times, and as many as 102 eggs have been deposited by a female in 1 minute. The total number of eggs laid by

a female varies to a considerable extent under laboratory conditions, but it is usually less than 300. The adults may live as long as 3 weeks.

NUMBER OF BROODS

It seems doubtful whether there are definite generations of the wax moth during different periods of the year in the Southern States. Rather it is probable that the moth is always present, that larvae in all stages, pupae, and adults may be found at any time, and that development goes on except during periods of low temperature.

FIGURE 4.—Adults of the wax moth: *A* and *B*, With wings folded; *C* and *D*, with wings spread; *A* and *C*, females; *B* and *D*, males. Note the deep clefts in the tips of the fore wings of the male.

OTHER MOTHS CAUSING DAMAGE TO STORED COMBS

Mention has been made of the lesser wax moth, but this moth does not cause so much damage to stored combs as does the wax moth. The work of the lesser wax moth is similar to that of the wax moth, but the tunnels are smaller, the webs finer, and feeding and webbing are more confined to the outer surface of the combs. The Mediterranean flour moth (*Ephestia kuehniella* Zell.) is a pollen feeder rather than a wax feeder but does some damage to combs by boring tunnels through the midrib. This moth also tunnels into brood cells and consumes the food intended for the developing bee larvae. These moths, also, may be controlled by the methods given in this circular.

NATURAL CONTROL

The bees are the greatest and most effective natural enemies of the wax moths. They will, when the colony is strong, carry them bodily out of the hive, and there is no better insurance against the ravages of the pest than to have the combs populated with a strong colony headed by a vigorous queen.

A small red ant (*Monomorium* sp.), which seems to have a wide range of foods, has been quite effective in controlling the wax moth in laboratory experiments by feeding on resting wax moths during daylight, but it has not been observed attacking larvae in combs under

apiary conditions. The ant seems more attracted to honey and pollen than to the wax moth in stored combs.

Comparatively little is known of the other insect enemies of the wax moth, and not much benefit can be expected from them.

Climatic conditions, particularly temperature, are effective in limiting the spread of the wax moth, and the rate of growth, and thereby the amount of damage done by the insect.

ARTIFICIAL CONTROL

CONTROL MEASURES UNDER APIARY CONDITIONS

Two phases of artificial control for the wax moth must be discussed, (1) the control measures for colonies under apiary conditions, and (2) control of the wax moth, or prevention of its damage, in stored equipment.

Under apiary conditions, the best control is in keeping colonies strong. Added to this should be cleanliness of hives—removal of propolis, bur combs, and refuse on the bottom board which provide protection for larvae of the wax moth. Even in strong colonies, developing larvae of the wax moth may often be found beneath the comb burs on the bottom board or in propolis and bur combs in the less accessible portions of the hives. Accidental loss of queens in such colonies late in the fall may mean the loss of colonies from wax moth damage before the first spring examination. Beekeeping practices and manipulations should be based on the assumption that the wax moth in some stage may be present in the hives at all times.

The box hive, or a hive in which the frames are not easily movable, gives the wax moth an opportunity to reproduce, and forms a breeding place from which other colonies may be attacked. From the standpoint of both productive beekeeping and wax moth control, such hives should be destroyed and replaced by modern equipment.

Control of the wax moth by trapping the adults at lights or by trap combs has not been successful. The adults are not attracted to lights and trap combs evidently are not more attractive.

CONTROL MEASURES IN STORED EQUIPMENT

For controlling the wax moth on equipment in storage, two methods of attack are possible. Some substance may be used which will kill the wax moth or some method adopted of repelling the adults so that eggs are not deposited on the stored equipment. Of the killing substances, fumigants (poisonous gases) have proved most satisfactory, but, with the exception of paradichlorobenzene, the gases do not remain in the supers long enough to have any distinct repellent action.

Fumigants for wax moth control are substances, whether liquid or solid, that form a killing gas that diffuses through the stored equipment and is taken in by the insect. Several different substances have been used with success against the wax moth, particularly paradichlorobenzene and carbon disulphide.

PARADICHLOROBENZENE

Paradichlorobenzene (" PDB ") is a white crystalline substance which changes slowly into a gas. The gas is not unpleasant to smell, is noninjurious to people at the concentration obtained when used as

directed, and is heavier than air. It is noninflammable and nonexplosive. It kills adults and larvae of the wax moth but is not effective against the eggs.

In fumigating with paradichlorobenzene, the supers should be stacked as tightly as possible and the cracks between supers covered with gummed paper strips (fig. 5). A generous handful of the crystals should be placed on the top of the frames of the top super and the cover put tightly in place. The crystals may be sprinkled directly on the top bars of the frames, as in figure 5, or put on a piece

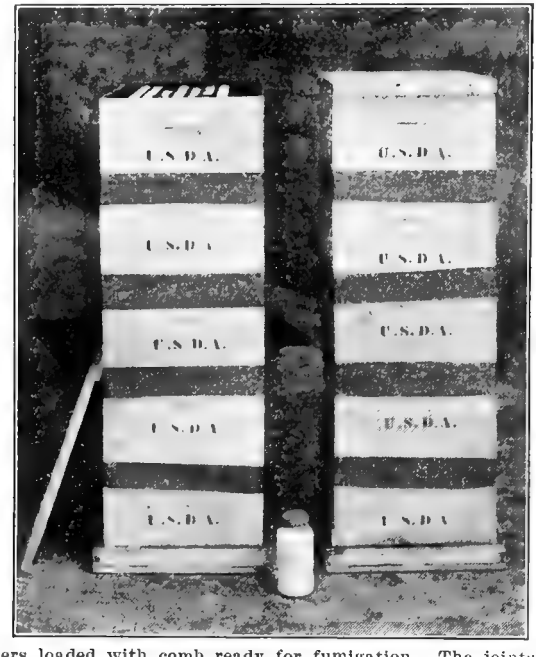

FIGURE 5.—Supers loaded with comb ready for fumigation. The joints are sealed with gummed paper tape, and the crystals of paradichlorobenzene have been sprinkled heavily over the top bars.

of paper laid on the top bars. Since the gas is nonpoisonous and not disagreeable, treatment may be made in ordinary storage without taking the infected material out of doors. At intervals during the storage season the covers of the stacks should be raised, and unless some are still present, more crystals added.

Paradichlorobenzene is at present as cheap as any of the materials mentioned in this circular, with the exception of sulphur, and is by far the easiest and least dangerous to use. The crystals last for some time, since they volatilize slowly, and not only kill the larvae and adults first present and the larvae as they hatch from the eggs, but repel moths from outside which might otherwise enter and start a fresh infestation. Paradichlorobenzene is most effective at temperatures above 70° F. and volatilizes more rapidly as the temperature rises. Inspections of stored materials should be made at intervals of 2 or 3 weeks, depending on the temperature of the storehouse and the prevalence of adult moths.

CARBON DISULPHIDE

Carbon disulphide has been a standard fumigant for wax moths and similar insects until recently, and with proper precautions is still satisfactory. As commonly sold commercially, it is a more or less yellowish, somewhat oily liquid that changes readily at ordinary temperatures into an ill-smelling gas. The liquid is about one-fourth heavier than water, and the gas is heavier than air. *It is highly inflammable, and the vapor is explosive when mixed with air in certain proportions, and therefore this chemical must not be handled around fire of any kind.* Preferably it should be used out of doors or in a well ventilated or open shed.

In using carbon disulphide the supers should be sealed in the same manner as for paradichlorobenzene. One ounce of liquid is sufficient for five supers, and more than this number of supers should not be placed in a single stack, since the weight of the gas carries it quickly to the bottom of the stack, and the top super may not be adequately fumigated. The stack should remain sealed for not less than 12 hours. Carbon disulphide is effective against larvae and adults but not against eggs; consequently, it may be necessary to repeat the treatment after any eggs have had time to hatch.

FUMIGANTS THAT ARE LESS EFFICIENT FOR WAX MOTH CONTROL

Other substances may be used for the control of the wax moth in stored equipment, but, as explained in the following paragraphs, they are not so efficient for this purpose as either paradichlorobenzene or carbon disulphide and are therefore not recommended.

The fumes from burning sulphur effectively control the larvae and adults of the wax moth but are ineffective against the eggs. Sulphur was one of the earliest of the substances used to control the wax moth in stored combs. The early method was to stack the supers over a pan of live coals over which was sprinkled powdered sulphur. About 2 ounces of powdered sulphur (flowers of sulphur) is sufficient for a stack of five supers. At least one empty super should be placed at the bottom of the stack so that the heat will not melt the combs. Present-day practice is to put the sulphur in a dish, wet it with denatured or wood alcohol, and ignite it directly. The work should be done in a well-ventilated room or out of doors, and precautions must be taken against ignition or overheating of the combs.

Calcium cyanide is effective against the larvae, pupae, and adults of the wax moth, but cannot be depended on to destroy the eggs. It is obtainable either as dust or as fine or coarse crystals. For use in fumigating bee equipment the crystals are preferable to the dust. In the presence of moisture (such as that found in the air) the crystals form a deadly gas, noninflammable and nonexplosive, *but extremely poisonous to people and animals. Care must be taken when using the substance, and the gas must not be breathed.* For use put one full tablespoonful of crystals on a sheet of paper and place the paper on the top of the frames in a super. Quickly place the other supers on top, using not more than five supers per stack, and tape the joints between supers with gummed paper tape. The fumigation should be done out of doors, or in a well-ventilated room.

Leave the stack for at least 12 hours before disturbing it, and air the supers well before storing them.

Carbon tetrachloride is effective against wax moths, but does not have enough penetrating power to kill larvae in cocoons or in thickly webbed refuse. It is a colorless liquid with a sweetish, disagreeable odor. The gas formed is heavier than that of carbon disulphide, and it is used in the same way. The gas is noninflammable and nonexplosive, but poisonous.

GENERAL DIRECTIONS AND SUMMARIZED INFORMATION ON THE USE OF FUMIGANTS

Use not more than five supers in a stack and seal the joints with gummed-paper tape to make the stack gas tight. With gases heavier than air, make sure that the base of the stack is tightly closed, since the gases sink to the bottom of the stack and may escape. A pad of newspapers placed beneath the stack will help to confine the gas.

Fumigate out of doors, if possible, or at least in a well-ventilated room. Read carefully the directions for using the selected fumigant and have everything in readiness before fumigation is begun, especially if cyanide is to be used.

CAUTION.—*Carbon disulphide gas is highly explosive, and any chance of ignition must be carefully guarded against. Both carbon disulphide and calcium cyanide and their gases are poisonous to people and to animals and must be stored and handled with extreme care.*

When using paradichlorobenzene, put the crystals directly on the top bars of frames of the top super, as shown in figure 5, or on a paper laid on the top bars, and renew them throughout the season whenever the crystals have disappeared.

As the other fumigants mentioned are not effective against reinfestation from hatching eggs, examinations must be made at intervals to see if any eggs have hatched, especially if the storage room is warm. If the temperature is above 70° F., repeat the treatment after 2 or 3 weeks. The stacks being fumigated must be kept sealed for at least 12 hours, and preferably for 24 hours.

Air the combs thoroughly before placing them on the hives.

Table 1 gives an outline for reference in fumigating against the wax moth.

CONTROL MEASURES IN STORED COMB HONEY

The control for wax moth damage to stored comb honey is the same as for other stored comb products. The supers should be removed from the colonies as soon as possible after the flow ceases and piled in tiers of not more than 8 to 10. All joints between supers should be covered with paper, and the bottom of the stack should be sealed to prevent leakage of gas. Paradichlorobenzene crystals should be sprinkled over the sections of each super as it is placed in the tier, as well as on the sections of the top super, since circulation of air is poor in such stacks. The treatment should be continued until the honey is graded and marketed. Carbon disulphide may be used according to the directions given, if desired.

TABLE 1.—*Summarized information concerning fumigants for the wax moth*

[Based on a stack of 5 supers (standard 10-frame)]

Substance	Characteristics of the gas	Quantity to be used	Approximate cost of first treatment (1934)	Length of treatment	Remarks
			Cents		
Paradichlorobenzene	Nonpoisonous to humans, noninflammable, nonexplosive.	3 ounces or 6 tablespoonfuls	4	Keep supply in stack throughout storage period.	Inspect supers at 2- to 3-week intervals.
Carbon disulphide	Poisonous to humans, highly inflammable, highly explosive.	1 ounce or 2 tablespoonfuls	4	Not less than 12 hours	Repeat once after 2 or 3 weeks.
Sulphur	Irritating	2 ounces [1]	3	do	Do.
Calcium cyanide	Extremely poisonous to humans, noninflammable, nonexplosive.	½ ounce or 1 tablespoonful	8	do	Do.
Carbon tetrachloride	Poisonous to humans, noninflammable, nonexplosive.	1 ounce or 2 tablespoonfuls	4	do	Do.

[1] The ordinary Mason-jar top holds about 1 ounce of sulphur flowers.

