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Bacteriological Methods

IN

Food and Drugs Laboratories

WITH AN

...duction to Micro-analytical Methods

BY

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PREFACE

The administration of the Federal Pure Food and Drugs Act and of the several State Pure Food and Drugs Laws has made the introduction of bacteriological methods into food and drugs laboratories a necessity. Because of the close relationship between the work of the bacteriologist and that of the micro-analyst, it is advised that, wherever possible, these two laboratory branches be combined in the most effectual coöperative manner. With such coöperation in mind, a brief introduction to micro-analytical methods is added. Fuller details on micro-technique will be found in special works on the microscopy of fibers, foods, spices, drugs, of water supplies, of sewage, etc.

As is more fully set forth in the text, the bacteriological as well as micro-analytical methods in our food and drugs laboratories are not yet fully worked out, and the present volume is submitted hoping that it will be instrumental in bringing about a unification of methods and that it will perhaps also serve as a guide to the working out of newer and inadequately tested older methods.

The volume is primarily intended as a guide to students who are interested in the bacteriological examination of foods and drugs. Its use as a laboratory guide presupposes a thorough knowledge of general bacteriology.

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San Francisco, California.

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I

OUTLINE OF MICRO-ANALYTICAL METHODS IN FOOD AND DRUGS LABORATORIES

1. Introduction

The value of the compound microscope in the examination of foods and drugs is as yet not generally recognized. Efficiency in this line of work depends very largely upon a long and wide range of experience, in this regard differing very markedly from efficiency in the field of chemical analyses. All that is required of the chemist, as far as routine analytical work is concerned, is a very close adherence to the methods laid down for him. He is pronounced skilled in direct proportion to his adherence to methods and skill shown in the manipulation of apparatus and reagents. The micro-analyst in order to be efficient must be very familiar with the appearance of the multitudinous forms of cells, tissues, cell-contents and with the behavior of certain microchemical reagents and this familiarity can be acquired only through long and careful observation.

2. Grouping of Substances to be Examined in Food and Drugs Laboratories

The analytical methods, as they apply to the critical examination of foods and drugs, are chemical, microscopical and bacteriological. The substances to be analyzed may be grouped as follows:

1. Vegetable drugs, crude and powdered, pharmacopœial and other simple and compound medicinal powders.
2. Spices and condiments, whole, ground and powdered. Prepared spices and condiments.
3. Coffee, tea, cocoa, chocolate, confections, candies.
4. Tobacco and preparations made from tobacco, as snuff, smoking tobacco, cigars, etc.
5. Chemicals, minerals, solutions of chemicals, etc.
6. Tablets, pills, powders.
7. Meats of all kinds, raw, cooked, canned, sausage meats, etc.
8. Dairying products, as milk, cream, cheese, butter, ice-cream, ice cream fillers, etc.
9. Insect powders, dusting powders, cosmetics.
10. Cattle and poultry powders.
11. Unknown powders, wholly or partly of vegetable origin.
12. Starches, dextrins, sausage meat binders (starches).
13. Vegetable foods, as jams and jellies; fresh, pickled, cooked, canned and preserved.
14. Flours and meals.
15. Breakfast foods, infant and invalid foods.
16. Breads and similar materials; biscuits, doughnuts, cakes, pies, pastries, etc.
17. Macaroni, spaghetti and similar preparations, noodles, etc.
18. Nuts and nut-like fruits and seeds, etc.
19. Beverages of all kinds, liquids generally.
20. Pharmaceuticals of all kinds.
21. Patent and proprietary medicines.
22. Unknown foods and medicines.

In the examination of some of these substances the chemical method is all important, as in chemicals generally; in the examination of others the microscopical method is all-important, as in meals, flours, spices; and again the bacteriological testing is all-important, as in sewage, contaminated water, contaminated milk, infected foods and drinks generally, etc. A properly equipped analytical laboratory, whether federal, state or private, should be prepared to apply all three methods. The bacteriological investigations should be made by the micro-analyst rather than by the chemist, because of the closer relationship between bacteriology and microscopy.

3. The Work of the Micro-analyst in Relationship to that of the Chemist and Bacteriologist

Just what work should or should not be done by the micro-analyst is as yet not definitely determined; at least, there is no uniformity as to scope of action in the different analytical laboratories. It is suggested that the following work be assigned to the micro-analyst:

1. Gross and net weight determination of all such samples as require it.
2. Moisture determination of substances which require it.
3. Ash and acid insoluble determinations of substances which are primarily subject to microscopical analysis, as vegetable drugs, pills, powders, vegetable compound powders, etc.
4. Use of certain special tests, as sublimation tests for benzoic acid, salicylic acid and boric acid; Grahe's cinchona test, wheat gluten test, color reactions for boric acid, capsicum, guaiac, salicylic acid, morphine, etc., tests for cholesterol and phytosterol crystals, and others which may prove useful.
5. Bacteriological testing of foods and drugs generally, of sera, vaccines, galenicals, syrups, milk, water, jams, jellies, catsups, etc., as may be required, following the method of the Society of the American Bacteriologists, and limiting the testing to determining the presence or absence of the colon bacillus and other sewage organisms, and the usual quantitative bacterial determinations for milk, water and other substances, of which the quality is usually based upon the quantitative bacterial content.

Substances subject to analysis in the laboratories mentioned should be grouped or classified according to the special or preferred methods of examination to be applied. It is, of course, evident that in the majority of cases chemical as well as microscopical methods should be used. In some cases even all three must be used in order that conclusive results may be obtained. The following grouping is suggested:

1. Substances in which the chemical analysis is of first importance. Chemicals generally, and chemicals in solution, alcohol, alcoholic drinks, flavoring extracts, syrups, oils, fats, etc.
2. Substances in which the microscopical analysis is of first importance—vegetable substances and preparations which are essentially of vegetable origin. Meats of all kinds, variously prepared, cooked, spiced, etc.
3. Substances in which the chemical and microscopical examinations are of equal

importance—assayable vegetable drugs, all prepared food substances with chemicals in solution, compound powders, pills, tablets.

4. Substances to which the microscopical examination is not generally applied —chemicals, liquids in which the insoluble particles are slight in amount, as wines, brandies, comparatively pure solutions, etc. Here the centrifuge plays an important part.

5. Substances in which the bacterial testing is of prime importance—milk, sewage or otherwise organically contaminated water supplies, and other liquids, beers, etc., contaminated foods generally. In this class of substances the microscopical and chemical examinations become necessary in addition to the bacteriological; in fact, a bacteriological test is incomplete without the use of a good compound microscope.

The work of the micro-analyst is, so to speak, on trial. The doubt in the minds of the critics is due, very largely, to the unsatisfactory results traceable to the efforts of those who are not sufficiently qualified. Even the most skillful analysts admit numerous defects in methods and shortcomings in results. For example, the quantitative estimates based upon optical judgment are approximate only, and with most workers there is a very marked tendency to make these estimates volumetric rather than gravimetric. This can in a measure be corrected by bringing into play the judgment of the relative weights of the several substances under comparison. For example, the amount of sand present in powdered belladonna root may be volumetrically estimated at 20 per cent. In this case the acid insoluble ash residue may show 35 to 40 per cent. of silica. An example like this also indicates why the micro-analyst should make the sand and ash determinations. The percentage estimates based upon microscopical examination may vary within 25 to 50 per cent. when the amounts of the admixtures are small or slight. For example, the actual amount of arrow-root starch in the so-called arrowroot biscuit is 2.5 per cent. The micro-analyst's estimates may range from a trace or small amount to 5 per cent. When the quantities of admixtures are large, from 30 to 90 per cent., the estimations may approximate within 10 or 15 per cent. of the actual amount present. These estimates can no doubt be

made much more accurate by uniform methods of technique, aided by certain mechanical devices. For example, in the examination of vegetable powders, spices, meals, flours and similar substances, the samples should be thoroughly mixed, and slide mounts should be of standard and uniform thickness and the relative amounts of the ingredients should be estimated by means of microscope slides having uniform ruled squares of definite measuring value in microns. These and other details in the methods should be more fully worked out.

Several micro-analysts have declared themselves as opposed to giving percentage estimates of the several ingredients of a compound. However, not to give the approximate percentages will cause great confusion and very materially lessen the value of the work done. For example, to report a pancake flour as composed of "buckwheat and wheat flour, the former predominating," instead of "buckwheat approximately 75 per cent. and wheat approximately 25 per cent.," would certainly be unsatisfactory.

The following examples will serve to explain the relative value of the chemical and microscopical analyses. Suppose the substance to be examined is a baby food. The microscope may reveal approximate percentages of oil globules, steam dextrinized wheat starch, unchanged wheat and arrowroot starch, wheat tissue and milk sugar. The chemical analysis will show a definite percentage of sugar, soluble starch, insoluble starch, fat, vegetable fiber and ash. This is a good example of a case where the two methods of analysis are of equal importance; one without the other would be unsatisfactory, incomplete and inconclusive. Again, the chemical assay may show that a sample of powdered belladonna leaf contains 0.35 per cent. of mydriatic alkaloids, and yet the microscopical examinations may prove the presence of 20 per cent. or more of some foreign leaf.

An adjunct in analytical work, much neglected by the chemist, is the organoleptic testing. This is especially important in the examination of unknown substances, fruit products, spices,

meats, etc., as it often gives a clue to the quality of the substances and to the means of getting quick results.

4. Equipment for Micro-analytical Work

The equipment and apparatus required by the micro-analyst is comparatively inexpensive, and it is very earnestly advised to secure only those appliances which are useful or essential for the work in hand. The following list is submitted without entering into detail, as it may be assumed that the microscopist does not require explanations:

1. Simple lens.
2. Compound microscope.
   a. Ocular with micrometer scale.
   b. Oculars, Nos. 2 and 4.
   c. Objectives, Nos. 3, 5 and 7.
   d. 1/12 in. oil-immersion objective for bacteriological work.
3. Slides and covers.
4. Section knife or razor, and strop.
5. Polarizer, for the study of starches, crystals and other substances. Should be convenient to use. The selenite plates are useful.
6. Thoma-Zeiss hemacytometer; for counting bacteria and yeast cells.
7. Stage mold and spore counter, as described in Part. II (Fig. 5).
8. Accurate metal or hard rubber millimeter ruler for measuring seeds (in fruit products), etc.
9. The required glassware and adjunct apparatus.
10. The required reagents.
11. Equipment for making moisture determinations.
12. Equipment for making ash determinations.
13. Equipment for the required bacteriological tests and determinations.

The laboratory in which the work is done should be roomy, well-lighted, provided with the necessary shelves, apparatus and supply cases, reference books, etc. The details need not be given here. The analyst must see to it that the necessary things are provided. A skillful and experienced worker should have the tools of his choice, not those selected for him by some one not qualified to judge.

The skilled micro-analyst has little difficulty in determining

the purity and comparative quality of the simple spices, as pepper, allspice, cloves, cinnamon and ginger. However matters are

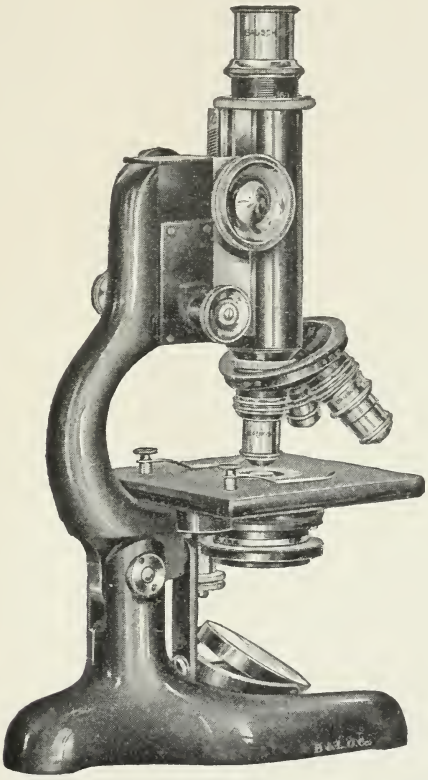


FIG. 1.—Form of compound microscope suitable for bacteriological and general microscopical work in food and drugs laboratories. Note the desirable and necessary accessories as given in the text. The form of polarizing apparatus convenient to be used with the compound microscope, sets into the substage diaphragm ring with the iris diaphragm opened to the maximum. The analyzer takes the place of the ocular. —(*Bausch & Lomb Co.*)

quite different when it comes to the examination of powdered vegetable drugs, compound vegetable powders and vegetable products of unknown composition. A thorough knowledge of,

and a wide familiarity with, cell-forms, tissue elements and formed cell contents is an absolute essential in order that accurately reliable and conclusive results may be obtained and serious confusion may be avoided. Differences in the reports of findings by micro-analysts are in part due to the personal equation, in part due to variable methods and differences of judgment in estimating the quantity of tissue elements present and also in part due to a lack of extensive and intensive experience.

5. Organoleptic Testing

The organoleptic tests are indeed valuable adjuncts to the microscopical work. There are, however, some differences of opinion regarding the interpretation and valuation which are to be placed on comparisons of color, odor and taste, even among those having had considerable experience and endowed with a fairly normal special sense development. Our color terminology is in great confusion, and so far as the olfactory sense is concerned, there are only comparatively few odors or flavors which admit of ready comparison such as tea flavor, coffee odor, vanilla odor, raspberry flavor, loganberry flavor, and the odor of such drugs as valerian, cubeb, fenugreek, asafetida, aloes, turpentine, camphor, the essential oils, calamus, etc., and the odor of the spices. Our comparative judgment of tastes is more reliable. Much experience is necessary to form fairly reliable estimates of flavors (associations of tastes and odors), though pure fruit flavors are, as a rule, readily distinguishable, as that of apples, dried apples, peach, dried peach, quince and strawberry. Manufactured fruit preparations generally lose much of their flavor due to many causes, as cooking, steaming, fermentative changes, presence of decayed (moldy) fruits, mixing of several kinds of fruits or fruit juices, etc., to say nothing of the wholly artificial or imitation fruit flavors and the flavors of the imitation fruit products which have little or no fruit in their composition.

6. Methods Useful in the Examination of Vegetable Drugs, Spices, Etc.

We shall give a few tests which have proven useful in the examination of drugs and food products. It will be found that many of the test results are largely approximate, and some of them are primarily intended to serve as aids or checks to the chemical examination.

1. **Mace Test.**—To a pinch of the powdered mace add a few drops of 10 per cent. sodium hydroxide solution. Banda or true mace changes color only slightly, whereas wild or Bombay mace turns a deep orange color.

2. **Conium Test.**—To the substance to be tested for the presence of conium fruits (as anise, caraway or other umbelliferous fruits), add 25 per cent. sodium or potassium hydroxide solution. In the presence of 1 per cent. or more of conium fruits a distinct mouse odor is developed in time (10 min. to ½ hr.). This test is not reliable with old umbelliferous fruits, as many of them develop a more or less marked mouse odor with alkalies.

3. **Lignin Test.**—The classic phloroglucin-hydrochloric acid test is useful in making estimates of the amount of lignified tissue present, as in old belladonna root, aconite roots and stems, lobelia herb, fruit products, spices, etc.

4. **Grahe's Cinchona Test.**—Drive the moisture from the inner surface of a small test-tube by holding it over a Bunsen burner. Into this dried test-tube place a pinch of finely powdered cinchona bark (No. 80) and heat rather carefully over an alcohol lamp or Bunsen burner. When the bark begins to char, red fumes begin to fill the tube and condense on the side of the tube as a reddish purplish liquid. The intensity of the reaction is approximately proportional (direct proportion) to the percentage of alkaloids present. Some skill and experience is necessary to perform this test well. The tube must not be heated too quickly or too much, and the powder should be uniformly fine.

5. Beaker Sand Test.—Pour a definite amount of the powdered spice or vegetable drug into a beaker, add water, stir until the sand is washed away from the vegetable particles and settles to the bottom of the beaker. Let a stream of water run into beaker so as to wash out the vegetable matter. The final washing and decanting must be done carefully so as not to lose the sand. Salt brine may be used instead of water, should the vegetable matter have a comparatively high specific gravity. Dry the sand and weigh to obtain the percentage of sand present.

6. Ash Determination.—According to the regulation method. The percentage of the acid-insoluble residue should also be determined. It should be borne in mind that the ash determination gives only approximate results as far as the presence of clay and dirt is concerned, since the organic matter of dirt is combustible. The ash percentage varies greatly in vegetable drugs, especially in herbs and leaves. The sand percentage is comparatively high in those herbs and leaves having abundant trichomes, especially if the drug plants (or herbaceous spices) bearing such trichomes are grown in dry sandy soil. Dirt (and sand) percentage is apt to be high in roots and rhizomes, particularly when rootlets are abundant and when the gathering, garbling and cleaning is carelessly done.

There are a number of chemical tests giving color reactions which can be done conveniently by the micro-analyst, as the boric acid reaction with curcuma, the H_2SO_4 color reaction with some barks, capsicum, guaiac, resin, cubeb, etc.; the H_2SO_4 plus formaldehyde color reaction with morphine; the ferric chloride color reaction with salicylic acid, etc. These tests should be used when, in the judgment of the analyst, they may serve to give better information regarding the identity, purity and quality of the drug.

7. Methods Useful in the Examination of Vegetable Food Products

1. Sublimation Test for Benzoic Acid.—Place a drop or two of the suspected liquid or semi-liquid food substance into a deep watch crystal of 1 in. diameter. Place over it a clean dry slide. Now hold the watch crystal over a flame (alcohol lamp[1]) until the substance (as wine, vinegar, catsup, jam, jelly, etc.), comes to an active boil. The steam vapor, carrying with it the benzoic acid, is condensed on the slide. Remove the slide and set it aside until the condensed moisture has evaporated; very moderate heat may be used to hasten evaporation. Examine under the microscope, whereupon the benzoic acid crystals may be seen, provided any were present. The test is delicate, very reliable and very few substances interfere with it. It is very pronounced in the presence of 0.01 per cent. of benzoic acid.

2. Sublimation Test for Salicylic Acid.—Made like the benzoic acid test. The crystal formation (plates) is very pronounced in dilutions of 1 : 1000. After having examined the crystals under the microscope, add a drop of weak solution of ferric chloride to the crystals upon the slide, whereupon a blue coloration develops. Boric acid is likewise deposited by sublimation, but the test is not as satisfactory as those for benzoic acid and for salicylic acid.

The sublimation test may also be extended to other crystalline substances which undergo sublimation on exposure to heat.

3. Curcuma Thread Test for Boric Acid.—Boil 5 grams of powdered curcuma in 10 cc. of alcohol. To the evaporated alcoholic extract add a little soda and several cc. of 50 per cent. alcohol. In this place paper (bast fiber), cotton or linen threads and bring to a brisk boil for a few moments. Remove threads and dry between blotting paper, lay them in a very weak solution of sulphuric acid and rinse in water. When dry the threads should be a golden yellow.

[1] Alcohol lamp is preferable because the flame is small and yet the heating is more quickly done.

The test for the presence of boric acid (also for borax) is made as follows: Dip the end of a prepared thread in a 10 per cent. solution of hydrochloric acid and allow to dry. Lay the thread on a slide, cover with cover glass and examine. It should be of a reddish-brown color. To the edge of cover glass apply a droplet of a 10 to 13 per cent. solution of sodium carbonate, followed by a droplet of the suspected solution. In the presence of boric acid, the thread is colored blue, which coloration remains for a longer or shorter period and then changes to gray and violet. The test is a very delicate one and is not hindered by the presence of sodium chloride, magnesium sulphate and aluminium sulphate. Strong solutions of phosphoric acid, silicic acid, calcium chlorite and magnesium chlorite, interfere with the reaction more or less.

4. Formaldehyde Test.—Concentrated hydrochloric acid added to weak solutions of formaldehyde (1 : 5000) or substances containing formaldehyde, forms stellate clusters having a somewhat crystalline appearance. The formaldehyde can be deposited on a slide by sublimation (as for benzoic acid) and the acid added. The stellate clusters appear upon evaporation of the hydrochloric acid. The test requires further verification to determine its value.

5. Sulphurous Acid Test.—Moisten starch paper with a very dilute solution of potassium-iodide iodine solution which colors it blue. In the presence of the merest trace of sulphurous acid the paper is decolorized. Do not use heat in this test.

6. Iodine Reaction.—The color reaction of starch with N/50 iodine solution is of great importance in the examination of fruit products, such as jams, jellies, catsups, etc., as it shows whether or not ripe or green fruits and juices of unripe fruit were used and whether or not starch paste may have been added as a filler or thickening agent. As is known, green fruits generally contain more or less starch, whereas ripe fruits are quite generally free from starch. The reaction may be observed only in the fruit pulp cells, indicating the presence of unripe fruit, or it may be

limited to the non-cellular portions of such substances as jams and jellies, indicating the use of fruit juices obtained from unripe fruits.

7. Microscopical Examination of Bacteria and Metals by Direct Sunlight.[1]—Very minute quantities of certain minerals as iron, copper, mercury, and a few others, can be detected in liquids and semiliquids (in the form of metallic hydroxides) when examined (on slide mounts) by means of direct sunlight. All transmissible light must be cut off.

Direct sunlight can also be used in making bacterial counts in liquids, using the Thoma-Zeiss hemacytometer (Turck ruling). The bacteria are readily recognizable on the dark background, standing out far more clearly than in the usual examination by transmitted light, because of the more pronounced color contrasts.