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TEXAS AGRICULTURAL EXPERIMENT STATION

BULLETIN NO. 231 JUNE, 1918

DIVISION OF ENTOMOLOGY

THE BEEMOTH OR WAXWORM

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COLLEGE STATION, BRAZOS COUNTY, TEXAS.

AGRICULTURAL AND MECHANICAL COLLEGE OF TEXAS

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*As of July 1, 1918.
**In cooperation with A. & M. College of Texas.
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CONTENTS

THE BEEMOTH OR WAXWORM

F. B. PADDOCK, M. S.,
STATE ENTOMOLOGIST, ENTOMOLOGIST IN CHARGE

INTRODUCTION

This paper is a revision of Bulletin 158 of this Station, now out of print. The continued interest manifested in the beemoth makes this revision and reprint necessary. Additional information has been derived from experiments in the practical control of this pest since the first treatise appeared in the aforementioned bulletin in 1913.

In modern works on apiculture the opinion is expressed that in the present age of modern beekeeping the beemoth cannot be judged a pest of the apiary. It is not only true that box hives favor this insect in its work of destruction, but that warm climates also favor it, even where movable frame hives are used. The long open winters in the southern states allows this insect to feed freely on stored combs. No doubt as the practice of producing extracted honey grows in the northern states, that section will experience more trouble with the work of this insect in the stored combs.

At the present time the beemoth or waxworm is a serious hindrance to the beekeeping industry in Texas, as well as in all of the southern states. Many beekeepers no longer dread the beemoth but keep every colony provided with a vigorous queen. Under such conditions it is difficult for the beemoth to enter the hive to deposit its eggs. The waxworm has become very largely an enemy of the box hive and a destroyer of stored combs and honey and is found usually around the bee hive and in piles of used comb. In large apiaries, the wax and comb that is carelessly left lying around affords ample food for the insect to breed in. In this way the pest is maintained in a yard, ready to infect any weak colony. With many beekeepers the beemoth is a source of constant trouble, for if the bees are not closely watched and become queenless the colony is certain to become infested in a short time. If the beemoth once becomes established, it is hard to exterminate. At present the beekeepers are not able more than to keep this pest in check. It is hoped that a more thorough knowledge of the habits and life history of the insect will result in a better control of this enemy and a reduction of the loss now suffered from its ravages.

Almost every beekeeper is acquainted with the work of the insect, generally known as the "web worm" or "miller," but it is not commonly known that the worm, following maturity, develops into a moth or miller. The worms or larvae feed in protected places, within the comb, which makes them difficult to fight successfully.

HISTORY

This species was known to observers and beekeepers in very early times. Perhaps the first mention was that made by Aristotle, who lived in 384-322 B. C. Later Virgil (70-19 B. C.) made mention of the beemoth in his writings. In the first century, A. D. Columella, a Roman writer on agricultural subjects, mentioned the beemoth as an enemy to the honey bee. Reamur (1685-1757) in France told of the damage done by the beemoth. In Holland, Swammerdam (1637-1680) refers to species of the beemoth, commonly called at that time the "bee wolf." Linnaeus (1707-1778) in Sweden tells of the presence of this pest among the beekeepers of that country. The introduction of the beemoth into America occurred about the beginning of the nineteenth century. It is said that the pest was found in Australia prior to 1878 and in New Zealand it was not noticed until 1904. When this pest was introduced into Texas is not known.

DISPERSION

It has been said that man is the spreading agency of this pest. It is undoubtedly true in the larger sense, for it is very doubtful if the pest spreads fast through its own flight. The carelessness of the beekeeper is almost wholly responsible for the maintenance and spread of the waxworm. As beekeeping has progressed from Asia through Europe and Northern Africa to the North American continent and to the islands of the Pacific, so has been the spread of the beemoth. Wherever beekeeping has been introduced, a few years later the presence of the pest is recorded. Within small areas the spread of the pest is largely through the exchange of infested combs. In Texas it has not been traced, nor is the first location known. The dispersion seems to be restricted by the climatic conditions of Colorado and other western states. In these localities it has been introduced often but had failed to establish itself.

DISTRIBUTION

The beemoth is now found in much of northern Asia and Africa north of the desert, throughout Europe, Great Britain, North America, Australia, New Zealand, Ceylon and India. The distribution of this pest in Texas includes the following counties: Anderson, Atascosa, Bandera, Bastrop, Bee, Bell, Bexar, Blanco, Bosque, Bowie, Brazoria, Brazos, Brooks, Brown, Burleson, Burnet, Caldwell, Callahan, Cass, Cherokee, Coleman, Collin, Colorado, Comanche, Concho, Cook, Coryell, Crockett, Dallas, Delta, Ellis, Erath, Falls, Fannin, Fayette, Franklin, Freestone, Gonzales, Gregg, Grimes, Guadalupe, Hamilton, Harrison, Hays, Henderson, Hill, Houston, Hunt, Jasper, Jefferson, Karnes, Kaufman, Kendall, Kerr, Kimball, Lamar, Lampasas, Lavaca, Lee, Leon, Liberty, Limestone, Llano, Madison, McCullough, McLennan, Mason, McMullen, Medina, Milam, Mills, Morris, Navarro, Nolan, Nueces, Panola, Parker, Polk, Rains, Red River, Robertson, Rockwall,

Runnels, Rusk, Sabine, San Jacinto, Schleicher, Shackelford, Smith, Stephens, Taylor, Travis, Trinity, Tyler, Uvalde, Val Verde, Waller, Ward, Washington, Wood, Wilson, and Williamson.

The dissemination of the beemoth in Texas has been very complete, for there are few counties in the State where bees are kept that are free from the pest today. The counties shown to be infested as reported by beekeepers include all of the important beekeeping counties of the State. There is no doubt that further inquiry will show the presence of this pest in still other counties.

SYSTEMATIC POSITION

The very early writers refer to the beemoth as *Tinea mellonella.* Later it was known as *Galleria cereana* Fabr, which genus was erected by Fabricius. A more careful search of the early records revealed that Linnaeus described two species of beemoths known as *Tinea mellonella* and *T. cereana.* Writers were then very confused and we find the beemoth called *G. cereana; G. alveria, T. cerella.* In early American literature this species is referred to as *G. obliquella* Walker. All recent publications on this insect refer to the species as *G. mellonella L.*

The name early applied to this species would indicate that it was placed in the family *Tineidae,* which contains many small fringe-winged moths, the larvae of which are often case-bearing. It can easily be seen how this might have been done, as the waxworms construct very substantial tunnels that might have been classed as cases. Today the beemoth is placed in the family *Galleriidae,* which is now included with the *Tineidae* as the micro-lepidoptera.

The lesser beemoth, a closely associated species, is now known as *Achroia grisella* Fabr. The two species of moths have often been confused by writers on beekeeping subjects. This species is not so widely distributed as the larger beemoth.

ECONOMIC IMPORTANCE

What this pest is costing the beekeepers of the State is hard to determine. The price of bees, honey and wax varies in the different sections of the State. Often the loss of colonies is attributed to other causes and frequently the presence of the beemoth is not detected. In the reports which have been received from beekeepers, no mention has been made of the loss of stored comb, but this must certainly be considerable.

The loss in some cases is very heavy. In the year 1911, 136 beekeepers reported losses varying from five per cent. to their colonies to as high as ninety-five per cent. Many more beekeepers reported the presence of the beemoth as "general," indicating that they suffered no small loss. In one very well kept apiary that has come under the observation of the writer, there has been an annual loss of three per cent. due to the beemoth. It is safe to say that in many of the larger

apiaries throughout the State this loss is not uncommon, while in the smaller apiaries and in boxhive apiaries the loss is much greater, as was indicated by the reports referred to above. The census of 1910 shows approximately 295,000 colonies of bees in the State, and it is generally conceded that these figures are below the actual number. Assuming that five per cent. is the average annual loss of colonies due to the waxworm, including the large losses in the poorly kept apiaries, it is seen that the annual loss amounts to at least 14,000 colonies. At an average valuation of $4.00 per colony, this amounts to $56,000 a year, a very considerable annual tax on the beekeeping industry of the State. There is no way to arrive at the loss of combs and honey.

HOSTS

In India, according to T. B. Fletcher, the beemoth attacks wild bees, as well as domesticated. He says, "In India, in districts where bees are not domesticated, it attacks the combs of the wild honey bees to such an extent that the bees often desert their nests in disgust and swarm off and found a new one, while it is very rare to find a deserted comb which does not bear traces of the ravages of this pest." This is the only reference to the attacks of the beemoth on wild bees. Observations of the writer have never disclosed the presence of the beemoth in the domiciles of wild bees.

In the hives of domesticated bees, the midrib of the comb seems to be the preferred food. The old brood-rearing combs are preferred to the new combs. This is probably due to the taste of the beemoth for the cast skins of the bee larvae. The larvae, however, are never eaten by the waxworm, although the bee larvae may die in a badly infested comb. This may be due to a lack of nourishment, as the waxworm will devour pollen wherever it is found, stored in cells or as food for the bee larvae. Small quantities of honey are consumed by the waxworms. Propolis is eaten in small quantities. Although the frames and hive are eaten out for pupation it is doubtful if the wood is a food, but probably it is used slightly in the construction of the cocoon.

In stored empty combs the waxworm shows a decided preference to the combs in which brood has been reared. Of course, any comb is readily attacked and destroyed. In the case of stored honey, the comb is preferred as food and the honey is only eaten in small quantities. Honey cappings are readily devoured whenever found and often serve as the only food of the waxworm. It has been said that if fed on pure wax the worms will die. The writer observed one instance in which two full sheets of medium brood foundation were riddled by the waxworm.

METHODS OF STUDY

For the purpose of observing the details of oviposition, small pieces of old brood comb were used. For the observations on the larvae larger pieces of old brood comb were used, the small pieces of comb being

placed in lantern globes having cheese cloth over the top. The habits of the moth were observed in the large rearing cages. Sometimes fresh cappings were supplied the moths for oviposition and for food for the larvae. There was no apparent difference in the activity of the adults with these two foods.

LIFE HISTORY

The larva ("webworm") upon reaching maturity, constructs a cocoon by means of silken threads which it is able to spin. After the cocoon is completed, the larva changes to the pupal stage. This is the stage in which the form of the larva is reconstructed to make the moth which will emerge later from the cocoon. The moths mate and the females deposit the eggs which hatch into the larva. This is called the "life cycle."

The life history of the moth has been assumed by almost every writer on the subject of beekeeping. No definite experiments, however, have been recorded of observations on the details of the life cycle of this insect in this country. The paper by Fletcher in 1911 gave data collected in India. In the United States it is certain that the details of the life cycle will vary much for there is evidence that even in this State there is a variation within the broods as well as a seasonal variation.

THE EGG

The egg is elliptical and pearly white in color. The shell is slightly roughened by wavy lines running across it diagonally at regular intervals. If the egg is not deposited on dark comb, it is very difficult to see and even then experience is necessary to detect all eggs present. There is a slight variation in the size of the egg, as is shown by the measurements given in the following table:

Table 1.—Measurements of eggs.

Date.	Number.	Length.	Width.
Jan. 15, 1913	9 eggs.....	.485 mm.	.440 mm.
Mar. 14, 1913	3 eggs.....	.473 mm.	.360 mm.
Mar. 25, 1913	9 eggs.....	.520 mm.	.400 mm.
April 2, 1913	10 eggs.....	.433 mm.	.378 mm

From the foregoing table the average length was .478 mm. and the average width was .394 mm. for the thirty-one eggs taken in the laboratory over a period of three months.

The embryonic development of the egg has not been studied, but a few observations have been made upon the incubation period. Throughout this period the egg gradually changes from a white to a yellow color. About four days before hatching, the developing larva becomes visible as a dark ring inside the shell. The perfectly formed larva can be distinctly seen for at least twelve hours before the shell bursts. During this time the larva is engaged in cutting an opening in the

shell and its final emergence from the egg is made through a ragged hole in the top. After the larva is out of the shell it appears white and clear.

INCUBATION PERIOD

The period of incubation varies greatly with the brood and in the cooler portions of the year this period is irregular within the brood. The low temperature at which the vitality of the eggs is destroyed has not been determined.

In table 2 the eggs were from moths placed in an unheated room and during this period the temperature averaged 65° F.

Table 2.—Duration of egg stage, spring 1912.

Laid.	Hatched.	Period.
Mar. 8, 1912	April 4, 1912	27 days
Mar. 9, 1912	April 4, 1912	26 days
Mar. 10, 1912	April 4, 1912	25 days
Mar. 13, 1912	April 4, 1912	23 days
Mar. 13, 1912	April 4, 1912	23 days
Mar. 14, 1912	April 4, 1912	22 days
Mar. 21, 1912	April 4, 1912	14 days
Mar. 27, 1912	April 7, 1912	11 days

The average period of incubation of this brood was 21.8 days.