8. Micro-gluten Test.—Mount a bit of the flour in water on a slide, being careful not to use too much water. Cover with cover glass and move cover glass to and fro a few times on the mounted material. The gluten separates into stringy fragments which may readily be seen under the low power of the compound microscope. The use of a weak solution of carbol-fuchsin, sofranin, or other stain, will bring out the gluten particles more clearly.

9. Hand Gluten Test.—Moisten wheat flour with water, making it into a dough. Knead constantly and carefully under a slow

[1] The optical principles of the ultra-microscope of Zsygmondy and Siedentopf depend upon the use of direct sunlight (or other intense light) combined with an absolutely dark field, with or without the use of a condenser, the rays of light being directed upon the object to be examined approximately at right angles to the optical axis of the compound microscope.

The limits of vision with the ultra-microscope are approximately 0.003μ, however, solid particles (as of metallic colloids) of not more than 0.003μ in diameter show no structure, they appear rather as points of light.

The limits of vision with the ordinary microscope are, for air (white light) about 0.30μ, for homogeneous immersion (white light) about 0.25μ, and for homogeneous immersion when rays of shorter wave length than white light (as the blue spectrum) are used, are about 0.15μ.

stream of water, washing out all of the starch. The gluten separates out as a tenacious gummy mass. With care fairly accurate quantitative results may be obtained. Weigh the dried flour and compare with weight of the dried gluten mass. With cereal flours other than wheat, the entire dough mass is gradually washed away, leaving no gluten.

10. Agar in Jams, Jellies and Similar Fruit Products.—The method generally recommended is to ash a sample of the jam or jelly at as low a temperature as possible, and to add weak hydrochloric acid for the purpose of decomposing the carbonates, etc. If agar has been added to the substance the silicious skeletons of diatoms will appear in the ash residue examined under a compound microscope.

A far better method is to dissolve (with heat) about 10 grams of the substance in 200 cc. of distilled water and centrifugalize (while still hot) for half an hour. Decant off the supernatant liquid and examine the residue microscopically. If agar has been added, characteristic agar diatoms (mostly *Arachnodiscus ehrenbergii* Baillon) will be found, also undissolved agar cell fragments and remnants of undissolved parasitic algal forms, which are quite universally found upon agar. The undissolved agar remnants and the algal parasites, which are in fact almost as characteristic as the diatoms, would be wholly destroyed by the ashing process. Furthermore, the ashing-acid process, no matter how carefully done, results in a comminution and destruction of some of the diatom shells. Finding one or more diatoms and one or more algal remnants in one slide mount (or in 5 to 20 fields of view) is conclusive evidence that agar has been added, though this does not indicate the exact amount that is present. If the characteristic structures (diatoms and algal remnants) are comparatively abundant then it is safe to conclude that agar has been added in considerable amount (2–4 per cent.) or that an impure grade of agar was used. The purer the grade of agar the fewer are the diatoms present, but no agar has yet been found on the

market which is wholly free from diatoms, undissolved agar cells and algal parasites.

The reason why distilled water should be used in making the solution for centrifugalizing is because ordinary hydrant water may contain diatoms, which might be confusing, especially to a beginner, although the marine diatoms are mostly quite different in form from the fresh water diatoms. With a high-speed centrifuge less material and less time need be consumed. Also, the more complete the solution the better the results.

8. Micro-chemical Color Reaction Tests

There are certain micro-chemical color reactions, other than those already mentioned, which are of great value in determining the presence of impurities or adulterants in liquids and semi-liquids. The methods as perfected by F. Emich depend upon the use of cotton fibers treated with certain chemicals which convert the metallic compounds into the sulphides. The prepared threads can be readily transferred to the several solutions used and the color and precipitation effects can be observed under the microscope. The following are the more important reagents and reactions:

1. **Cotton Threads for Metal Tests.**—Dip absorbent cotton threads alternately into 15 per cent. solutions of sodium sulphide and zinc sulphate, pressing between blotting paper, and air-dry each time.

The threads thus prepared should assume a deep black color with a 1 per cent. solution of silver nitrate. They may be kept for a long time and are used to demonstrate the presence of As, Sb, Au, Pt, Cu, Hg, Pb and Bi, in various chemical compounds.

2. **Ammonium Sulphide Vapor Test.**—Place a few fibers of absorbent cotton into a drop of the suspected solution and allow the moisture to evaporate. Suspending the threads in the vapor of ammonium sulphide will indicate the presence of Cd, Hg, Ag, Fe, Co and Ni (dark to black coloration).

The prepared threads are used in the following tests:

a. **Arsenical Test.**—Dip a sodium sulphide thread into the suspected solution and allow to dry. In the presence of 0.008 per cent. arsenic there is a distinct yellowish coloration, due to the sulphide of arsenic formed in and upon the threads. The arsenical threads will also show the characteristic reactions with hydrochloric

acid, ammonia and ammonium sulphide by bringing a drop of the reagent in contact with the thread upon the slide. (See also Biological Test for Arsenic in Part II.)

b. **Zinc Test.**—Dip cotton fibers into the suspected solution, allow the moisture to evaporate, and then dip the threads into a solution of gold chloride. A violet coloration develops which remains in the presence of acids but vanishes in the presence of chlorine water, indicating the presence of zinc chlorite. The reaction is appreciable in the presence of 0.003 μg of zinc chlorite, whereas in the form of the sulphite, 0.1 μg of zinc is required to show the reaction.

c. **Antimony Test.**—Dip a sulphide thread into the solution, allow solution to evaporate and then expose the thread to the vapor of ammonium sulphide. If the solution to be tested contains considerable hydrochloric acid, sulphide of antimony is formed upon evaporation.

d. **Gold Test.**—Gives a brown coloration with the sulphide thread, which color disappears upon prolonged exposure to ammonium sulphide, more quickly on exposure to chlorine, bromine and sodium hypochlorite. The threads which have been decolorized with chlorine are colored blue to black with iron chlorite and violet to red with zinc chlorite.

e. **Silver Test.**—A neutral or faintly acid silver nitrate solution gives a brown to black coloration with the sulphide thread, the depth of the reaction depending upon the concentration of the solution. The fibers can be decolorized by placing in sodium hypochlorite, and the color can be restored by means of zinc chlorite or an alkaline solution of grape sugar. Sulphuric acid will again decolorize.

f. **Mercuric Chloride.**—Cotton threads dipped into a solution containing mercuric chloride and exposed to the vapors of ammonium sulphide or ammonia, are colored black. The color is quite permanent in the presence of acids. A sulphide thread is colored yellow in neutral solution of mercuric chloride, changing to black in the ammonium sulphide vapor.

g. **Lead Test.**—Neutral lead solutions (lead nitrate) turn the sulphide threads yellow and black on prolonged exposure to ammonium sulphide. In acid solutions the color reaction with the sulphide thread is black. The yellow coloration is promptly changed to black upon exposure to ammonium sulphide, or when placed in weak sulphuric acid (1 : 15). The latter reaction distinguishes between lead and mercury, as the yellow coloration of the mercury is changed very slowly with dilute sulphuric acid.

h. **Bismuth Test.**—Solutions color the sulphide thread reddish-brown. Bromine causes the color to disappear. Potassium dichromate causes a yellow coloration, while alkaline solutions of zinc chlorite produce a black coloration. Lead solutions are not reduced by alkaline solutions of zinc chlorite.

i. **Iron Test.**—Ammonium sulphide vapor gives a black precipitate which is soluble in weak solutions of hydrochloric acid. Potassium ferrocyanide gives a blue coloration.

j. **Copper Test.**—Solutions of copper sulphate give a brown coloration to the sulphide thread, which color persists in 10 per cent. hydrochloric acid, but disappears on exposure to bromine vapor. The threads which have been bleached with bro-

mine give the copper ferrocyanide reaction when placed in an acidulated solution of potassium ferrocyanide.

The following table from the work by Koenig gives the relative sensitiveness of the tests above described:[1]

Elements in combination valency	Reaction	Limit (mg. × 10^6)	Comparative sensitiveness
Bo'''	Curcuma thread	0.1	1 in 33,000
As'''	Sulphide thread	10.0	1 in 2,500
Sb'''	Sulphide thread	1.0	1 in 40,000
Sn''	Violet color with sulphide thread	3.0	1 in 20,000
Au'''	Sulphide thread—brown, purple	3.0	1 in 22,000
Pt''''	Sulphide thread	8.0	1 in 6,000
Cu''	Sulphide thread + ferrocyanides.	8.0	1 in 4,000
Ag'	Sulphide thread + Ag	5.0	1 in 22,000
Hg'	NH_3 vapor	8.0	1 in 25,000
Hg''	Sulphide thread	5.0	1 in 20,000
Pb''	Sulphide + $PbCrO_4$	8.0	1 in 13,000
Bi'''	Sulphide + chromate + Bi	8.0	1 in 9,000
Cd''	(NH_3SH) vapor	6.0	1 in 9,000
Fe''	(NH_3SH) — blue	8.0	1 in 3,500
Co''	NH_3SH or nitroso—beta—naphthol	0.3	1 in 100,000
Ni''	NH_3SH	0.3	1 in 100,000

9. Making Analytical Reports

The methods of micro-analysts, whether in private, commercial or government laboratories, should be uniform. Much could be

[1] The comparative degree of sensitiveness of the different chemical compounds concerned in the color reactions above described and tabulated is indicated by the number of cubic centimeters in which 1 gram of the substance in solution is still appreciable. The actual limit, determined experimentally, is indicated in terms of milligrams, that is 0.001 mg., represented by μg. Expressing the comparative sensitiveness (CS) in a formula we have

$$CS = \frac{\mu g \text{ limit}}{\text{amount limit}} \times \frac{\text{molecular weights}}{\text{combination valency}}$$

or to give the example for boron, we have

$$CS = \frac{0.00001}{0.00000006} \times \frac{59}{3} = 33{,}000.$$

done to bring this about if the analysts were to meet for the purpose of comparing methods and results. Uniform blank report forms should be adopted and used in the micro-analytical laboratories, somewhat like those used by chemists. It cannot, however, be denied that the efficiency in the work done depends largely upon the ability, judgment and experience of the analyst.

The reports of the micro-analysts may be made according to the following groups:

I. Drugs and foods of vegetable origin, including dry or solid products of both animal and vegetable origin.

II. Liquid or moist products of animal and vegetable origin (canned and preserved products generally).

III. Bacterial examinations of liquids, foods and drugs.

There should be a special blank report card for each group of substances, arranged as follows:

FORM NO. I

No..... (I. S., laboratory or other serial number).
Label.....
.....
Sample received.....Sample examined.....
Condition of wrappings and seals.....
Organoleptic tests.....
Consistency of feel.....
Color.....
Odor .....
Taste.....
Adjunct tests .....
Sand (beaker test).....Per cent.
Ash.....Per cent.
Acid-insoluble ash.....Per cent.
Special tests.....
.....
Microscopical findings.....
.....
.....
.....
Conclusions.....
.....
.....
.....Analyst.

FORM NO. II

(No., label, dates, condition of seal and organoleptic tests, as for form I.)