Table 3.—Duration of egg stage, fall 1912.

Laid.	Hatched.	Period.
Aug. 27, 1912	Sept. 3, 1912	7 days
Aug. 27, 1912	Sept. 3, 1912	7 days
Aug. 28, 1912	Sept. 3, 1912	6 days
Aug. 30, 1912	Sept. 5, 1912	6 days
Sept. 1, 1912	Sept. 7, 1912	6 days
Sept. 3, 1912	Sept. 10, 1912	7 days
Sept. 3, 1912	Sept. 11, 1912	8 days
Sept. 4, 1912	Sept. 12, 1912	8 days
Sept. 5, 1912	Sept. 14, 1912	9 days

These eggs were kept in the laboratory and the temperature averaged about 95° F. The average period of incubation of this brood was 7.1 days.

Table 4.—Duration of egg stage, winter 1912.

Laid.	Hatched.	Period.
Dec. 6, 1912	Dec. 11, 1912	5 days
Dec. 6, 1912	Dec. 12, 1912	6 days
Dec. 4, 1912	Dec. 13, 1912	9 days
Dec. 7, 1912	Dec. 14, 1912	7 days
Dec. 7, 1912	Dec. 15, 1912	8 days
Dec. 7, 1912	Dec. 16, 1912	9 days
Dec. 6, 1912	Dec. 18, 1912	12 days
Dec. 7, 1912	Dec. 20, 1912	14 days
Dec. 10, 1912	Dec. 23, 1912	13 days
Dec. 14, 1912	Dec. 26, 1912	12 days

These eggs were kept in the laboratory with artificial heat which

averaged 80° F., although it was quite irregular. The average period of incubation of this brood was 9.5 days.

THE LARVA

DESCRIPTION

When first hatched the young larva (worm) is only one to three millimeters (1/25 to 1/8 of an inch) in length. They have a dirty white, waxy color. The head is slightly yellow and smaller than the prothoracic segment, which is decidedly prominent. The true or thoracic legs are especially well developed and the pro-legs or abdominal legs are not apparent when the larva is first hatched, and only appear normal when the larva is about three days old.

FEEDING HABITS

After emerging from the shell through a ragged hole, the larvae are quiet for a short time while they are apparently drying in preparation for their work of destruction. Soon they become active, but only upon close examination is it possible to detect them hurrying over the comb in their attempt to gain an entrance. Within a short time after hatching, the first meal is taken by the larvae. This consists of scales of wax which they can loosen from the comb in their attempts to gain an entrance. The larvae do not enter the comb near the eggs from which they hatched. In fact, several entrances are attempted before one is finished. This may be due to the extra hard comb in such areas or it may be that they are frightened and never return to continue. It was not observed whether entrances started are taken up by other larvae. The entrance is made at the top of the comb between the walls of adjoining cells. It is during this short period of an hour or more that the larvae are at the mercy of the bees but no doubt few if any are killed at this time in the dark hives.

The entrance is extended by the larvae into tunnels directed toward the center of the comb or the bottom of the cells. The presence of the larvae is readily noticeable as soon as the tunnels are well started. In making these tunnels the larvae push back of them and out of the tunnel bits of chewed wax not used for food. This makes the surface of the comb appear rough and poorly kept. These bits of chewed wax contain strands of the web of the larvae. It is evident that the web is secreted continually by the larvae. Some larvae leave their tunnels considerably and may even work two or three tunnels down to the midrib of the comb. During this period of reaching the center of the comb the growth of the larvae is very slow. During this time only a small proportion of the wax is consumed for food. These tunnels extending to the bottom of the cell are increased in size to accommodate the growth of the larvae, in which case only the thinnest wall protects them. The time consumed in extending the tunnels to the center of the comb varies greatly, from four to eight days.

When the center of the empty comb is reached, holes are cut through the bottoms of the cell walls and the larvae leave their tunnels and wander along the mid-rib from cell to cell. At this time the old tunnel serves as a shelter and it is enlarged. The material thrown out falls on the bottom of the cells. This refuse is about four-fifths chewed and webbed wax and one-fifth excrement. When the comb is disturbed, the larvae may run through three or four cells to their tunnels. At first only holes are eaten through the cells, but in a few days lines of web can be seen outlining the passageway from cell to cell. The holes are enlarged to admit free passage of the growing larvae. The silk spun by the larvae is numerous enough in the course of a few days to form a gallery which gives very good protection. If the larvae are shaken from the comb they seem lost for a time but shortly proceed in the direction of the comb. What causes them invariably to seek the comb it is impossible to say. If they encounter refuse before reaching the comb, they will avail themselves of such shelter. At all times the larvae avoid light as much as possible. When disturbed, the larvae will often drop by means of a thread. In this way they return to the exact place of feeding.

From this central gallery the feeding is extended out along the bottoms of the cells or the middle of the comb. The silk is spun wherever the larvae go, so that very soon the bottoms of the cells are replaced by a layer of silk thread covered with excrement of the larvae and particles of chewed wax. The time required for this varies, of course, with the temperature.

After the mid-rib has been eaten, the larvae start on the walls of the cells, the ones farthest away from the light being the first destroyed. As this feeding continues along the cell walls, the threads of silk are extended to cover the new feeding ground, and not only serve to protect the larvae but also act as a scaffold to support the damaged cells. Soon the center of the comb appears as a mass of tangled refuse and discarded wax. The feeding is at times that of a colony all working comparatively close together. At various places through the comb there are constructed false cocoons that serve as a protection to the larvae while they are resting during the day. When small pieces of comb were used, the larvae would leave the comb during the day and remain in well constructed galleries in the refuse under the comb. After the larvae were three-fourths grown, they worked practically none during the day although the cages were darkened. At night the gnawing of the larvae was distinctly audible. Dark comb is preferred for feeding to light comb. When small pieces of comb were used and additional food was necessary, another piece of empty comb was placed under the old comb. The larvae would immediately attack the new comb, going to the bottom of the cells, eating the mid-rib, lower cells, then upper cells, exactly as the first piece of comb was eaten.

The feeding continued until the walls were entirely eaten, but the top of the cells was never eaten, perhaps because this would expose them

to outside influences and enemies. The area of feeding was gradually extended from the point of infestation finally to include the entire comb. If the comb does not furnish sufficient food the larvae begin to feed on the refuse under the comb in which there is considerable wax in small pieces. In this they construct such a large amount of web that they are absolutely protected from enemies.

In a few cages, balls of fresh cappings, containing normal amounts of honey, were supplied as food for the larvae. The entrance was usually made on the top side of the cappings. A tunnel was made as in the case of empty comb, directed toward the center of the ball. This tunnel, however, was not extended to the center but was extended around the mass, keeping a uniform distance from the surface. The refuse was thrown back and out of the tunnel as was done when feeding on empty comb. When the tunnel was extended around to a point just above the point of contact with the bottom of the cage, an exit was made. Here much refuse was accumulated and the larvae seemed to prefer to eat here. The growth of larvae feeding on cappings was slower than when feeding on comb and there was a greater variation in the growth.

When feeding on stored combs of honey, the worms apparently fed only on the comb, which allowed the honey to drip. The amount of chewed wax was not so great as when feeding on empty comb, nor were there so many webs visible.

LENGTH OF PERIOD

There was a great variation in the length of the larval period even within the brood when food and climatic conditions were apparently the same in all cages. Throughout the observations made on the feeding habits this fact was readily noticeable. In some cages this variation in the size of the larvae was apparent as early as seven days after hatching. This variation is much more apparent during the later part of the period, and during the colder portion of this year the variation is still more pronounced. As indicated above, the food has an effect upon the length of this period, it being greater when cappings were supplied than when empty comb was used.

Table 5.—Length of larval period, spring 1912.

Hatched.	Matured.	Period.
April 8, 1912	May 25, 1912	47 days
April 4, 1912	May 29, 1912	52 days
April 4, 1912	May 27, 1912	43 days
April 4, 1912	May 29, 1912	55 days
April 3, 1912	May 25, 1912	52 days
April 12, 1912	May 25, 1912	43 days
April 3, 1912	May 21, 1912	48 days
April 5, 1912	May 27, 1912	52 days

These larvae were kept in the laboratory at normal temperature with

empty combs for food. The average length of the larval period of this brood was forty-nine days.

Table 6.—Length of larval period, fall 1912.

Hatched.	Matured.	Period.
Aug. 26, 1912	Sept. 25, 1912	30 days
Aug. 25, 1912	Sept. 25, 1912	31 days
Aug. 25, 1912	Sept. 27, 1912	33 days
Aug. 26, 1912	Sept. 28, 1912	33 days
Aug. 26, 1912	Sept. 30, 1912	35 days
Aug. 26, 1912	Oct. 2, 1912	37 days
Aug. 26, 1912	Oct. 4, 1912	39 days
Aug. 24, 1912	Oct. 5, 1912	42 days

These larvae were kept in the laboratory at normal temperature, with empty comb for food. The average length of the larval period of this brood was thirty-five days.

In the fall brood many of the larvae do not mature and pupate until cold weather occurs and a few larvae feed throughout the winter. At any time a comb was inspected there was a vast variation in the size of the larvae from eggs deposited at the same time.

Table 7.—Length of larval period, winter 1912-13.

Hatched.	Matured.	Period.
Sept. 4, 1912	Dec. 10, 1912	97 days
Sept. 9, 1912	Dec. 15, 1912	101 days
Sept. 7, 1912	Dec. 17, 1912	101 days
Sept. 5, 1912	Dec. 17, 1912	103 days
Sept. 3, 1912	Dec. 10, 1912	98 days
Sept. 6, 1912	Dec. 20, 1912	105 days
Sept. 14, 1912	Jan. 27, 1913	135 days
Sept. 13, 1912	Jan. 31, 1913	140 days

These larvae were kept in the laboratory with normal artificial heat, with empty comb as food. The average length of the larval period of these was 110 days.

No observations were made on the number of moults of the larvae.

DESCRIPTION

On September 15, 1912, ten specimens of mature larvae were measured and the average length was twenty millimeters. The head is small and pointed, reddish brown in color, with a light v-shaped line on top, this "v" opening towards the front of the head. The body is larger than the head, long, cylindrical, smooth except for a few short hairs. The general color is a dirty gray with the prothoracic shield brown and having a broad band across it.

PUPATION

Having completed its growth, the larva seeks a place in which to pupate, though sometimes the end of the feeding gallery may be enlarged and closed to serve as a cocoon. The cocoon may also be spun

in the refuse under the comb and this mass of webs affords an excellent protection to the pupa. The most common places are cracks or corners about the hive, or between the frames and the hive or in the "bee space" at the end of the top bars. The larva prefers to get into a place which it can chew in order that a cavity may be constructed and the cocoon thus be better protected.

Having found the location for the cocoon, the larva begins the spinning of the silk thread about itself, starting just above the head and working backward more than the length of the body. A thin layer of silk is spun in the general shape of the cocoon and this frame work is covered with fine silk from the inside. The larva is able to reverse itself within the cocoon, which it does many times during its construction. The outer layer, upon hardening, becomes very tough, and even like parchment, while the inner layer remains soft and fluffy.

CONSTRUCTION OF COCOON

The time consumed in the spinning varies with the season and varies most in the cooler portions of the year. Within the brood there is some variation.

Table 8.—Period of cocoon construction, summer 1912.

Started.	Completed.	Period.
Aug. 10, 1912	Aug. 11, 1912	1 day
Aug. 10, 1912	Aug. 12, 1912	2 days
Aug. 10, 1912	Aug. 12, 1912	2 days
Aug. 10, 1912	Aug. 13, 1912	3 days
Aug. 10, 1912	Aug. 13, 1912	3 days
Aug. 10, 1912	Aug. 14, 1912	4 days
Aug. 10, 1912	Aug. 11, 1912	1 day
Aug. 11, 1912	Aug. 15, 1912	4 days
Aug. 11, 1912	Aug. 15, 1912	4 days
Aug. 11, 1912	Aug. 16, 1912	5 days
Aug. 11, 1912	Aug. 13, 1912	2 days
Aug. 11, 1912	Aug. 13, 1912	2 days
Aug. 12, 1912	Aug. 14, 1912	2 days
Aug. 12, 1912	Aug. 14, 1912	2 days
Aug. 12, 1912	Aug. 13, 1912	1 day
Aug. 12, 1912	Aug. 13, 1912	1 day
Aug. 12, 1912	Aug. 13, 1912	1 day
Aug. 13, 1912	Aug. 15, 1912	2 days
Aug. 13, 1912	Aug. 15, 1912	2 days
Aug. 13, 1912	Aug. 15, 1912	2 days

The average period of construction of the cocoon of this brood was 2.25 days. The cocoons were spun in cages in the laboratory with normal temperatures.

Table 9.—Period of cocoon construction, fall 1912.