Adjunct tests.

Sublimation tests for.....

Benzoic acid.....

Salicylic acid.....

Boric acid (curcuma thread).....

Iodine reaction.....

Intracellular.....

Extracellular.....

Special tests.....

.....

Microscopical findings.

General.....

.....

.....

Cytometric counts.

Dead yeast cells.....per cc.

Living yeast cells.....per cc.

Bacteria.....per cc.

Mold (hyphal fragments and hyphal clusters)...per cc.

Mold spores.....per cc.

Conclusions.....

.....

.....

.....Analyst.

FORM NO. III

Bacteriological Examination

(No., label, dates, condition of seals as for form I.)

1. Direct count. (Thoma-Zeiss hemacytometer with Turck ruling.)
   1. Bacilli per cc.....
   2. Cocci per cc.....

II. Plate and tube cultures. (Lactose-litmus-agar.)
   1. Temperature differential test.
      a. (20° C.) colonies per cc.....
      b. (38° C.) colonies per cc.....
   2. Color differential test.
      a. Pink or yellow colonies per cc.....
      b. Not pink or yellow colonies per cc.....
   3. Gelatin liquefying colonies per cc.....
   4. Indol reaction (±).....
   5. Neutral red reduction (±).....
   6. Gas (hydrogen) formula.....
   7. Gram stain behavior (±).....
   8. Presumptive colon bacillus test (±).
      a. Amounts used.....
      b. Number of tests.....
      c. Rating.....

III. Special tests.....
.....
.....

IV. Conclusions.....
.....
.....
.....Analyst.

We may give an example of a report as follows:

FORM NO. II

Lab. No. 462.

Label: *Pure currant jelly. Made by Smith, Jones & Co., Nantucket, Wis.*

Sample received *August* 5, 1914. Sample examined *August* 5, 1914.

Condition of seals: *Good, unbroken sample.*

Organoleptic tests: *Not conclusive.*

Consistency or feel: *Poorly jellied.*

Color: *Normal for currant jelly.*

Odor: *Faint, somewhat disagreeable.*

Taste: *Not characteristic, bitterish, quite acid.*

Adjunct tests.

Sublimation tests for

Benzoic acid: *Negative.*

Salicylic acid: *Very marked.*

Boric acid (curcuma thread): *Negative.*

Iodine reaction: *Very marked.*

Intracellular: *Negative.*

Extracellular: *Positive, very marked.*

Special tests: *Salicylic acid color reaction, with ferric chloride, very marked.*

Microscopical examination.

General. *Some apple tissue (window cells and pulp cells) and currant tissue (sclerenchyma) present. Added wheat starch about 5 per cent.*

Cytometric counts.

Dead yeast cells, 80,000,000.....per cc.

Living yeast cells, *none*.....per cc.

Bacteria, 600,000,000.....per cc.

Mold (hyphal fragments and clusters), 84,000 per cc.

Mold spores, 5,000,000.....per cc.

Smut spores, *none*.....per cc.

Conclusions: *Misbranded. Adulterated with apple and with wheat starch and made from fermented and decomposed material, preserved with salicylic acid. Not fit for human consumption because of the quantity of yeast, mold and bacteria present.*

John Doe, Analyst.

The great advantage of the micro-analytical work as compared with chemical work lies in the fact that small amounts of the substances are used for analysis, the equipment is comparatively inexpensive and the results are quickly attained. From twenty to forty and even sixty samples of simple spices can be examined in one day, from five to twelve samples of powdered vegetable drugs, cocoas, chocolates, flours, meals, etc., and perhaps an equal number of jams, jellies, etc.

Because of the very close relationship between the microscopical and bacteriological work, as already explained, certain essentially micro-analytical methods will be given under bacteriological methods, more especially in Chapter 2 of Part II which deals with the direct bacterial counts, and also under milk analysis, water analysis and meat analysis.

DESCRIPTION OF PLATE I

FIG. 1.—**Types of Pollen Grains.**—1. Saffron flower. 2. Flax. 3. Pink. 4. Pumpkin and squash. 5. Cloves. Mature pollen grain. 6. Cloves. Immature pollen grain. 7. Onagraceæ. *Circea lutitiana* (Enchanter's Nightshade). 8. Scutellaria. 9. Mallow. Distended by moisture. 10. Mallow. Normal form. 11. Albuco. 12. *Lobelia inflata.* 13. Compositæ, showing one mature and two immature pollen grains. 14. Hibiscus. 15. Pine pollen. 16. Santonica. 17. Mentha species. 18. *Hyoscyamus niger.*

FIG. 2.—**Potato Starch.**—The granules are large and the markings (hili, lamellations) are distinct. The cross bands under the polarizer are very distinct. Potato starch, mounted in water, makes a good test object for judging the resolving power of objectives. Dried and ground potatoes and potato parings are sometimes used for adulterating purposes.

FIG. 3.—**Starches.**—1. Sago starch from *Cycas revoluta* (Cycadaceæ). The commercial article known as sago is usually in the form of small granules (pearl sago). There are many false sagos made from other than Cycad or Palm starch. Much of this false sago is made from corn starch.

2. Canna starch from several species of Canna. The markings are very distinct, the hili being at the larger end as a rule. Also called arrowroot (*tous le mois* arrowroot).

3. Cassava or tapioca starch from the tuberous roots of *Manihot utillissima* and other species of Manihot. Simple and compound granules; the granules are largely separated in the processing, thus giving the appearance of simple granules. Their compound origin is, however, recognizable by the contact facets.

4. Maranta starch (Arrowroot starch) from *Maranta arundinaceæ* (Marantaceæ). The granules have many of the structural characteristics of potato starch.

5. Yam starch from several species of Dioscorea (Dioscoreaceæ).

FIG. 4.—**Dextrinized Starch.**—The process of baking and cooking causes the starch granules to undergo marked structural changes. They become much enlarged, the outline becomes quite indistinct and the hili and lamellations are distorted and correspondingly indistinct. 1. Normal wheat starch granules. 2. Normal rye starch granules. 3. Dextrinized wheat and rye granules. 4. Normal and dextrinized corn starch. 5. Normal and dextrinized bean starch. 6. Normal and dextrinized ginger starch.

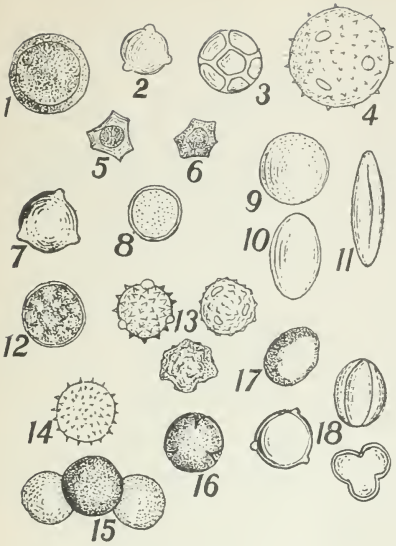


FIG. 1.

FIG. 2.

FIG. 3.

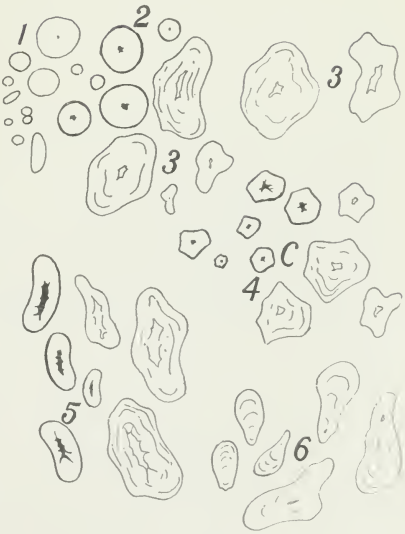


FIG. 4.

DESCRIPTION OF PLATE II

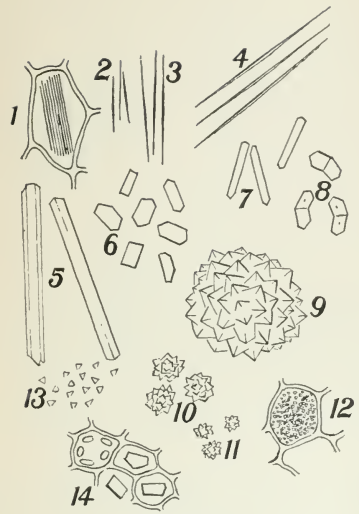
FIG. 5.—**Types of Crystals of Calcium Occurring in Different Plants.**—1. A parenchyma cell containing a bundle of needle shaped (acicular) crystals of calcium oxalate (raphide). 2, 3, 4, Acicular crystals differing in length, as they occur in Scilla and in other representatives of the liliaceous groups of plants. 5. Much elongated prismatic crystals as they occur in Quillaja and in *Iris florentina*. 6. Prismatic crystals very widely distributed in the plant kingdom. 7. Elongated prismatic crystals. 8. Twin crystals as they occur in Ulmus bark. 9. Very large aggregate crystals as they occur in Rheum and Polygonum species. 10, 11. Smaller aggregate crystals very widely distributed in the vegetable kingdom. 12, 13. Very minute prismatic (pyramidal) crystals as they occur in Belladonna. 14. Prismatic crystals as they occur in Hyoscyamus and in other plant groups.

Calcium oxalate crystals are among the highly diagnostic structural characteristics of drug plants and should be studied not only as to form but also as to size. They are not dissolved in the usual mounting media and are not destroyed by heat. They dissolve slowly in the stronger acids (hydrochloric acid).

FIG. 6.—**Types of Bast Cells as They Occur in Barks and in Other Plant Parts.**—1. Shorter bast cell as they occur in the cinnamon barks. 2. Typical bast cell (showing a portion of a cell only) as they occur in willow bark, in Ulmus, in Mezereon, etc. 3. Branching bast cells as they occur in Quillaja and in Prunus bark. 4. Greatly thickened sclerenchymatous bast cells as they occur in the Cinchonas.

FIG. 7.—**Types of Sclerenchyma (Stone) Cells.**—1. Typical sclerenchyma cells as they occur in the endocarp of drupaceous fruits and nuts. 2. Elongated bast-like sclerenchyma cells. 3. Thin-walled typical sclerenchyma cell. 4. Sclerenchyma cell with unequally thickened walls as they occur in the cinnamons. 5. Large thin-walled sclerenchyma cells as they occur in the seed coat of Amygdala. 6. Branching sclerenchyma cells as they occur in tea leaves and in peanut exocarp. 7, 8, 9. Forms of sclerenchyma cells.

FIG. 8.—**Typical Sclerenchyma Cells** (in groups) as they occur in the pulp of the pear.



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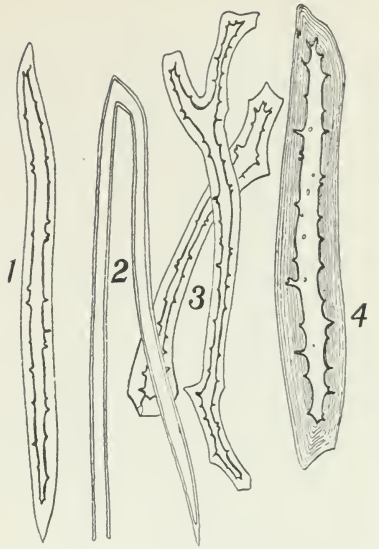

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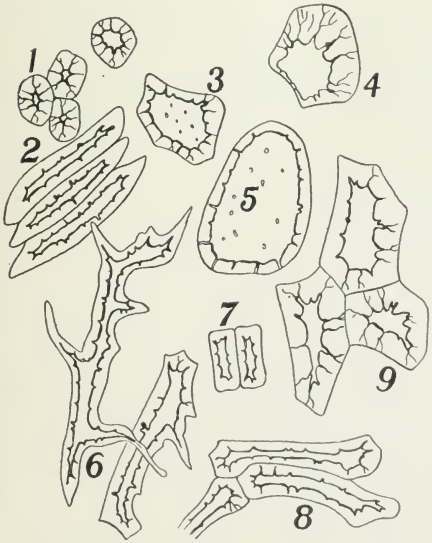


FIG. 7.

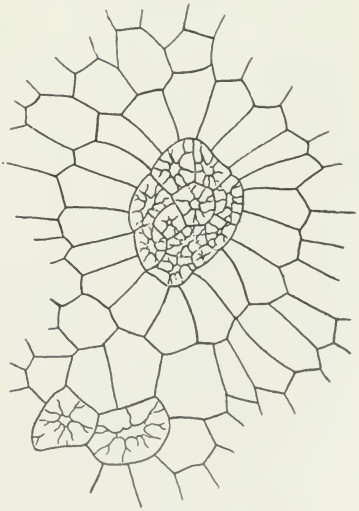


FIG. 8.

DESCRIPTION OF PLATE III

FIG. 9.—**Buckwheat.**—1. Proteid-bearing tissue. 2. Starch-bearing endosperm tissue. Cell walls are very thin and the entire cell lumen is packed with starch granules. 3. Starch granules. The granules resemble those of corn, being somewhat smaller. 4. Sclerenchymatous fibers.

Buckwheat is the predominating ingredient of the buckwheat pancake flours and is occasionally used as an adulterant of spices.

FIG. 10.—**Tissues of the Pine.**—1. The characteristic tracheids with bordered pits. 2. Bast-like fibers of the bark. 3. Crystal-bearing bark parenchyma cells. 4. Tracheids in radial view. 5. Medullary ray cells in radial view. Pine wood (pulp) is much used in making paper.

FIG. 11.—**Sclerenchyma Cells of Olive Pits.**—Ground olive pits were, until recently, extensively employed as an adulterant of spices and drugs.

FIG. 12.—**Clove Stems.**—A very common adulterant of cloves and of allspice. 1. Typical sclerenchyma cells. 2. Sclerenchyma cells with unequally thickened walls. 3. Sclerenchymatous bast fibers.

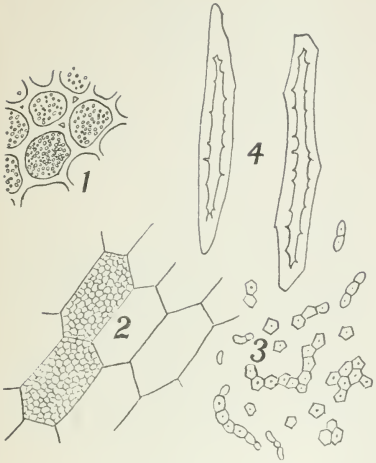


FIG. 9.

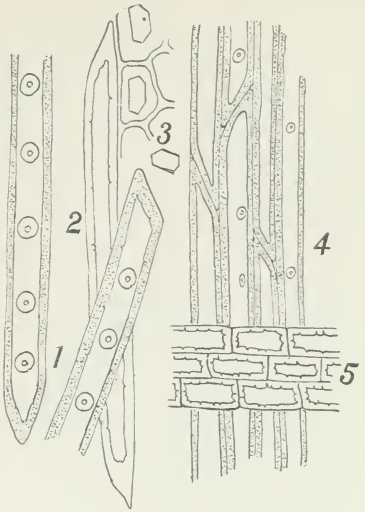


FIG. 10.

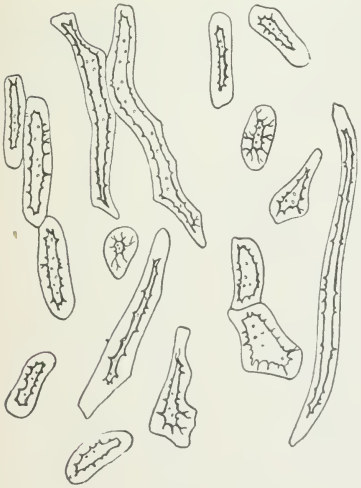


FIG. 11.

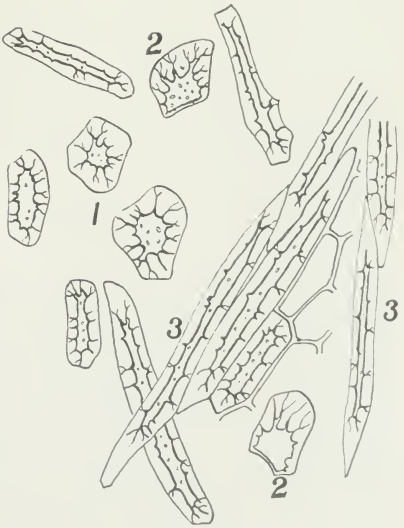


FIG. 12.

DESCRIPTION OF PLATE IV

FIG. 13.—**Cassia Buds and Cassia Stems.**—1. Sclerenchymatous fibers of the cassia stems. 2. Bast fibers of cassia stems. Parenchymatous cells of the buds. 4. Trichomes of buds. 5. Thick-walled parenchyma cells. Cassia buds and cassia stems are frequently used in adulterating cloves, allspice and cinnamon.

FIG. 14.—**Coffee Adulterants.**—1. Sclerenchyma cells of date pits. 2. Sclerenchyma cells of the walnut shell. 3, 4, 5. Tracheids and inulin-bearing parenchyma cells of chicory. Figs and prunes are also much used as coffee adulterants, also cereals, fleshy roots, acorns, etc.

FIG. 15.—**Wheat Tissues.**—1. Wheat starch. 2. Trichomes from the bran. 3. Starch-bearing parenchyma. 4. Epicarp cells. 5. Proteid-bearing cells from middlings. Rye histology is similar to that of wheat. Wheat flour is used in macaroni, spaghetti, noodles, etc. Wheat flour, bran and middlings are much used for adulterating purposes. Rye starch differs from that of wheat in the larger size of the granules and the greater prominence of the hili.

FIG. 16.—**Rice Tissues.**—1. Starch. Single granules and aggregates. These aggregates are characteristic of rice and of oats. 2. Starch-bearing endosperm cells. 3, 4, 5. Epicarp and pericarp cells. In form the starch granules of rice, oat, corn, darnel, millet, fox-tail, buckwheat and chess resemble each other. The size varies very much.

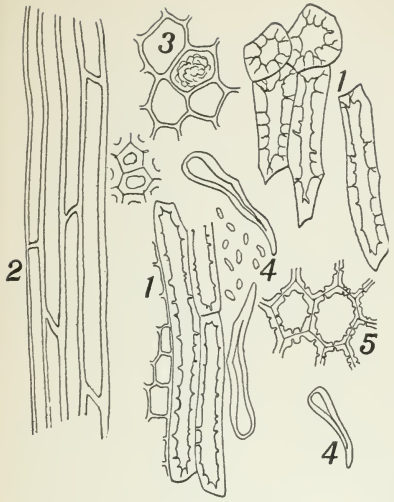
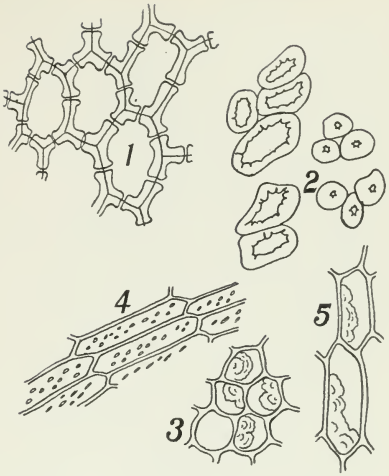


FIG. 13.



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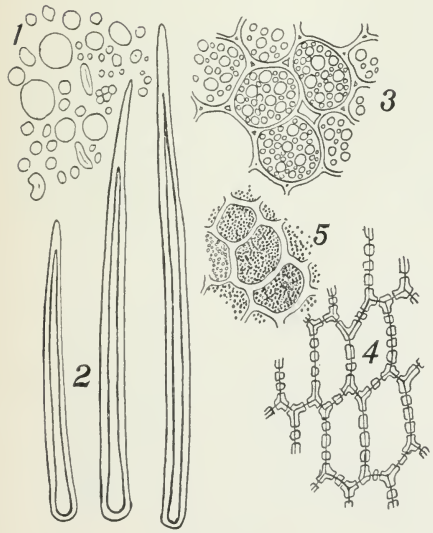

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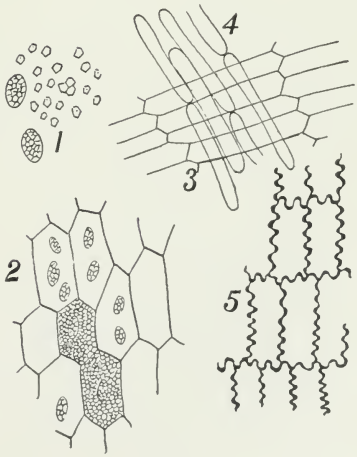


FIG. 16.

DESCRIPTION OF PLATE V

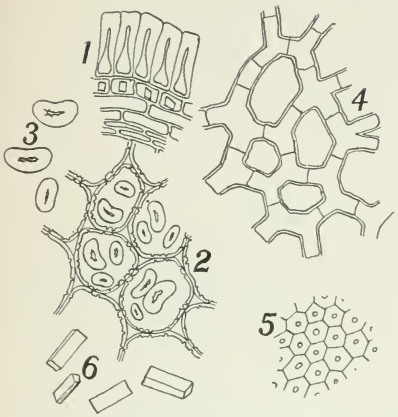
FIG. 17.—**Bean Tissues.**—1. Epidermal palisade tissue with the crystal-bearing hypoderm. 2. Starch-bearing endosperm tissue. 3. Starch granules with prominent fissured hili. 4. Spongy tissue. 5. Epidermal palisade cells in vertical view. 6. Prismatic crystals of calcium from hypoderm.

Ground beans, peas and lentils are used for adulterating purposes.