Started.	Completed.	Period.
Oct. 25, 1912	Oct. 30, 1912	5 days
Oct. 24, 1912	Oct. 30, 1912	6 days
Oct. 25, 1912	Oct. 30, 1912	5 days
Oct. 25, 1912	Oct. 30, 1912	5 days
Oct. 27, 1912	Oct. 30, 1912	3 days
Oct. 29, 1912	Oct. 30, 1912	3 days
Oct. 26, 1912	Oct. 31, 1912	5 days
Oct. 26, 1912	Oct. 31, 1912	5 days
Oct. 26, 1912	Oct. 31, 1912	5 days
Oct. 26, 1912	Oct. 31, 1912	5 days

These cocoons were spun in cages in the laboratory with normal artificial heat. The average period of construction of this brood was 4.73 days.

THE PUPA

TRANSFORMATION

As the cocoon nears completion, the larva becomes very sluggish, and the body shortens. The last act of the larva is to make an incision in the cocoon near the head end which provides for the easy emergence of the moth at maturity. The time required in the transformation from the larva to the pupa varies with the broods and somewhat within the broods.

Table 10.—Transformation period, summer 1912.

Larva.	Pupa.	Period.
Aug. 13, 1912	Aug. 15, 1912	2 days
Aug. 7, 1912	Aug. 15, 1912	8 days
Aug. 10, 1912	Aug. 15, 1912	5 days
Aug. 13, 1912	Aug. 15, 1912	2 days
Aug. 14, 1912	Aug. 16, 1912	2 days
Aug. 13, 1912	Aug. 16, 1912	3 days
Aug. 11, 1912	Aug. 20, 1912	9 days
Aug. 17, 1912	Aug. 20, 1912	3 days
Aug. 14, 1912	Aug. 20, 1912	6 days
Aug. 16, 1912	Aug. 21, 1912	5 days
Aug. 19, 1912	Aug. 21, 1912	2 days
Aug. 18, 1912	Aug. 21, 1912	3 days
Aug. 18, 1912	Aug. 22, 1912	4 days
Aug. 19, 1912	Aug. 22, 1912	3 days
Aug. 19, 1912	Aug. 22, 1912	3 days
Aug. 19, 1912	Aug. 22, 1912	3 days
Aug. 19, 1912	Aug. 23, 1912	4 days
Aug. 19, 1912	Aug. 23, 1912	4 days
Aug. 21, 1912	Aug. 23, 1912	2 days
Aug. 21, 1912	Aug. 23, 1912	2 days

This transformation took place in the cages in the laboratory at normal temperature. The average period of transformation of this brood was 3.75 days.

Table 11.—Transformation period, fall 1912.

Larva.	Pupa.	Period.
Oct. 23, 1912	Oct. 29, 1912	6 days
Oct. 23, 1912	Nov. 3, 1912	11 days
Oct. 23, 1912	Oct. 29, 1912	6 days
Oct. 22, 1912	Oct. 28, 1912	6 days
Oct. 22, 1912	Oct. 27, 1912	5 days
Oct. 21, 1912	Oct. 27, 1912	6 days
Oct. 24, 1912	Nov. 3, 1912	10 days
Oct. 20, 1912	Oct. 25, 1912	5 days
Oct. 20, 1912	Oct. 25, 1912	5 days
Oct. 23, 1912	Oct. 27, 1912	4 days

This transformation took place in the laboratory with normal artificial heat. The average period was 6.40 days.

DESCRIPTION

The newly formed pupa is white. At the end of the first twenty-four hours it turns to a straw color, very light at first, deepening slowly. By the end of the fourth day the pupa is light brown and this color gradually deepens, so that by the end of the pupal period the insect is dark brown. The male pupae average fourteen millimeters (about two-thirds of an inch) in length and the female pupae are fully sixteen millimeters in length. A row of spines arises just back of the head and extends to the fifth abdominal segment; the body line is somewhat curved downward.

DURATION OF PUPAL STAGE

The pupal stage varies greatly with the seasons, being especially long during the fall and winter.

Table 12.—Duration of pupal stage, 1912.

Pupated.	Emerged.	Period.
Jan. 17, 1912	Mar. 9, 1912	52 days
Jan. 18, 1912	Mar. 3, 1912	45 days
Jan. 18, 1912	Mar. 7, 1912	49 days
Jan. 21, 1912	Mar. 11, 1912	51 days
Jan. 20, 1912	Mar. 9, 1912	49 days
Jan. 20, 1912	Mar. 15, 1912	55 days

These pupae were in cages in the laboratory with no artificial heat. The average length of the period was fifty days.

Table 13.—Duration of pupal stage, 1912.

Pupated.	Emerged.	Period.
Aug. 12, 1912	Aug. 20, 1912	8 days
Aug. 13, 1912	Aug 19, 1912	6 days
Aug. 12, 1912	Aug. 19, 1912	7 days
Aug. 12, 1912	Aug. 18, 1912	6 days
Aug. 11, 1912	Aug. 20, 1912	9 days
Aug 14, 1912	Aug. 21, 1912	7 days
Aug. 13, 1912	Aug. 21, 1912	8 days
Aug. 13, 1912	Aug. 24, 1912	9 days
Aug. 14, 1912	Aug. 23, 1912	8 days
Aug. 15, 1912	Aug. 23, 1912	9 days
Aug. 16, 1912	Aug. 24, 1912	8 days
Aug. 16, 1912	Aug. 24, 1912	8 days
Aug. 17, 1912	Aug 25, 1912	8 days
Aug. 17, 1912	Aug. 25, 1912	8 days
Aug. 18, 1912	Aug. 24, 1912	6 days
Aug. 18, 1912	Aug. 26, 1912	8 days
Aug. 19, 1912	Aug. 29, 1912	10 days
Aug. 18, 1912	Aug. 26, 1912	8 days
Aug. 20, 1912	Aug. 27, 1912	7 days
Aug. 20, 1912	Aug. 27, 1912	7 days

These pupae were in cages in the laboratory with normal temperature. The average length of the period was 7.85 days.

Table 14.—Duration of pupal stage, 1912.

Pupated.	Emerged.	Period.
Oct. 31, 1912	Nov. 20, 1912	21 days
Nov. 1, 1912	Nov. 20, 1912	19 days
Nov. 2, 1912	Nov. 22, 1912	19 days
Nov. 3, 1912	Nov. 24, 1912	21 days
Nov. 1, 1912	Nov. 22, 1912	21 days
Nov. 6, 1912	Nov. 24, 1912	18 days
Nov. 6, 1912	Nov. 24, 1912	18 days
Nov. 1, 1912	Nov. 15, 1912	15 days
Nov. 7, 1912	Nov. 25, 1912	18 days
Nov. 7, 1912	Nov. 25, 1912	19 days

These pupae were in cages in the laboratory with normal artificial heat. The average length of the pupal period was 18.9 days.

Table 15.—Duration of pupal stage, 1912.

Pupated.	Emerged.	Period.
Nov. 3, 1912	Nov. 30, 1912	27 days
Nov. 1, 1912	Nov. 30, 1912	30 days
Oct. 26, 1912	Dec. 1, 1912	35 days
Oct. 28, 1912	Dec. 5, 1912	38 days
Nov. 10, 1912	Dec. 20, 1912	40 days
Nov. 1, 1912	Dec. 15, 1912	45 days
Nov. 1, 1912	Dec. 15, 1912	45 days

These pupae were kept under the same conditions as those recorded in table 14. From this it is obvious that a portion of the pupae have a prolonged period. For those recorded in table 15 the average length of the period was 35.5 days.

ADULTS

DESCRIPTION

Although very familiar to many beekeepers, the beemoth is yet not definitely known to many who should be acquainted with it in order that they might more readily combat it. Having been a pest for such a long time, it is remarkable that more beekeepers are not acquainted with this pest of the apiary. Perhaps the reason that these moths are not more commonly known is due to the fact that they are seldom to be seen on the wing, except at dusk, unless frightened from their hiding places.

The adult beemoth is about five-eighths of an inch (fifteen millimeters) in length, with a wing expanse of about one and one-quarter inches (thirty to thirty-two millimeters). The moth with its wings folded appears ashy gray in color but the back third of each front wing is bronze colored. This wing is thickly covered with fine scales which rub off easily when the moth is touched. On the outer and rear margins of the forewing is a scanty row of short hairs. The hind wings are uniform in color, usually gray, with traces of a few black lines

extending from the outer margin inward toward the base; on the outer and rear margins is a thick fringe of hairs on which is a dark line running parallel with the border of the wing. The body is brown, the shade varying with a covering of scales. These scales rub off easily and are not always present on the older moths. The male is slightly smaller than the female. A difference between the sexes is noticed in the forewing, which in the case of the male is deeply scalloped on the outer margin. This scallop carrier a heavy fringe of hairs, almost black in color. Another difference is in the mouth parts, the palpi of the male being rudimentary.

EMERGENCE

The moths emerge during the first part of the evening. In the cages emergence started just before sundown (6:30 p. m.) and no moths emerged after 9 p. m. They at once sought some protected place in which to expand their wings and dry, and by the next morning they were able to fly.

The first and last emerging individuals of the brood are smaller in size than the average, regardless of sex. The quantity of the food has a great deal to do with the size of the adults. The last larvae of the brood are always undersized, but are almost always able to pupate and reach maturity.

DURATION OF LIFE

In no instance were the moths of either sex seen to feed during their existence. In many cages fresh cappings were supplied for oviposition but the honey in those cappings did not serve as food for the moths. No other attempts were made to supply food to the moths. The duration of the life of the adults varies greatly, apparently depending upon several conditions, retarded fertilization and oviposition, brood and temperature conditions. The males usually live longer than the females, as shown in table 16. The average length of the life of a female was twelve days and the average length of the life of the male was twenty-one days. The average time that the male lived longer than the female was nine days.

Table 16.—Length of adult life.

Date.	Female lived.	Male. lived.
Jan. 5, 1912.....	21 days	39 days
Sept. 5, 1912.....	6 days	18 days
Sept. 9, 1912.....	11 days	21 days
Sept. 9, 1912.....	12 days	18 days
Sept. 10, 1912.....	12 days	21 days
Sept. 19, 1912.....	21 days	21 days

PROPORTION OF SEX

On October 25, 1912, 182 larvae were placed in separate vials to determine sex and rate of emergence. The results are shown in table 17.

Table 17.—Proportion of sex.

Date.	Males.	Females
Nov. 20, 1912	3	0
Nov. 21, 1912	5	4
Nov. 22, 1912	5	5
Nov. 23, 1912	4	4
Nov. 24, 1912	3	3
Nov. 25, 1912	1	1
Nov. 26, 1912	2	3
Nov. 27, 1912	2	5
Nov. 29, 1912	1	1
Nov. 30, 1912	5	6
Dec. 1, 1912	0	2
Dec. 2, 1912	13	14
Dec. 3, 1912	3	9
Dec. 4, 1912	0	4
Dec. 5, 1912	1	2
Dec. 6, 1912	2	3
Dec. 7, 1912	4	6
Dec. 8, 1912	3	9
Dec. 9, 1912	2	0
Dec. 10, 1912	1	0
Dec. 11, 1912	7	6
Dec. 12, 1912	11	16
	78	104

The results that are given would seem to indicate a preponderance of females and a tendency of the males to emerge somewhat ahead of the females.

Another collection of eighty-five larvae was made on November 29, 1912, and each larva was placed in a separate vial. The emergence record of this collection is shown in table 18.

Table 18.—Proportion of sex.

Date.	Males.	Females.
Dec. 1, 1912	2	0
Dec. 11, 1912	7	6
Dec. 12, 1912	14	13
Dec. 19, 1912	3	19
Dec. 26, 1912	3	3
Jan. 18, 1913	0	2
Jan. 19, 1913	2	1
	31	44

In this case there were more females than males and the early emergence of the males is more pronounced than in table 16.

On November 30, 1912, another collection of sixty-one larvae was made. These larvae were placed in separate vials to observe the emergence of the adults. The results are shown in table 19.

Table 19.—Proportion of sex.

Date.	Males.	Females
Dec. 26, 1912	7	6
Dec. 30, 1912	6	7
Jan. 3, 1913	10	10
Jan. 6, 1913	2	12
Jan. 18, 1913	0	1
	25	36

The females predominate in this collection, as in those of the first two shown in tables 16 and 17. Of the 318 larvae observed 184 developed into females and 134 into males.

HABITS

During the day the moths seek a sheltered place away from light and enemies, where they apparently settle down and draw their wings around them, remaining very quiet. Usually they are well protected by their color, which resembles weatherbeaten wood. If disturbed during the day, the moths will make a dart or short flight, acting as though blinded by light. When an object is met, the moth quickly settles down and seems very anxious to avoid flight. That the moths are hard to disturb in the daytime is shown by the fact that in several of the cages used in the experiments small ants attacked the moths and killed them without any apparent struggle on the part of the latter. Only by close examination could it be detected that the moths were dead and not resting in the usual manner. It is only during the later part of the oviposition period that the females are active during the daytime. The male moths are very active throughout their existence.

PERIOD BETWEEN EMERGENCE AND COPULATION

The moths under normal conditions probably mate soon after emergence. No cage observations were recorded but a series of unmated females were killed and examined to determine the condition of the eggs. The first moth was killed one hour after emergence. Many of the eggs were full size but were not close to the ovipositor. The second moth was killed fourteen hours after emergence. In this moth fully one-third of the eggs were fully developed and a very few were close to the ovipositor. The third moth was thirty-eight hours old when killed. Four eggs were extruded before death. The eggs were crowded close to the oviduct and were well rounded, the immature eggs being somewhat flattened on the ends. A count was made of 1128 eggs, 700 of which were full sized. The fourth moth was sixty-two hours old when killed. Fully two-thirds of the eggs were full sized, the remainder varying in size. The fifth moth was eighty-six hours old when killed. In this moth fully three-fourths of the eggs were full sized and they were closely packed in the lower portion of the reproductive organ. The next female was 110 hours old when killed. The eggs were of the same comparative size and condition as in the previous moth. Those eggs nearest the ovipositor, however, assumed a yellowish color. The next moth killed was 134 hours old. The proportion of full sized eggs was the same as in the two previous cases noted but the smaller eggs were increased in size.

LIFE OF UNMATED FEMALES

Several observations were made on the length of life of the unmated females, which period is very irregular. The results of these observations are shown in table 20.

Table 20.—Life of unmated females.

Emerged.	Died.	Period.
Mar. 19, 1912	Mar. 27, 1912	8 days
Mar. 18, 1912	Mar. 26, 1912	8 days
Aug. 14, 1912	Aug. 22, 1912	8 days
Aug. 25, 1912	Aug. 31, 1912	6 days
Aug. 20, 1912	Sept. 9, 1912	19 days
Aug. 30, 1912	Sept. 9, 1912	10 days
Aug. 30, 1912	Sept. 9, 1912	10 days
Aug. 30, 1912	Sept. 9, 1912	10 days
Sept. 1, 1912	Sept. 16, 1912	15 days
Aug. 31, 1912	Sept. 19, 1912	19 days
Nov. 20, 1912	Dec. 11, 1912	20 days

MATING

During the mating period the males are more active than the females, and at this time can be noticed "drumming" with their wings, the vibrations of which are at times sufficient to produce a low hum.

Mating takes place at night, as would naturally be expected from the nocturnal habits of the species. In one case a pair of moths were observed *in coitu* early in the morning, but this was no doubt an abnormal condition and the female died in a short time. Another case was observed when the moths were *in coitu* from 7 p. m. till 10:30 p. m. The next morning no eggs were deposited, but the following night the female began ovipositing. This was an exceptional case, as the female had been confined for a week after emergence before having the opportunity to mate.

AGE AT BEGINNING OF OVIPOSITION

The females begin to oviposit in a comparatively short time after emergence. This period, of course, varies with the brood and within the brood. The comparative age of the male and of the female undoubtedly has an influence on the length of this period. The results of the observations on the age of the female when the first eggs are deposited are shown in table 21.

Table 21.—Age of female.

Emerged.	First eggs.	Age.
Mar. 8, 1912	Mar. 14, 1912	6 days
Mar. 6, 1912	Mar. 21, 1912	15 days
Aug. 29, 1912	Sept. 1, 1912	3 days
Aug. 26, 1912	Sept. 2, 1912	7 days
Aug. 31, 1912	Sept. 2, 1912	7 days
Aug. 29, 1912	Sept. 3, 1912	3 days
Aug. 29, 1912	Sept. 3, 1912	5 days
Aug. 28, 1912	Sept. 3, 1912	5 days
Aug. 30, 1912	Sept. 3, 1912	6 days
Aug. 28, 1912	Sept. 5, 1912	6 days
Sept 7, 1912	Sept. 6, 1912	9 days
Sept. 4, 1912	Sept. 10, 1912	3 days
Sept. 3, 1912	Sept. 11, 1912	7 days
Sept. 3, 1912	Sept. 11, 1912	8 days
Nov. 23, 1912	Dec. 1, 1912	8 days
Nov. 26, 1912	Dec. 1, 1912	8 days
Nov. 24, 1912	Dec. 4, 1912	8 days
Dec. 2, 1912	Dec. 4, 1912	10 days
Dec. 2, 1912	Dec. 5, 1912	3 days
Dec. 2, 1912	Dec. 5, 1912	3 days
Nov. 27, 1912	Dec. 5, 1912	8 days
Nov. 25, 1912	Dec. 5, 1912	10 days
Nov. 22, 1912	Dec. 5, 1912	13 days
Dec. 3, 1912	Dec. 7, 1912	4 days
Nov. 30, 1912	Dec. 7, 1912	7 days
Nov. 30, 1912	Dec. 9, 1912	9 days
Dec. 7, 1912	Dec. 11, 1912	4 days
Dec. 10, 1912	Dec. 14, 1912	4 days
Dec. 9, 1912	Dec. 14, 1912	5 days

PROCESS OF OVIPOSITION

While depositing eggs the female seems mindful only of the task she is performing and is not easily disturbed. Some of the females appear nervous while ovipositing but work steadily. In the cages relatively small pieces of comb were supplied for oviposition. The female usually went over the top and down the sides of the comb, repeating this course continuously. In going over the top of the comb the ovipositor is extended and appears to be dragged along. Apparently the interior of every cell was inspected by the ovipositor but never were any eggs deposited in these empty cells. From the top of the comb the female went to the sides, where suitable places for oviposition were readily found. A very thorough inspection was made of the crevice before the eggs were deposited, sometimes a situation would not be accepted one time but an egg would later be placed there. When a suitable location was found, the moth exerted a tremendous force backward, such as to bend the abdomen, perhaps to force the ovipositor into the crevice as far as possible. Then there was a moment of quiet when the body was rigid, then a quick jerk and the female was on her journey again. During the inspection work the antennae were vibrating continuously but they were motionless while the egg was passing down the oviduct.

The eggs are always securely fastened to whatever object they are laid upon. The eggs are always laid in cavities. In the cage experiments these were on the side of the comb, often where the walls of cells had been turned in. Only one egg is deposited at a time, although in

working over the comb, the female often places the eggs close together. On the smaller pieces of comb, furnished to moths confined in cages, as many as seven eggs were found in a single cavity. The number of eggs actually deposited by one female has not been determined. In the cages, under artificial conditions, if the comb was not supplied for the female she would deposit her eggs in any rough place detected by her ovipositor. In many instances the female would refuse to oviposit on cappings which were furnished in some of the cages, but would go around the base of the lamp globe in which they were confined and fill every crevice with eggs. Sometimes these eggs would be fastened on the outside of the glass, and in such cases the globe would be fastened to its resting place.

TIME OF OVIPOSITION

Oviposition usually takes place at night, beginning at early dusk. In every cage the most of the ovipositing was completed by 9:30 p. m. On the last day the female may oviposit during the afternoon, especially if the day is cloudy or the cage is not directly exposed to light.

HABITS DURING OVIPOSITION

Whenever freed from the cages, the females always started immediately for the windows of the laboratory, but the males, when turned out, sought protection in darkened places. The female was active as soon as darkness started, but upon turning on the electric lights in the room, sought the darker places. If a bright light, such as a candle or reading lamp was placed close to the cage, however, the female at once attempted to reach the light. The male was not so readily affected by light, seeming to prefer quiet and protection.

During the cool evenings of early fall, the moths are active only on those nights when no breeze is blowing. At this latitude the usual breeze stops during the later part of the evening, and the moths may become active for a short period.

The male is never found on the food during the oviposition period, and rarely is the male found on food preceding the period. The female is not found around the food before the oviposition period, but may often be found in the better protected places during the period.

PERIOD OF OVIPOSITION

The period of oviposition of the beemoth varies considerably within the brood as well as with broods. During the last part of the egg laying period the female appears to be in a great hurry, and during the last few days she deposits during the day, as well as during the night, at times stopping to rest. If disturbed during the resting period, she vigorously resumes her egg laying. The females usually die while ovipositing and the last three or four eggs are barely extruded from

the ovipositor. If a female is being killed or injured, she will attempt to oviposit even after she is unable to walk.

The cage records on the period of oviposition are shown in table 22.

Table 22.—Period of oviposition.

First eggs.	Last eggs.	Period.
Mar. 9, 1912	Mar. 13, 1912	4 days
Mar. 12, 1912	Mar. 17, 1912	5 days
Mar. 20, 1912	Mar. 26, 1912	6 days
Mar. 21, 1912	Mar. 28, 1912	8 days
Mar. 27, 1912	April 7, 1912	13 days
Sept. 3, 1912	Sept. 6, 1912	3 days
Sept. 5, 1912	Sept. 8, 1912	3 days
Sept. 5, 1912	Sept. 8, 1912	3 days
Sept. 5, 1912	Sept. 9, 1912	4 days
Sept. 6, 1912	Sept. 9, 1912	3 days
Sept. 9, 1912	Sept. 18, 1912	9 days
Sept. 12, 1912	Sept. 20, 1912	8 days
Dec. 11, 1912	Dec. 19, 1912	8 days
Dec. 14, 1912	Dec. 17, 1912	3 days
Dec. 14, 1912	Dec. 18, 1912	4 days
Dec. 14, 1912	Dec. 20, 1912	6 days
Dec. 16, 1912	Dec. 23, 1912	7 days

EFFECT OF AGE OF SEX

The age of the sex apparently does not have any constant effect on the fertility of the eggs. Females of excessive age were mated with freshly emerged males. Eggs were deposited which hatched. Females that had just emerged were mated with males of excessive age and the eggs that were deposited hatched. In one cage a female was mated with a male that had mated in another cage the previous night. Mating took place and all eggs deposited hatched.

The females will deposit their eggs even when they have not had the opportunity to mate. In all cases when the sexes were not properly paired the females would finally oviposit, the period of oviposition being, however, much shorter than the natural one. Although many females which did not mate were confined in cages, and although they deposited eggs, none of these unfertilized eggs ever hatched. It seems a fairly safe conclusion that parthenogensis does not occur with this species.

RATE OF OVIPOSITION

In many instances females have been observed depositing their eggs at the rate of one every minute for a period of thirty minutes, and then after a short rest have continued again at the same rate.

EFFECT OF HUMIDITY ON DEVELOPMENT

Experiments were planned to determine the effect of a low humidity on the hatching of the eggs and development of the larvae and pupae. For this purpose a large egg incubator of the hot water type was secured. The temperature of ninety degrees F. was decided upon and a humidity of approximately thirty-five per cent. A recording hygro-

thermograph was placed inside the incubator and the records of the temperature and the humidity maintained in the experiments are shown in table 24.

The eggs were deposited by the moths in cages in the laboratory and the following morning they were placed in the incubator. The larvae were supplied with ample food in the form of fresh cappings. The larvae that hatched in a twenty-four hour period were kept together.

The results of this experiment are shown in table 23. The eggs of one batch were placed in the incubator over a period of one day. After the first twenty-four hour period, the hatching was very slow, only four to six larvae from a batch. The prolonged exposure in the incubator dried the eggs badly and after a short time the eggs would not hatch. The larvae started their work in the normal manner but this condition was soon changed.

Table 23—Effect of humidity on development.