FIG. 18.—**Histology of Mallow Leaf.**—1. Transverse section of leaf showing stellate trichome, epidermal, palisade and spongy tissue cells. Aggregate crystals of calcium oxalate are present. 2. Stellate or aggregate trichomes. 3. Epidermal cells (lower) showing stomata. Mallow leaves are extensively employed for adulterating leafy spices and drugs.

FIG. 19.—**Histology of Corn.**—1. Corn starch. 2. Starch-bearing endosperm of corn kernel. 3. Trichomes of the chaff of the corn cob. 4. Sclerenchymatous cells of the corn cob. Ground corn cobs are used for adulterating purposes and also in the manufacture of artificial maple syrup flavor.

FIG. 20.—**A Few Types of Trichomes.**—1. Branching trichome of mullein. 2. Many-celled simple trichome of henbane showing wart-like marking on outer surface. 3. Simple single-celled trichome as of rye and wheat. 4. Glandular trichome with two secreting cells. 5. Glandular trichome with one secreting cell. 6. Many-celled glandular trichome. 7. Simple, single-celled trichome of Indian hemp. 8. Much elongated and twisted single-celled trichome, as of sage. 9. Sessile glandular trichome (Eriodictyon). 10. Indian hemp. 11. Pyrethrum. 12. Simple trichome.



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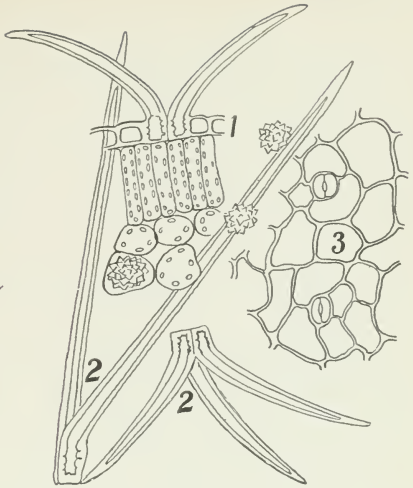

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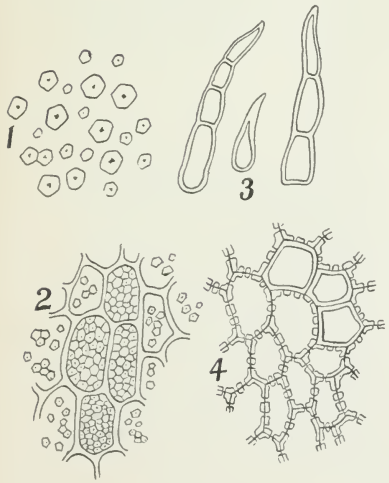


FIG. 19.

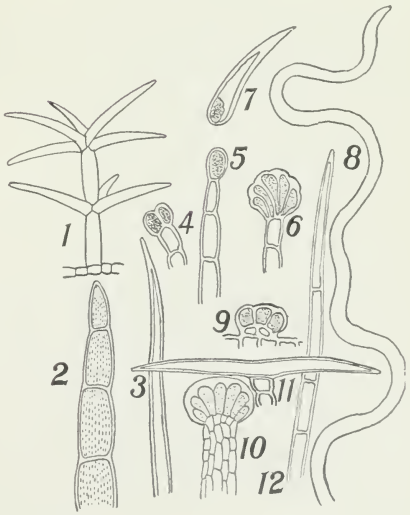


FIG. 20.

DESCRIPTION OF PLATE VI

Illustrating the Histology of a Typical Bark Showing all of the Tissues Which May be Found in a Bark.—A, Longitudinal section in the radial direction but not showing the medullary rays. B, Transverse section. 1. Outer bark. The demarkation between outer and inner bark is not always distinct. 2. Inner bark. 3. Beginning of wood tissue. *a*, Epidermis. Always wanting in tree trunks and older branches. *b*, Cork tissue. *c*, Bark parenchyma. Cell-walls are usually not suberized and the cells may contain various inclusions such as crystals of calcium oxalate, tannin, starch granules and resin. *d*, Groups of sclerenchyma cells. These, when present, normally predominate in the outer bark. *e*, Crystal-bearing fibers which usually accompany the bast fibers. *f*, Bast fibers. These, when present, normally predominate in the inner bark. The fibers may occur singly or in groups. *g*, Cambium. *h*, Wood fibers. *i*, Ducts. Usually of the typically porous type. *k*, Medullary rays.

An excellent typical bark having all of the histological elements indicated in Plate VI is *Rhamnus purshiana*. The demarkation between outer bark and inner bark is well defined in Ulmus and Quillaja.

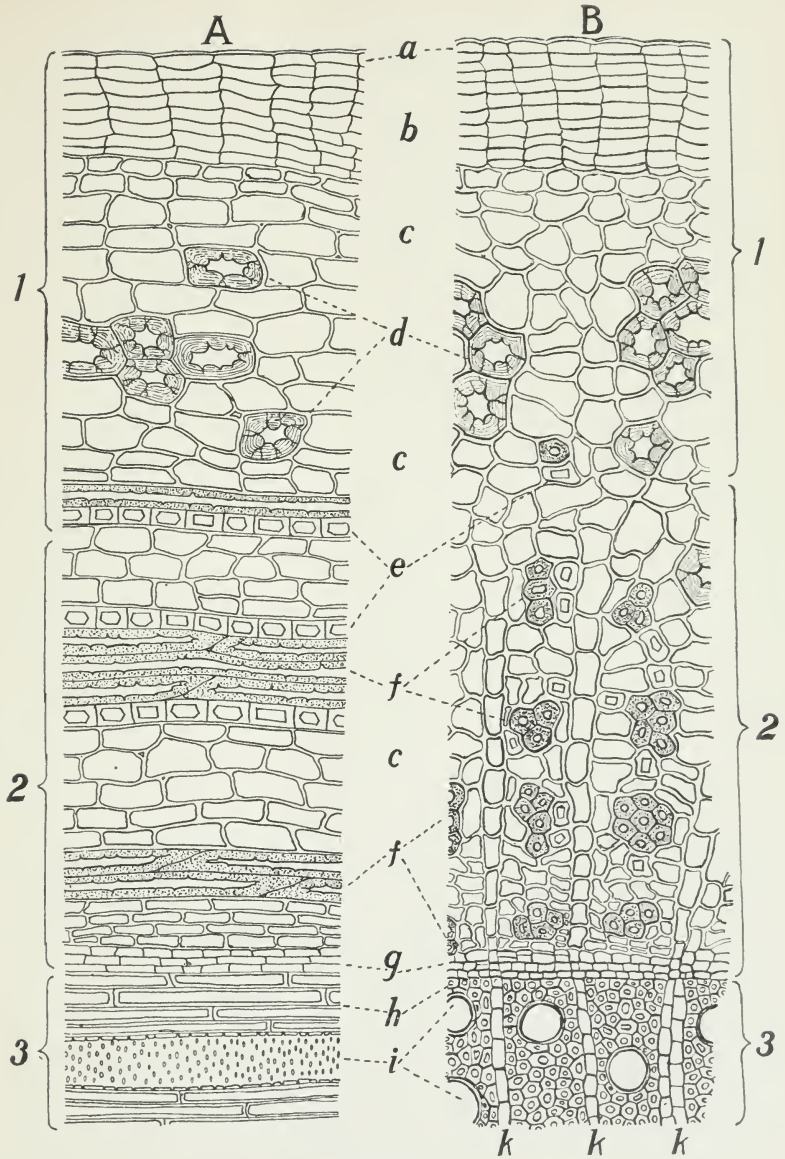


FIG. 21 .

II

BACTERIOLOGICAL METHODS IN FOOD AND DRUGS LABORATORIES

1. Introduction

The study of the significance of bacteria in foods of all kinds is one of the most important and interesting of scientific subjects and one which has received much attention ever since the science of bacteriology has become more highly developed as the result of the perfection of the compound microscope. For a long period of time the popular notion has prevailed that bacteria were essentially harmful and to designate any substance as bacterially contaminated was to pronounce it dangerous and to condemn it without trial. We now know that many, in fact most bacteria, are beneficent rather than harmful, and that many different species of bacteria are concerned in the preparation of food substances. It cannot be denied, however, that many kinds of bacteria as well as other organisms are concerned in the production of changes in food substances which we know to be highly detrimental to the well being of the human race. It is the duty of modern sanitary science to guard against disease and the contamination of food substances through the invasion of pathogenic and otherwise objectionable organisms. It is the work of the food bacteriologist to detect objectionable contaminations in foods and to aid in developing those processes and methods of food preparation and manufacture which will prevent the recurrence of such contamination. The food bacteriologist will center his attention on the following:

1. Chemical (decomposition) changes in foods and drugs induced by the various organic infecting agents, as bacteria and other living organisms, which render such substances unfit for human use or which render them dangerous for human use.

2. Foods and drugs as actual or possible carriers of infecting agents which are or may be dangerous to life or which may or might be injurious to the physical well-being of the human species.

It goes without saying that the food bacteriologist must proceed carefully in order that there may be no hasty decisions resulting in the condemnation of food products which are not injurious. There is, however, little excuse for hasty or unjustifiable passing of judgments as regards the quality of food. Bacteriological and toxicological methods have been sufficiently perfected so that the careful analyst need not make unfair or unwarranted decisions. The men entrusted with the critical examination of foods and drugs as to their fitness for human use should be investigators of authority and should have had wide range of practical as well as laboratory experience, and they should furthermore be possessed of good judgment. While the condemnation of food materials should not be hasty it should on the other hand not be too tardy or conservative. The prime object of the work by the food bacteriologist is to protect the consumer, not the dealer or manufacturer. This very important point is most unfortunately not properly heeded with the result that some of the work done in the administrative laboratories is, or appears to be, in the interests of the dealer or manufacturer.

A goodly number of infections enter the human system by way of the mouth with the ingested foods and drinks. Food substances form excellent pabula for the bacteria and other parasitic agents which enter the digestive tract or which may already have entered. Foods and drinks are exposed to infection in a great variety of ways. For purposes of illustration we may cite bread, the so-called "staff of life," as one of the foods which is liable to infection. It may be assumed that the loaf of bread, when it is taken from the oven, is entirely sterile and free from living organisms of all kinds. Just as soon as the loaf is cool enough to permit it,

the promiscuous manipulation begins and is continued until the bread enters the digestive tract of the consumers. The loaves are handled by the dirty, sweaty and oftentimes diseased hands of the baker or his helper. Basketfuls of uncovered bread are dragged over the dirty floors, over sidewalks, and through the filthy alleys. The uncovered loaves are repeatedly handled by the bakery drivers whose hands and clothing are generally very filthy. The uncovered loaves are left on doorsteps and other exposed places on the premises of the consumer. This much-handled bread is finally eaten, crust and all, without any attempt at sterilization. Such bread may be contaminated with a great variety of disease germs. Infections from hands, disease-bearing dust from the streets and alleys, excreta from disease-carrying flies, excreta from the intestinal tract of man and of animals are among the deposits which have been found on the exterior of bread. Miss Katherine Howell has traced an epidemic of typhoid fever to the consumption of contaminated bread and she has demonstrated the presence of typhoid fever germs and of intestinal bacteria on numerous loaves of bread. Edward Bartow, director of the Illinois State Water Survey, has also demonstrated a bread-borne typhoid epidemic in Rockford, Illinois. Colon bacilli are usually found in considerable numbers on every loaf of unwrapped bread. Every loaf of bread from the public bakeries should be wrapped in sterilized paper bags just as soon as it leaves the oven and it should remain in these bags until ready to be placed before the consumer.