Eggs deposited.	Eggs hatched.	Larvae died.	Remarks.
Dec. 3, 1912	Dec. 13, 1912	Mar. 24, 1913	Work very unnatural.
Dec. 3, 1912	Dec. 15, 1912	April 12, 1913	Variation in size.
Dec. 3, 1912	Dec. 16, 1912	Mar. 5, 1913	Very little growth.
Dec. 3, 1912			No eggs hatched.
Dec. 3, 1912	Dec. 16, 1912	April 5, 1913	Larva died.
Dec. 3, 1912	Dec. 16, 1912	Mar. 24, 1913	Larva one-third grown.
Dec. 3, 1912	Dec. 17, 1912	April 12, 1913	Larva one-fourth grown.
Dec. 3, 1912	Dec. 18, 1912	Mar. 24, 1913	Larva one-fourth grown.
Dec. 3, 1912	Dec. 19, 1912	April 5, 1913	Larva one-half grown.
Dec. 3, 1912	Dec. 20, 1912	Mar. 24, 1913	Very little work.
Dec. 3, 1912	Dec. 22, 1912	April 12, 1913	Larva one-fourth grown.
Dec. 3, 1912	Dec. 23, 1912	Mar. 15, 1913	Very little work.
Dec. 3, 1912			No eggs hatched.
Dec. 9, 1912	Dec. 17, 1912	Mar. 24, 1913	Larva one-third grown.
Dec. 9, 1912	Dec. 16, 1912	April 12, 1913	Very little growth.
Dec. 9, 1912	Dec. 17, 1912	Mar. 24, 1913	Larva one-half grown.
Dec. 9, 1912	Dec. 18, 1912	Mar. 24, 1913	Very little growth.
Dec. 9, 1912			No eggs hatched.
Dec. 9, 1912	Dec. 26, 1912	Mar. 24, 1913	Larva one-half grown.
Dec. 9, 1912	Dec. 24, 1912	Mar. 24, 1913	Very little growth.
Dec. 9, 1912	Dec. 24, 1912	April 5, 1913	Larva two-thirds grown.
Dec. 9, 1912	Dec. 26, 1912	April 5, 1913	Larva two-thirds grown.
Dec. 4, 1912	Dec. 12, 1912	April 12, 1913	Larva one-third grown.
Dec. 4, 1912	Dec. 16, 1912	Dec. 31, 1912	Very little work.
Dec. 4, 1912	Dec. 17, 1912	Mar. 24, 1913	Larva one-third grown.
Dec. 4, 1912	Dec. 18, 1912	April 12, 1913	Larva one-half grown.
Dec. 4, 1912			No eggs hatched.
Dec. 5, 1912	Dec. 17, 1912	Mar. 24, 1913	Larva one-third grown.
Dec. 5, 1912	Dec. 18, 1912	Mar. 12, 1913	Larva one-third grown.
Dec. 5, 1912	Dec. 20, 1912	April 12, 1913	Larva one-third grown.
Dec. 5, 1912	Dec. 24, 1912	Mar. 5, 1913	Larva one-third grown.
Dec. 5, 1912			No eggs hatched.
Dec. 5, 1912	Dec. 20, 1912	Mar. 24, 1913	Very little work.
Dec. 5, 1912	Dec. 21, 1912	Feb. 26, 1913	Very little work.
Dec. 5, 1912	Dec. 22, 1912	April 5, 1913	Larva one-fourth grown.
Dec. 5, 1912	Dec. 23, 1912	April 5, 1912	Larva one-fourth grown.
Dec. 5, 1912			No eggs hatched.
Dec. 5, 1912	Dec. 20, 1912	April 5, 1913	Very little work.
Dec. 5, 1912	Dec. 21, 1912	April 5, 1913	Larva dried.
Dec. 5, 1912	Dec. 27, 1912	April 5, 1913	Larva one-third grown.
Dec. 5, 1912	Jan. 2, 1913	Feb. 21, 1913	Very little work.
Dec. 5, 1912			No eggs hatched.
Dec. 9, 1912	Dec. 22, 1912	Mar. 12, 1913	Larva one-fourth grown.
Dec. 9, 1912	Dec. 23, 1912	April 5, 1913	Larva one-fourth grown.
Dec. 9, 1912	Dec. 26, 1912	Mar. 12, 1913	Very little work.
Dec. 9, 1912	Dec. 28, 1912	April 5, 1913	Very little work.
Dec. 9, 1912	Jan. 1, 1913	Mar. 24, 1913	Larva two-thirds grown.
Dec. 11, 1912			No eggs hatched.
Dec. 11, 1912	Dec. 23, 1912	Feb. 21, 1913	Larva one-third grown.
Dec. 11, 1912	Dec. 24, 1912	Feb. 26, 1913	Very little work.
Dec. 11, 1912	Dec. 25, 1912	Mar. 24, 1913	Very little work.
Dec. 11, 1912	Dec. 26, 1912	Mar. 24, 1913	Very little work.
Dec. 11, 1912	Dec. 28, 1912	Mar. 24, 1913	Very little work.

Table 23.— Effect of humidity on development—continued.

Eggs deposited.	Eggs Hatched.	Larvae died.	Remarks.
Dec. 11, 1912	Dec. 29, 1912	Mar. 21, 1913	Larva two-thirds grown.
Dec. 11, 1912	Jan. 2, 1913	Mar. 21, 1913	Larva two-thirds grown.
Dec. 11, 1912	Jan. 9, 1913	Feb. 21, 1913	Very little growth.
Dec. 11, 1912	.....	.....	No eggs hatched.
Dec. 11, 1912	Dec. 23, 1912	April 12, 1913	Larva two-thirds grown.
Dec. 11, 1912	Dec. 21, 1912	Mar. 21, 1913	Very little work.
Dec. 11, 1912	Dec. 25, 1912	Mar. 21, 1913	Very little work.
Dec. 11, 1912	Dec. 26, 1912	April 12, 1913	Larva one-half grown.
Dec. 11, 1912	.....	.....	No eggs hatched.
Dec. 3, 1912	Dec. 15, 1912	Mar. 31, 1912	Larva two-thirds grown.
Dec. 3, 1912	Dec. 17, 1912	Mar. 31, 1913	Very little work.
Dec. 3, 1912	Dec. 19, 1912	Mar. 31, 1913	Very little work.
Dec. 3, 1912	Dec. 22, 1912	Mar. 31, 1913	Very little work.
Dec. 3, 1912	Dec. 26, 1912	Mar. 31, 1913	Very little work.
Dec. 3, 1912	Dec. 31, 1912	Mar. 31, 1913	Very little work.
Dec. 3, 1912	Jan. 2, 1913	Mar. 31, 1913	Very little work.
Dec. 3, 1912	.....	.....	No eggs hatched.
Dec. 5, 1912	Dec. 21, 1912	Mar. 31, 1913	Very little work.
Dec. 5, 1912	Dec. 22, 1912	Mar. 31, 1913	Very little work.
Dec. 5, 1912	Dec. 26, 1912	Mar. 31, 1913	Very little work.
Dec. 5, 1912	.....	.....	No eggs hatched.
Dec. 14, 1912	Dec. 30, 1912	Mar. 31, 1913	Very little work.
Dec. 14, 1912	Dec. 31, 1912	Mar. 31, 1913	Very little work.
Dec. 14, 1912	Jan. 1, 1913	Mar. 31, 1913	Very little work.
Dec. 14, 1912	Jan. 2, 1913	Mar. 31, 1913	Very little work.
Dec. 14, 1912	Jan. 3, 1913	Mar. 31, 1913	Very little work.
Dec. 14, 1912	Jan. 4, 1913	Mar. 31, 1913	Very little work.
Dec. 14, 1912	Jan. 5, 1913	Mar. 31, 1913	Very little work.
Dec. 14, 1912	Jan. 7, 1913	Mar. 31, 1913	Very little work.
Dec. 14, 1912	.....	.....	No eggs hatched.

For the temperature maintained, the work of the larvae was much prolonged. The table shows that in no case did a larva ever reach more than two-thirds normal size when it died. Very quickly after death the larva would become dry and hard. Usually the larva grew but little in the long period of exposure and a point was reached where growth was not possible and death ensued.

In table 24 is shown the mean atmospheric humidity prevailing at College Station over the period of months when the experiment was conducted. Compared with this is the mean humidity of the incubator where the experiments were conducted.

Table 24.—Mean atmospheric humidity prevailing at time of experiment.

Date.	Incubator.						Room.		
	Humidity.			Temperature.			Temperature.		
	Max.	Min.	Mean.	Max.	Min.	Mean.	Max.	Min.	Mean.
Dec. 1, 1912	43	37	40.0	92	90	91.0	.....	.....	.....
Dec. 2, 1912	41	38	39.5	92	91	91.5	.....	.....	.....
Dec. 3, 1912	41	40	40.5	92	91	91.5	.....	.....	.....
Dec. 4, 1912	45	44	44.5	93	92	92.5	.....	.....	.....
Dec. 5, 1912	44	37	40.5	93	91	92.0	.....	.....	.....
Dec. 6, 1912	37	36	36.5	91	89	90.0	.....	.....	.....
Dec. 7, 1912	38	35	36.5	93	91	92.0	.....	.....	.....
Dec. 8, 1912	38	35	36.5	91	90	90.5	.....	.....	.....
Dec. 9, 1912	35	31	33.0	93	90	91.5	.....	.....	.....
Dec. 10, 1912	39	35	37.0	92	90	91.0	.....	.....	.....
Dec. 11, 1912	39	33	36.0	92	88	90.0	.....	.....	.....
Dec. 12, 1912	36	31	33.5	92	89	90.5	.....	.....	.....
Dec. 13, 1912	32	30	31.0	92	90	91.0	.....	.....	.....
Dec. 14, 1912	34	32	33.0	93	90	91.5	.....	.....	.....

Table 24.—Mean atmospheric humidity prevailing at time of experiment—continued.

Date.	Incubator.						Room.		
	Humidity.			Temperature.			Temperature.		
	Max.	Min.	Mean.	Max.	Min.	Mean.	Max.	Min.	Mean.
Dec. 15, 1912	34	33	33.5	91	89	90.0	.....	.....	.....
Dec. 16, 1912	47	42	44.5	93	89	91.0	.....	.....	.....
Dec. 17, 1912	41	36	38.5	91	90	90.5	.....	.....	.....
Dec. 18, 1912	43	35	39.0	90	88	89.0	.....	.....	.....
Dec. 19, 1912	42	38	40.0	91	90	90.5	.....	.....	.....
Dec. 20, 1912	43	42	42.5	91	89	90.0	.....	.....	.....
Dec. 21, 1912	42	41	41.5	90	89	89.5	.....	.....	.....
Dec. 22, 1912	40	38	39.0	89	88	88.5	.....	.....	.....
Dec. 23, 1912	35	33	34.0	91	89	90.0	73	58	65.0
Dec. 24, 1912	34	32	33.0	92	90	91.0	72	60	66.0
Dec. 25, 1912	35	33	34.0	92	90	91.0	69	62	65.5
Dec. 26, 1912	35	30	32.5	94	91	92.5	73	59	66.0
Dec. 27, 1912	33	31	32.0	93	90	91.5	77	60	68.5
Dec. 28, 1912	36	32	34.0	93	91	92.0	68	62	65.0
Dec. 29, 1912	37	34	35.2	93	90	91.5	72	60	66.0
Dec. 30, 1912	34	33	33.5	90	86	88.0	75	56	70.5
Dec. 31, 1912	35	33	34.0	90	89	89.5	73	61	67.0
Jan. 1, 1913	37	31	34.0	94	89	91.5	78	64	71.0
Jan. 2, 1913	34	31	32.5	92	89	90.5	74	59	66.5
Jan. 3, 1913	33	30	31.5	92	89	90.5	74	60	67.0
Jan. 4, 1913	39	32	35.5	93	90	91.5	72	71	71.5
Jan. 5, 1913	41	33	37.0	92	85	88.5	75	55	65.0
Jan. 6, 1912	33	30	31.5	93	89	91.0	80	62	71.0
Jan. 7, 1913	32	30	31.0	92	89	90.5	73	60	66.5
Jan. 8, 1913	31	30	30.5	92	88	90.0	75	59	67.0
Jan. 9, 1913	31	29	30.0	93	89	91.0	80	64	72.0
Jan. 10, 1913	41	31	36.0	92	90	91.0	75	67	71.0
Jan. 11, 1912	38	32	35.0	93	89	91.0	80	62	71.0
Jan. 12, 1913	33	32	32.0	90	88	89.0	69	60	64.5
Jan. 13, 1913	34	33	38.5	91	88	89.5	80	66	73.0
Jan. 14, 1913	37	33	35.0	92	90	91.0	80	69	64.5
Jan. 15, 1913	42	37	39.5	92	90	91.0	79	72	75.5
Jan. 16, 1913	44	42	43.0	93	90	91.5	80	74	77.0
Jan. 17, 1913	45	42	43.5	92	90	91.0	80	73	76.5
Jan. 18, 1913	45	42	43.5	92	90	91.0	83	73	78.0
Jan. 19, 1913	45	44	44.5	90	89	89.5	77	73	75.0
Jan. 20, 1913	42	33	37.5	93	89	91.0	82	67	74.5
Jan. 21, 1913	33	33	33.0	92	89	90.5	81	67	74.5
Jan. 22, 1913	35	34	34.5	92	89	90.5	80	67	73.5
Jan. 23, 1913	35	33	34.0	91	90	90.5	78	69	73.5
Jan. 24, 1913	34	32	33.0	93	90	91.5	79	69	74.0
Jan. 25, 1913	34	32	33.0	92	90	91.0	79	69	74.0
Jan. 26, 1913	33	31	32.0	91	89	90.0	73	65	69.0
Jan. 27, 1913	30	28	39.0	91	87	89.0	77	61	69.0
Dec. 28, 1913	30	28	29.0	91	89	90.0	73	67	70.0
Jan. 29, 1913	31	27	29.0	91	89	90.0	75	66	70.5
Jan. 30, 1913	31	26	28.5	91	89	90.0	73	66	69.5
Jan. 31, 1913	26	24	25.0	92	89	90.5	80	65	72.5
Feb. 1, 1913	27	23	25.0	90	87	89.5	75	65	70.0
Feb. 2, 1913	27	26	26.5	92	88	90.0	75	60	67.5
Feb. 3, 1913	33	32	32.5	92	90	91.0	72	62	67.0
Feb. 4, 1913	39	33	36.0	91	89	90.0	74	66	70.0
Feb. 5, 1913	39	38	38.5	91	90	90.5	74	68	71.0
Feb. 6, 1913	39	36	37.5	92	89	90.5	78	65	71.5
Feb. 7, 1913	37	33	35.0	90	88	89.5	71	59	65.0
Feb. 8, 1913	35	33	34.0	90	87	88.5	74	68	71.0
Feb. 9, 1913	37	33	35.0	89	88	88.5	67	60	63.5
Feb. 10, 1913	41	38	39.5	92	91	91.5	73	66	69.5
Feb. 11, 1913	41	36	38.5	92	91	91.5	77	63	70.0
Feb. 12, 1913	37	35	36.0	93	90	91.0	74	63	68.5
Feb. 13, 1913	36	35	35.5	91	89	90.0	70	61	65.5
Feb. 14, 1913	35	35	35.0	90	89	89.5	67	60	63.5
Feb. 15, 1913	35	34	34.5	91	88	89.5	72	63	68.5
Feb. 16, 1913	37	35	36.0	91	89	90.0	71	64	67.5
Feb. 17, 1913	41	38	39.5	92	90	91.0	76	68	72.0
Feb. 18, 1913	47	41	44.0	92	90	91.0	75	68	71.5
Feb. 19, 1913	47	45	46.0	92	90	91.0	73	70	71.5
Feb. 20, 1913	45	42	43.5	91	90	90.5	71	70	70.5
Feb. 21, 1913	43	36	39.5	91	89	90.0	75	65	70.0
Feb. 22, 1913	38	35	36.5	92	89	90.5	85	65	75.0
Feb. 23, 1913	37	32	34.5	91	89	90.0	70	62	66.0
Feb. 24, 1913	32	28	30.0	92	88	90.0	83	64	73.5
Feb. 25, 1913	38	30	34.0	92	92	92.0	83	70	76.5
Feb. 26, 1913	40	31	35.5	92	89	90.5	74	67	70.5