Polluted water may carry the germs of dysentery, of cholera, of typhoid fever and the larvæ of intestinal and other parasites. Clams and oysters have caused typhoid epidemics. Fruits and vegetables are frequently polluted with fertilizer, especially where human fertilizer is used, as is the custom with the Chinese truck gardeners not only in China but also in other lands where the Chinese are found. Using human excrement as a fertilizer of soil should be prohibited by law. American army surgeons at

the time of the American occupation of Cuba made the filthy farming customs of the Chinese the object of a special report but apparently nothing ever came of the recommendations made. The Chinese also import dried human feces and dried human urine for medicinal purposes and a recommendation was made to Washington to prohibit such importations but apparently nothing has ever been done about it.

Pollution of fruits and berries of all kinds may come from the hands of pickers. Gathering of fruit is usually done by the very ignorant, those who have no proper conception of personal cleanliness and of sanitation. Entire families, men, women, and children, migrate to the fields and work during the hottest part of the season. They live in the open or in tents or perhaps in covered wagons. The environment of these temporary abiding places is anything but sanitary. Sickness often prevails in these camps, such as typhoid fever, scarlet fever, measles and dysentery, to say nothing of the more common body and intestinal parasites which infest many of the laborers. These multitudinous infections are brought in contact with the fruit, berries, peas, beans, lettuce, cabbage, cucumbers, etc., etc. The products of the field are then carried to the consumer by a driver who disseminates the contamination by mixing and frequent handling. And in spite of all this there are those who insist on eating berries unwashed because they might lose some of the natural flavor.

Next to bread, milk is the most popular food substance. Most unfortunately milk is also one of the best food substances for all manner of germs, harmful and harmless. Sickness in those employed about the dairying establishment has time and again caused epidemics, such as diphtheria, typhoid fever, scarlet fever, tuberculosis, dysentery, and streptococcic tonsillitis. Diseased animals transmit infection to humans, as will be more fully explained in the chapters following.

It is generally believed that the usual processes of baking and cooking as practised in the household are a sure guarantee that

the foods so prepared are entirely free from living bacterial infection of all kinds. This is true of some foods but not by any means of all of them. Dr. W. A. Sawyer, Director of the California State Hygienic Laboratory, in reporting upon an epidemic of 93 cases of typhoid fever (at Hanford, California) due to a single carrier, traced the source of the infection to cooked Spanish spaghetti, prepared by the typhoid carrier. The following tests were made in the California State Hygienic Laboratory to ascertain the effects of baking on the presence of typhoid fever germs in the interior of a mass of spaghetti.

"A large hot-air sterilizer was heated and kept between 160° and 170° C. (320° and 338° F.). The pan of spaghetti was introduced and subjected to this heat for 30 min. When the dish was removed the surface was of a golden brown color. The appearance and aroma suggested that the spaghetti was thoroughly cooked and very hot. The temperature near the top was 54° C. (129.2° F.) and at the middle, 23° C. (73.4° F.). Ten minutes later the temperature at the middle was 24° C. (75.2° F.) and the dish was then returned to the oven. Cultures taken at various levels showed that the typhoid bacilli were alive even close to the surface.

"In the next baking the oven was kept at temperatures ranging between 207° and 214° C. (405° to 417° F.). After half an hour the pan was removed. The surface was dark brown and the points sticking up from it were charred. The liquid around the margin was boiling vigorously and the whole dish was sizzling. The temperature just under the surface was 83° C. (181.4° F.). At the middle it was 28° C. (82.4° F.) and near the bottom it was 48° C. (118.4° F.). An hour later the temperatures had become nearly equalized and were 46° C. (114.8° F.) near the top, 42.5° C. (108.5° F.) at the middle, and 43° C. (109.4° F.) near the bottom. This showed that the interior of the dish did not reach even a pasteurizing temperature.

"Cultures taken at the surface soon after the pan had been

removed from the oven showed no typhoid colonies and very few of other kinds. Cultures taken at a distance of half an inch from the surface showed a few colonies of the typhoid bacillus, most of the organisms having been killed. Cultures from a depth of 2½ in. showed abundant colonies of typhoid bacilli. In these cultures the typhoid colonies were identified by their appearance on Endo medium and Russell medium and also by agglutination by anti-typhoid serum."

Dr. Sawyer sums up the experimental evidence as follows:

"The laboratory experiments completed the explanation of the Hanford outbreak by showing that the sauce used in making the Spanish spaghetti was a good culture medium and that the dish had not been sterilized after leaving the house of the typhoid carrier.

"Moreover, it was demonstrated that cooked dishes must be considered as possible conveyors of infection unless it can be shown that the method of cooking would produce complete sterilization. The slowness with which heat penetrates dishes like the Spanish spaghetti shows that very prolonged heating would be necessary for sterilization of large dishes of such food. Ordinary baking merely incubates the interior of these masses of food."

This report by Dr. Sawyer is of special significance to the food bacteriologist as it illustrates two very important factors concerned in the study of food sanitation: First, the possible contamination of food materials through carriers of disease, and secondly, the necessity of studying more carefully our present methods of sterilization (of food materials) through the agency of heat. As will be more fully set forth in subsequent chapters, the examination of canned foodstuffs shows that sterilization is far from complete in the great majority of cases.

In addition to the more or less acute infections traceable to the consumption of contaminated food products, there are the multitudinous infections which are of slow development or

chronic in character. In many of these cases it is not possible to acertain definitely how the infection entered the system. There are numerous so-called autointoxications which are said to be due to autolytic changes in the ingested food substances resulting in the formation of toxins which often give rise to very serious and even fatal poisoning. As is generally known, certain toxin-forming bacteria after once gaining access to the intestinal tract may remain there for years feeding upon the contents of the intestines and producing enough of the toxin to give rise to symptoms of poisoning of a more or less chronic character. In some instances the toxin-forming bacteria are not present in sufficient numbers or do not multiply in sufficient numbers to give rise to any marked symptoms, and in still other cases the originally pathogenic or toxin-forming bacteria lose their virulency after having lived in the intestinal tract for some time. As is known, there is constant warfare in the intestinal tract between the harmful and the really beneficent bacteria, and it is this discovery which has led Metschnikoff and other bacteriologists to find germs which upon being introduced into the intestinal tract would overcome or crowd out the objectionable toxin formers.

Food poisoning has received considerable attention in recent years. Vaughan and Novy have suggested a nomenclature applicable to certain recognizable forms of poisonings traceable to foods, as:

Bromatotoxismus or food poisoning.
Galactotoxismus or milk poisoning.
Tyrotoxismus or cheese poisoning.
Kreatoxismus or meat poisoning.
Ichthyotoxismus or fish poisoning.
Mytilotoxismus or mussel poisoning.
Sitotoxismus or cereal poisoning.

The poisonings mentioned are generally due to toxins or related products elaborated by bacteria, but in some instances the exact species responsible for such toxin formation have not yet been determined. The identification of the species of bacteria

responsible for the poisoning of foods and drinks is of minor importance. What is of prime importance to the food bacteriologist is to find the poison and if possible to ascertain the manner in which the poison gained access to the food substance, in order that methods may be devised to guard against the recurrence of such contamination. It may also be stated that in the great majority of cases of food poisoning the nature of the poison and its source have already been determined and means are available to protect the consumer. If the manufacturers of foods and of food products would give proper attention to the modern methods of manufacture, then poisonings due to the eating of such products will be a rare occurrence indeed. It is regrettable that so many of the smaller establishments engaged in the manufacture of food products are not better informed regarding the available modern methods of preparing and storing food substances in such a manner as to guard against infection and contamination. It is also regrettable that the various pure food and drugs laws and regulations intended to protect the consumer are not more efficiently and more strictly enforced.

We have already suggested a more efficient coordination of the chemical, microscopical and bacteriological methods of analysis in our food and drugs laboratories—federal, state, municipal and private. The following are the bacteriological methods applicable in the examination of foods and drugs as to quality and purity. It is hoped that the suggestions offered may serve as a basis for establishing more complete practical working methods and at the same time indicate lines for further research.

Just what bacteriological analyses and tests should be made in pure food and drugs laboratories has as yet not been decided upon. However, based upon the present purpose and scope of such laboratories, we submit the following outline as covering the field fairly well and which outline will be followed quite closely in the text, however not necessarily adhering to the same sequence of the subject-matter.

Quantitative and Qualitative Determinations of Organisms in Foods and Drugs

- Substances to be analyzed.
  - Liquids of all kinds.
  - Semiliquids and semisolids miscible with water.
  - Solids of all kinds.
- Numerical and quantitative limits of contamination in different substances.
  - For molds—quantity of spores and hyphæ.
  - For yeasts—number and kind.
  - For bacteria—number and kind.
  - For pus, dirt, sand, etc.
- Methods.
  - Making concentrations.
  - Making dilutions.
  - Making the counts and estimates.
    - Bacteria.
    - Yeasts.
    - Mold spores and mold hyphæ.
    - Algæ, in drinking waters, etc.
    - Protozoa.
    - Pus cells, in milk, etc.
    - Dirt, sand, etc.
  - Plate counts—Petri dish cultures.
    - Culture media used.
    - Optimum temperature.
    - Time of incubation.
- Qualitative determinations.
  - Apparatus.
  - Culture media.
  - Stains.
  - Special methods.
    - Colon group of bacilli.
    - Presumptive colon bacillus test.
    - Sewage streptococci.
    - Dysentery bacilli and amebæ.
    - Bacillus typhosus.
    - Paratyphoid group.
    - Cholera vibrio.
    - Yeasts.
    - Molds.
    - Animal parasites.
    - Larvæ, ovæ, etc.

Biological water analysis.
- Bacteria, number and kind.
- Diatoms.
- Desmids.
- Nostoc.
- Other algæ.
- Molds; significance of.

Bacteriological milk analysis.
- Quantitative.
  - Standards for different geographic areas.
  - Summer and winter standards—temperature standards.
- Qualitative.
- Pus and blood corpuscles; significance of.
- Milk diseases.
  - Blue milk.
  - Ropy milk.
  - Bad odors, bad taste, etc.
- Sour milk.
- "Buttermilk" tablets.
- Kefir, koumys, etc.

Bacteriological examination of shellfish.