Table 24.—Mean atmospheric humidity prevailing at time of experiment—continued.

Date.	Incubator.						Room.		
	Humidity.			Temperature.			Temperature.		
	Max.	Min.	Mean.	Max.	Min.	Mean.	Max.	Min.	Mean.
Feb. 27, 1913	33	30	31.5	90	90	90.0	72	66	69.0
Feb. 28, 1913	32	27	29.5	91	88	89.5	69	59	64.0
Mar. 1, 1913	30	23	26.5	91	90	90.5	82	61	71.5
Mar. 2, 1913	26	23	24.5	92	90	91.0	69	60	64.5
Mar. 3, 1913	32	29	30.5	91	90	90.5	80	72	76.0
Mar. 4, 1913	32	28	30.0	93	91	92.0	78	65	71.5
Mar. 5, 1913	30	25	27.5	92	91	91.5	71	66	68.5
Mar. 6, 1913	32	25	28.5	94	85	89.5	80	67	73.5
Mar. 7, 1913	28	24	26.0	93	91	92.0	83	74	78.5
Mar. 8, 1913	28	25	26.5	91	91	91.0	79	73	76.0
Mar. 9, 1913	30	28	29.0	91	91	91.0	77	65	71.0
Mar. 10, 1913	33	30	31.5	92	91	91.5	82	67	74.5
Mar. 11, 1913	36	31	33.5	92	91	91.5	85	68	76.5
Mar. 12, 1913	39	32	35.5	92	91	91.5	80	70	75.0
Mar. 13, 1913	32	22	27.0	93	91	92.0	90	66	78.0
Mar. 14, 1913	24	21	22.5	92	89	90.5	90	60	75.0
Mar. 15, 1913	21	19	20.0	93	90	92.0	80	60	70.0
Mar. 16, 1913	19	19	19.0	91	90	90.5	76	58	67.0
Mar. 17, 1913	24	20	22.0	92	88	90.5	74	62	68.0
Mar. 18, 1913	28	24	26.0	93	89	91.0	70	64	67.0
Mar. 19, 1913	33	28	30.5	94	92	93.0	69	68	68.5
Mar. 20, 1913	33	25	29.0	94	89	91.5	80	63	71.5
Mar. 21, 1913	25	25	25.0	92	89	90.5	80	63	71.5
Mar. 22, 1913	30	26	28.0	90	90	90.0	71	63	67.0
Mar. 23, 1913	33	30	31.5	92	90	91.0	74	71	72.5
Mar. 24, 1913	46	44	45.0	93	90	91.5	77	73	75.0
Mar. 25, 1913	46	40	43.0	92	91	91.5	.....	.....	.....
Mar. 26, 1913	40	32	36.0	92	90	91.0	.....	.....	.....
Mar. 27, 1913	36	28	32.0	92	88	90.0	.....	.....	.....
Mar. 28, 1913	34	30	32.0	93	90	91.5	75	65	70.0
Mar. 29, 1913	39	33	36.0	91	89	90.0	74	68	71.0
Mar. 30, 1913	38	37	37.5	91	90	90.5	70	64	67.0
Mar. 31, 1913	40	38	39.0	93	90	91.5	82	71	76.5
April 1, 1913	48	38	43.0	93	91	92.0	78	72	75.0
April 2, 1913	50	45	47.5	95	86	90.5	76	74	75.0
April 3, 1913	47	38	42.5	91	86	88.5	74	69	71.5
April 4, 1913	40	34	37.0	92	88	90.0	72	65	68.5
April 5, 1913	34	30	32.0	92	90	91.0	75	68	71.5
April 6, 1913	38	32	35.0	90	88	89.0	74	70	72.0
April 7, 1913	42	38	40.0	92	90	91.0	77	70	73.5
April 8, 1913	45	42	43.5	92	91	91.5	74	70	72.0
April 9, 1913	43	39	41.0	91	88	89.5	72	65	68.5
April 10, 1913	41	31	36.0	91	89	90.0	67	63	65.0
April 11, 1913	33	30	31.5	92	90	91.0	67	62	64.5
April 12, 1913	32	22	27.0	93	90	91.5	67	61	64.0
April 13, 1913	22	18	20.0	91	89	90.0	68	62	65.0

Table 25.—Comparison of humidity.

Year.	Atmosphere.			
	December.	January.	February.	March.
1914	78.1	64.6	72.0	70.5
1915	74.2	72.8	69.6	72.6
1916	64.5	78.9	66.7	61.8
1917	62.8	70.8	58.7	63.0
Average	69.9	71.8	66.8	66.7

Year.	Incubator.			
	December.	January.	February.	March.
1912	36.7	.....	.....	.....
1913	.....	34.2	35.7	30.0

SEASONAL HISTORY

From the work which was done in trying to identify the different broods or generations of this insect, it appears that there are three broods in the extreme southern part of the United States. The third brood is not nearly so large as the first two, due to the fact that some of the second brood of larvae do not pupate until late fall. There is a decided overlapping of the generations, which makes it difficult to determine the exact number of broods a year. At almost any time, from early spring until December, examination of a colony of bees is likely to reveal this insect in all stages. It is often assumed from this that the life history is short and there are several generations each year.

In well protected hives the development may continue throughout the year without interruption. Usually the winter is passed with about one-third of the insects in the pupal stage and the remainder in the larval stage. Warm spells during the winter cause some of the moths to emerge from their cocoons; in the laboratory many moths emerged when the temperature was maintained constantly at sixty degrees F. It is not unusual to see moths on the windows of the honey house, trying to escape during the warm spells in December and January. Their presence may be accounted for on the supposition that they have just emerged from their cocoons or that they may have been in hibernation as adults and become active with the rise in temperature. Such moths do not reproduce in localities where freezing temperatures are frequent. Even the most vigorous moths cannot withstand a freezing temperature for more than three days. Moths in well protected places can survive an outside temperature as low as twenty-six degrees F. for as long as five days. The moths are never active during the day when the temperature is below fifty degrees F., so at such times reproduction does not take place.

For College Station, Texas, the following life history and duration of broods has been carefully determined:

The maximum number of moths which mature from the over-wintering larvae and pupae appear about the first of April. These moths are active for some time before any eggs are deposited and it is the middle of April before the eggs are laid for the first brood of larvae. Usually twelve days are required for the eggs of this brood to hatch, so that by the first of May most of the first brood of larvae are out. The larval period of this brood is quite long, most of the larvae feeding at least forty-five days before completing their growth. A majority of the larvae of the generation are ready to pupate by the middle of June, but there is a considerable variation in the rate of growth, for some of these larvae feed for six weeks longer before attaining full size. The pupation of the first brood takes place during the last two weeks in June, and by July 1 some of the moths of the second generation are to be seen.

The moths of this generation emerge at about the same time and give the impression of constituting a very large brood. Most of the eggs are laid very soon after emergence of the moths and by the middle

of July all of the eggs of the second generation are deposited. The high temperature at this time of the year shortens the egg period, only ten days being required for these eggs to hatch. There is a considerable variation in the maturing of this brood of larvae. Normally the larval period is shorter than for the first brood and by the first of September many of the larvae are full grown. Some of the larvae may continue to feed for four weeks longer and then pupate.

Some of the larvae which mature early in September may pass through a short pupal stage and soon emerge as moths. This accounts for the appearance of the number of moths about the first of October. This brood is usually small and scattered and few of the larvae which result from the eggs of these moths reach full size. Some of the larvae of the second generation do not pupate during the fall, but live over the winter in the larval stage and pupate the following spring.

The following summary shows the stages which normally occur during each month of the year at College Station, Texas:

April: Moths reach maturity from the over-wintering larvae and pupae.
Eggs are deposited.
May: Eggs hatch.
Larvae are about three-fourths grown.
June: Larvae reach maturity.
Some pupae.
July: Pupae.
Adults of the second generation.
Eggs deposited by the second generation of moths.
August: Larvae of the first generation.
Pupae of the first generation.
Moths of the second generation.
Eggs of the second generation.
Larvae of the second generation.
September: Pupae of the first generation.
Moths of the second generation.
Eggs of the second generation.
Larvae of the second generation.
Moths of the third generation.
Eggs of the third generation.
October: Larvae of the second generation.
Pupae of the second generation.
Moths of the third generation.
Eggs of the third generation.
November: Larvae of the second generation.
Pupae of the second generation.
Larvae of the third generation.
December: Same stages as during November.
January: Same stages as during November.
February: Same stages as during November.
March: Pupae.

NATURAL CONTROL

PREDACEOUS ENEMIES

Of the natural enemies of the beemoth, the most important is the honey bee itself. It is a well established fact that if the colony be kept strong, healthy, and with a vigorous queen, it will defend itself against the beemoth. This is particularly true in the case of "Italian" bees. In the *Ohio Cultivator* for 1849, page 185, Micajah T. Johnson says, "One thing is certain: if the bees, from any cause, should lose their queen and not have the means in their power of raising another, the miller and the worms soon take possession. I believe no hive is destroyed by worms while an efficient queen remains in it." This seems to be the earliest published notice of this important fact by an American observer.

This fact is of vital importance in the fight against the beemoth, for if the pest can be kept from its favorite food control measures are made much easier. The fact that the bees under natural conditions are able to defend themselves should leave the problem of control to such means as will destroy the pest in places other than the hives. Recently it has been found advantageous to introduce Italian blood into the colony, as the workers of this race seem to be more efficient fighters of the beemoth. In most cases this is sufficient for the control of the pest in the colonies, but it must be remembered that the colony cannot be kept under close observation and maintained at full strength unless domiciled in a frame hive.

A small red ant, *Solenopsis geminata* Fab. was found to be an enemy of the beemoth, as many of the cage experiments were destroyed by these ants killing the moths and larvae. The attack is made on the moths during the day or when they are at rest. Usually the ant crawls under the wings of the moth, and begin the attack on the abdomen. There is no apparent struggle on the part of the moth, for close examination was necessary to determine that the moth was dead and not resting. The abdomen seems to be all that is desired, and this is carried away in small pieces to the nest of the ants. The same species of ant also destroyed moths which had recently been prepared for exhibits. At such times only the abdomen was taken by the ants. In their attacks on the larvae the ants entered the cages and crawled over the comb and wax in search of their prey and if any larvae were exposed, they were attacked. The larger larvae are more frequently attacked, as they are less active and usually feed in more exposed places than do the smaller ones. Unless the larvae were well protected by webs in the refuse, they were destroyed by the ants. Apparently there are days and even parts of days when the ants are most active in their destruction. Never were the ants present in sufficient numbers to attempt tracing them to their nests. No observations have been made upon this ant in or about the apiary and while it proved very destructive under artificial conditions, the moths and larvae might be better able to protect themselves under natural conditions.

PARASITES

Three hymenopterous parasites have been recorded from the beemoth. One is chalcid, *Eupelmus cereanus,* found by Roudani in Italy; another is *Bracon bravicornis,* which was found by Marshall in France, and a third species, *Apenteles lateralis,* was recently found by A. Conté in France. The last species was found near Lyons, where it spread very rapidly. It is apparently of considerable importance since it has also been reported to attack the larvae of several other moths in England and Germany. The adult parasite is about one-sixth of an inch (4 millimeters) in length, very lively, and avoids light. The body is black and the wings are transparent, with black specks. The larvae of the beemoth are attacked while quite young and never attain a large size. A single parasite develops in each larva. The bees are said to pay no attention to the presence of the parasite, so that it can easily enter the hive in search of the beemoth larvae. It was artificially introduced into hives by Conté with very satisfactory results.

CLIMATE

Cold is perhaps the greatest climatic factor in the control of the beemoth. Young and vigorous moths when exposed were able to withstand a temperature of a few degrees below freezing. When a temperature of twenty-eight degrees F. occurred, all exposed moths were killed. Even with some protection all moths did not survive this temperature. The young moths may withstand this temperature for one night, but not for a longer period of time.

The larvae are greatly susceptible to cold. Larvae of all ages even up to two-thirds grown, were placed out-of-doors, and with only the meager protection of light tunnels and small pieces of comb. Whenever a temperature of thirty-two degrees F. was reached, all larvae were killed. Under natural conditions the larvae are better protected to pass through the winter by better built feeding galleries. The natural mortality was observed in an infested hive that was allowed to remain untouched throughout the winter. The results are shown in table 26. It will be seen that the high mortality was among the larvae. Of the 208 larvae examined, 146 or seventy per cent. were dead, and of the 158 pupae examined, eight or five per cent. were dead.

Table 26.—Mortality of waxworms.

Date.	Alive.	Larvae.	Pupae.
April 9, 1913	216	66	150
	Dead.	Larvae.	Pupae.
	150	142	8

ARTIFICIAL CONTROL

Unfortunately, the only natural enemy of the beemoth that is present to any great extent in Texas is the honey bee itself. In the absence of any natural enemies of importance, the measure of artificial control must be made all the more effective if the beekeeper is to free his apiary of the pest. If the moths are driven from the hives by strong colonies of Italianized bees, they will surely seek scraps of comb and wax about the ground and stored comb and honey in the honey house. It seems quite likely that in such cases the eggs are deposited as near to the comb as possible, as along the cracks between the supers, and the larvae, after hatching, find their way to the comb through crevices much smaller than the moth can enter.

TRAP LIGHTS

Trap lights were employed to learn if the moths were attracted to them. On September 10, 1913, a large lantern and an acetylene lamp were placed in an apiary where the beemoth had been present constantly. The lights were so placed as to throw the rays across the apiary over a great number of hives. The night was warm, clear, and still. The lights were run from 7:30 to 10 p. m. Not a single beemoth was ever present at either light.

Again on September 27, 1913, the trap lights were put in operation in the same apiary. The day had been rainy and it was still misty in the evening, which was also very dark. No moths were ever at the lights.

DECOY BOXES

Decoy boxes containing pieces of comb were placed in and around the apiary. These were put out during September, 1913, and remained under observation until December. None of the twelve boxes was ever infested during this period.

FUMIGATION

One of the methods of artificial control, and one upon which many beekeepers depend, is fumigation of combs and honey. Gas is able to penetrate material that it is not possible to treat in any other manner. The fumigation process is not difficult, for when once started no further attention is necessary until the treatment is complete. It is not necessary to watch the entire process. Stored material, such as comb honey and empty combs, should be examined from time to time and at the first evidence of the waxworm they should be fumigated. Stored material of this kind should be examined at least once every week during the summer and once every month during the winter season, so as to detect the infestation at the start.

In the present investigation two materials have been used in the fumigating experiments. These were selected because almost every beekeeper is acquainted with them and they can be obtained in practically

every locality at a reasonable price. They are sulphur and carbon bisulphide, or "high-life."

Sulphur

Dry powdered sulphur, or "flowers of sulphur," is a light yellowish powder with which everyone is familiar. When sulphur is burned, it unites with the oxygen of the air and forms a poisonous gas known as "sulphur dioxide." This gas is effective in killing some kinds of insects, including the waxworm. A common method of burning the sulphur is to place it on a pan of red hot coals and immediately tier up the infested supers over the burning sulphur. The bottom super should not contain any infested material and the pile should be covered as quickly as possible. A number of experiments were made with sulpher fumigating combs containing waxworms. The result of these experiments are given in table 26.

Table 26.—Results of fumigating infested combs with sulphur dioxide.

Stage of beemoth.	Amount of sulphur used per cubic foot.	Time the combs were confined to fumes.	Effect.
Larvae	One-fourth ounce	One hour	Killed
Larvae	One-half ounce	One hour	Killed
Larvae	Two-thirds ounce	One hour	Killed

The larvae which were used for these experiments were from ten to twenty days old and in every case they were well protected by the webs and refuse. The larvae which were used in the experiments were of different ages and some better protected than others. When the larvae are not very well protected, they are quite susceptible to the gas, but the larger larvae, which are often enclosed in a mass of webs, were not killed except when extremely large doses of sulphur were used.

From the experiment with sulphur dioxide, it is evident that only extremely large doses will effect the eggs of the beemoth; so large, in fact, that such fumigation would not be practical.

These results seem to indicate that the sulphur fumes are not ordinarily penetrating enough to effect the eggs, and only when the larvae are young and not well protected will the gas effect them. While the method is simple, there are minor details upon which the success of the operation depends. The sulphur must be burned at a high temperature in order to generate the most effective gas. While the method is generally effective under proper conditions, it cannot be recommended in preference to fumigation with carbon bisulphide.

Carbon Bisulphide ("High Life")

The commercial bisulphide is an oily liquid, very volatile and exceedingly foul smelling. It is cold to the touch and because of its rapid evaporation it produces a freezing sensation when dropped on the skin. When exposed to air at ordinary temperatures the bisulphide rapidly changes to a gas or vapor which is a little more than two and one-half

times as heavy as air. This is a point to be remembered in its use, since it goes first to the bottom of whatever it is confined in. When mixed with air, it becomes highly inflammable and sometimes explosive. Such a mixture of air and bisulphide gas may be exploded by even a spark such as might be made by hitting a nail with a hammer. The liquid, upon evaporation, leaves a residue of impurities. Its rate of evaporation is in proportion to the temperature and the area of the exposed surface. Its efficiency is the greatest with the rapid evaporation, and this is secured in relatively warm weather, but artificial heat must *never* be used to hasten its changes into gas. Carbon bisulphide is obtainable from practically every druggist.

When carbon bisulphide is to be used for fumigation of infested material, the greatest precaution should be used to keep all fire, such as lights, cigarettes, etc., away from the liquid and where it is being used. For this reason it is well to take the material that is to be fumigated out-of-doors and at least 100 feet away from any building. The infested material should be placed in supers or hive bodies if possible. These are piled as high as is convenient and all cracks between the containers made as nearly gas-proof as possible. Especially should the bottom be tight. A good plan is to place an inverted hive cover on the ground, lay a piece of canvas over it and then tier up the supers on this. After the pile has been completed, an empty super should be put on top, in which should be placed a large shallow pan. Into the latter the bisulphide is to be poured. When all is in readiness, pour the bisulphide into the pan, and immediately put a hive cover on the top of the tier to confine the gas. This operation is best performed in the evening, and the pile of supers should be left intact the following morning. When the supers are taken down the confined gas will escape immediately, even before they can be carried separately into a building.

The results of fumigating infested material with carbon bisulphide is shown in table 27.

Table 27.—Results of fumigating infested combs with carbon bisulphide.

Stage of beemoth.	Amount of liquid carbon bisulphide used per cu. ft.	Time of confinement.	Effect	Remarks.
Moth	One-half ounce	15 minutes	Killed	
Moth	Two-thirds ounce	20 minutes	Killed	The moth was unable to walk within 10 minutes after being confined.
Moth	Three-eighths ounce	20 minutes	Killed	The moth was unable to walk within 10 minutes. Not all the bisulphide evaporated.
Moth	One-fourth ounce	20 minutes	Killed	The moth was dead before all the bisulphide evaporated.
Pupae	One-sixth ounce	24 hours	Killed	Several larvae in cocoons were also killed.
Pupae	One-fourth ounce	24 hours	Killed	Several larvae in cocoons were also killed.
Pupae	Three-eighths ounce	24 hours	Killed	Several larvae in cocoons were also killed.
Pupae	One-half ounce	24 hours	Killed	Several larvae in cocoons were also killed.
Larvae (in cocoons)	One-eighth ounce	24 hours	Killed	Some died in one hour in cocoon.
Larvae (in cocoons)	One-fourth ounce	24 hours	Killed	Some died in one and one-half hours in cocoon.
Larvae (in cocoons)	Five-eighths ounce	24 hours	Killed	
Larvae (exposed)*	One-sixth ounce	24 hours	Killed	Larvae 10 days old and well protected.
Larvae (exposed)*	One-eighth ounce	24 hours	Killed	These larvae were five days old and well protected in webs.
Larvae (exposed)*	One-eighth ounce	24 hours	Killed	Larvae were 25 days old, and protected.
Larvae (exposed)*	One-fourth ounce	24 hours	Killed	These were 20 days old and fairly well protected.
Larvae (exposed)*	One-fourth ounce	24 hours	Killed	These were 20 days old and exposed.
Larvae (exposed)*	One-fourth ounce	24 hours	Killed	These were 12 days old and exposed.
Larvae (exposed)*	One-half ounce	24 hours	Killed	These were 15 days old and fairly well protected.
Larvae (exposed)*	Three-fourths ounce	24 hours	Killed	Eggs were present which hatched afterward.
Larvae (exposed)*	Three-fourths ounce	24 hours	Killed	Eggs were present; hatched afterward.
Larvae (exposed)*	One ounce	24 hours	Killed	Eggs were present, hatched afterward.
Larvae	One ounce	24 hours	Killed	Eggs were present; hatched afterward.
Larvae (exposed)*	One ounce	24 hours	Killed	

*These larvae were feeding in empty combs.

In all the experiments conducted, the eggs of the beemoth were uninjured by the fumes of carbon bisulphide. It is possible that in cases of extremely large doses the eggs may be injured.

A number of experiments were conducted to determine the effect of the fumes of carbon bisulphide upon the larvae. Comb containing larvae of various ages and different degrees of protection was fumigated. Many experiments were made with the larvae in cocoons, and these showed that the carbon bisulphide was very effective. The larvae which are hardest to kill are those about three-fourths grown and well protected in a mass of webs and refuse. Ordinarily the larvae succumb to the average dose of carbon bisulphide in a comparatively short time. The outcome of the experiments demonstrated the effectiveness of carbon bisulphide for the destruction of the larvae.

Several experiments were conducted to determine the effect of carbon

bisulphide upon the pupae. It was found that they are quite susceptible, but a long exposure to the fumes is necessary as the pupae do not consume air very fast.

From the experiments conducted with the moths, it was found that they are very susceptible to the fumes of carbon bisulphide. With the average dose the moths are overcome in from ten to fifteen minutes and are killed in from fifteen to twenty minutes after being confined.

All fumigation should be allowed to continue for at least twelve hours, for those larvae which are best protected by webs and refuse will not be killed unless plenty of time is given for the gas to penetrate the material. The liquid will evaporate in a few hours, but the resulting gas will be effective for several hours.

The following table has been prepared to show at a glance how much liquid carbon bisulphide is required for effective fumigation of ten frame supers and hive bodies containing infested material.

Table 28.—Amount of carbon bisulphide to use in fumigating ten frame supers for the waxworm.

Number of supers in the tier.	Cu. ft. contained in tier.	Amount of liquid bisulphide required.
2	1.74	½ ounce
3	2.61	½ ounce
4	3.48	¾ ounce
5	4.35	1 ounce
6	5.22	1¼ ounce
7	6.09	1½ ounce
8	6.96	1¾ ounce
9	7.83	2 ounces
10	8.70	2¼ ounces
11	9.57	2½ ounces
12	10.44	2¾ ounces

Table 29.—Amount of carbon bisulphide to use in fumigating ten frame hive bodies for the waxworm.

Number of bodies in the tier.	Cu. ft. contained in tier.	Amount of liquid bisulphide required.
2	2.90	¾ ounce
3	4.35	1 ounce
4	5.80	1¼ ounce
5	7.25	1½ ounce
6	8.70	2 ounces
7	10.15	2¼ ounces
8	11.60	2½ ounces

For eight-frame supers and hive bodies, use eighty per cent. as much bisulphide as is given in the foregoing table for the corresponding number of supers or bodies.

Example: Suppose that the beekeeper has six ten-frame shallow extracting supers containing combs which he wishes to fumigate. All are tiered up as previously directed and an empty super is placed on top. This makes seven supers in all. Reference to the preceding table shows that this tier of seven supers contains 6.09 cubic feet of space and that for the destruction of all of the waxworms in it one and one-half ounces of the liquid bisulphide are required.