The bacteriological and toxicological examination of meat and meat products.

The bacteriological examination of eggs and of egg products.

Bacteriological examination of mineral waters.

Bacteriological examination of pharmaceuticals.

Bacteriological examination of sera, vaccines, bacterins, etc.

The microscopical and bacteriological examination of syrups.

Standardization of disinfectants.
- Phenol coefficient.
- Albumen coagulation coefficient.
- Toxic coefficient.
- The efficiency value of disinfectants.

Biological toxicity tests.

Upon first consideration it would appear that the bacteriological methods in food and drugs laboratories might be closely similar to those in hygienic laboratories. Such is the case in a general way, however, with certain well-defined differences. Whereas the bacteriological work in hygienic laboratories pertains to the prevention of disease and finding the primary causes of disease, the work in the food and drugs laboratories has to do with the

investigation of the biological factors influencing the quality of food and drugs and the significance of pure food and drugs in the maintenance of the public health and the physical well-being of the human race, as against the pernicious effects of contaminated foods and drugs.

The question for first consideration is what bacteriological methods are necessary and practicably applicable in testing foods and drugs? This phase of the subject is comparatively new and accordingly there are but few food and drugs bacteriologists who have had any considerable general range of experience, and as a consequence there are comparatively few methods fully worked out. Most of the bacteriological investigations and researches pertaining to foods have been along special lines, and indeed much valuable information and useful data have been brought together. Within the last 10 years the work on the sanitary examination of milk and of water supplies has become monumental in volume as well as in importance. Numerous methods have been tried, some to be entirely abandoned after being for a time heralded as the final word in determining the potability of water supplies. The same may be said of the development of the bacteriological examinations of milk supplies.

Quite recently bacteriologists have given considerable attention to the sanitary examination of shellfish, more especially with reference to sewage contamination. In this investigation American bacteriologists have taken the lead. European bacteriologists have done an enormous amount of work in the examination of sewage and of sewage disposal, to say nothing of the classical researches on yeasts and on fermentation in general. However, the general bacteriology of foods and of drugs is as yet an unexploited field. It is true, the Bureau of Chemistry of the Department of Agriculture has, within recent years (since 1906), done considerable work on the sewage contamination of oysters and other shellfish (Bulletin No. 136, Bureau of Chemistry. U. S. Department of Agriculture, by George W. Stiles) and in

the quantitative estimation of the microbic contamination of certain food supplies, and still more recently the laboratory division of the U. S. Public Health Service has done much efficient work on the standardization of disinfectants. We must also mention the work on milk, meat inspection, etc., by the Bureau of Animal Industry and the work on sanitation and related subjects by the U. S. Public Health Service, not forgetting to mention the vast amount of routine analyses in state and municipal health laboratories and the sporadic research work in the biological and bacteriological laboratories of our colleges and universities and the individual investigations of food and drug contamination on the part of a few of the more enterprising state and municipal health officers. Very recently the sanitary study of mineral waters has received a great deal of attention on the part of individual workers. The Committee of the Laboratory Section of the American Public Health Association has prepared a report covering the general conclusions regarding some of the methods of analysis.

The purely microscopical examination of food substances and of drugs, with reference to contamination by mold, yeast and bacteria, should be a part of the work of the bacteriologist rather than that of the chemist. Therefore, for the sake of completeness, this phase of the subject is included in the present report. We shall now proceed with the discussion of the bacteriological method applicable in food and drugs laboratories, giving only the essential details, however adding certain suggestions intended as a guide for further investigation with a view to the improvement of the present working methods. Detailed description of apparatus and of technique will be given only when thought necessary.

For all practical purposes, the examination of foods and drugs for the presence of biologic contamination (inclusive of bacteria, yeasts, molds, protozoa, ova and larvæ of higher animal parasites, etc., etc.) is either made directly or indirectly. That is, the substance is either placed on a slide or counting apparatus and the

quantitative or qualitative determinations made directly under the suitable power of the compound microscope; or, certain quantities of the substances are placed in or upon certain culture media (Petri dish cultures, tube cultures, etc.) in order to bring out the biological and biochemical characteristics of the contaminating organisms, whereupon the cultural products are examined microscopically. In this latter instance the microscopical examination may even be entirely omitted.

The direct microscopical method has some very marked advantages and should be carried out whenever feasible, particularly when purely quantitative results or estimates is the main object sought after. In other instances the direct method must be combined with cultural tests and the two are often checks upon each other.

2. Direct Bacteriological Examinations—Quantitative Tests

Substances to be examined include waters and liquids of all kinds; sewage; milk[1] and cream; ice cream, liquid pharmaceuticals and medicamenta, oils, catsups, beverages of all kinds, all semisolids such as pastes, jams, jellies, etc., other semiliquids and semisolids which may be readily diluted with water if necessary; solids as powders, pills, tablets, soils, clays, meats, starches, dextrins, flours, meals, dried fruits, dried eggs, dried albumen, sugar, etc. In fact all substances which are in any way liable to contamination by micro-organisms.

The following is an outline of the methods of making determinations of the number of organisms in food and drugs.

a. Substances Requiring Concentration.—Certain substances which contain comparatively few micro-organisms, as drinking waters, mineral waters, beverages generally, tinctures, fluid ex-

[1] In the case of milk, the centrifuge is first used to separate out the fat as much as may be necessary to make the ready counting of the organisms possible. (See also Chapter on Milk Analysis.)

tracts, aquæ, etc., must be subjected to processes which will concentrate the organisms, as by passing the liquid through a filter in which the pores are sufficiently small to leave the organisms behind, as for example a Berkefeld or Chamberland clay tube. In addition to the filter, the centrifuge will be found useful as will be explained later.

Any liquid containing not more than from 100 to 1,000,000 organisms per cc. does not lend itself to direct examination quantitatively without concentration. The amount or volume of substance (liquid) to be passed through the filter will depend upon the degree of concentration required. Since the Thoma-Zeiss hemacytometer (with Turck ruling) is to be used in making the counts, the organisms should average at least 4 to 5 in the $\frac{1}{250}$ c.mm. areas of the counting apparatus, or 1,000,000 to 1,250,000 organisms per cc. Let us suppose that a direct count is to be made of a drinking water which is very pure, having not more than from 50 to 500 bacteria per cc. In order to make direct counting with the hemacytometer possible, it would be necessary to pass from 20 to 30 liters of the water through the clay filter and thoroughly mix the organisms left in the tube with 10 or even 1 cc. of filtered sterile water. To filter that amount of water requires too much time unless a large specially constructed apparatus is installed. For practical purposes, 1 liter is the largest amount of liquid that it will be necessary to filter and reduce to 1 cc., making a concentration of 1000. For special purposes the 1 cc. may be further concentrated in the centrifugal tube described in Fig. 3. Weaker concentrates may answer the purpose in some cases, as ten or one hundred, as in sewage, badly contaminated milk and in other liquids in which the number of organisms present may range from 100,000 to 1,000,000 per cc.

The special centrifugal tube described in Fig. 3 is used as follows: After passing a liter of the liquid to be examined through the clay filter tube and thoroughly washing out the organisms and other particles left in the tube, pour the contents into the special

Fig. 2.

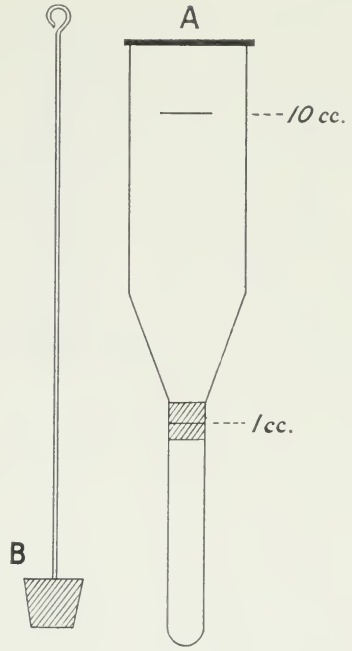


Fig 3.

Fig. 2.—Kitasato filtering outfit ready to be attached to the exhaust pump. A two-opening flask or bottle is interpolated to receive the backflow water, should there be any. Various types of clay bougies may be used with this filter. The rubber tubing for the connection must be heavy so as to prevent collapse by the exhaust pressure.—(*Pitfield.*)

Fig. 3.—Special centrifugal tube (*A*) for concentrating bacteria and other microorganisms in liquids and also used in isolating or separating motile bacteria from those which are not motile, as is explained under water analysis. The tube has a capacity of 15 cc. with 1 cc. and 10 cc. marks. The tube is in two parts. The lower narrowed end, having a capacity of 1 cc., is attached to the larger part by means of a rubber-band ring. Centrifugalization is done at high speed. After centrifugalization, the 1 cc. tube is removed and the contents thoroughly mixed by means of a platinum wire loop. To avoid loss of the contents of the tube during the mixing, attach the rubber ring. After the mixing the material is ready for the microscopical counting and other examination.

A suitable stopper attached to a brass or other metal rod (*B*) may be inserted into the narrowed portion of the upper part of the tube in order to prevent mixing of contents when removing the 1 cc. tube. These tubes will also be found useful in measuring the amount of sediment in milk, water and other liquids. For this purpose the 1 cc. portion should be graduated into tenths and hundredths.

centrifugal tube. For washing use about 10 cc. of filtered sterile water, adding up to the 10 cc. mark if necessary. Centrifugalize at high speed for 30 min., which will throw the bacteria and other solids down into the narrow 1 cc. end of the tube.

The following is a brief outline of the method of procedure: Use a Kitasato filter with the usual hydrant suction pump attachment. Pass a liter of the liquid to be examined through the filter, continuing suction until nearly all of the liquid has passed through. Remove the clay bougie and wash down the organisms clinging to the sides of the tube with not more than 10 cc. of distilled water which has been filtered and boiled. Place thumb over the opening of the tube and mix the contents thoroughly by shaking for 20 sec., then pour the thoroughly mixed contents into a sterile cylindrical graduate and add sterile distilled and filtered water up to the 10 cc. mark, shake thoroughly and make the counts at once by means of the hemacytometer. This procedure gives a concentration of 100. By means of the special centrifugal tube the concentration may be increased to 1000, as already explained. The method gives approximate results only, the counts as a rule being less than the actual number of organisms present in the liquid, a difference due to three chief sources of error: First, a small number of bacteria (especially the smaller motile forms) will pass through the clay filtering tube; second, some of the smaller bacteria are caught and held in the pores of the clay tube; and third, some organisms will remain clinging to the inner surface of the tube after the mixed contents are poured out for counting purposes. These sources of error are, however, not great, perhaps not exceeding 8 to 10 per cent., and are on the side of conservative estimates. The clay bougies used should be of the finest quality and should be of uniform and standard thickness. The sources of error by the direct method are perhaps not as great, certainly not greater, than by the usual plating methods and offer some very decided advantages. The concentrates show, in addition to the bacteria, other organisms

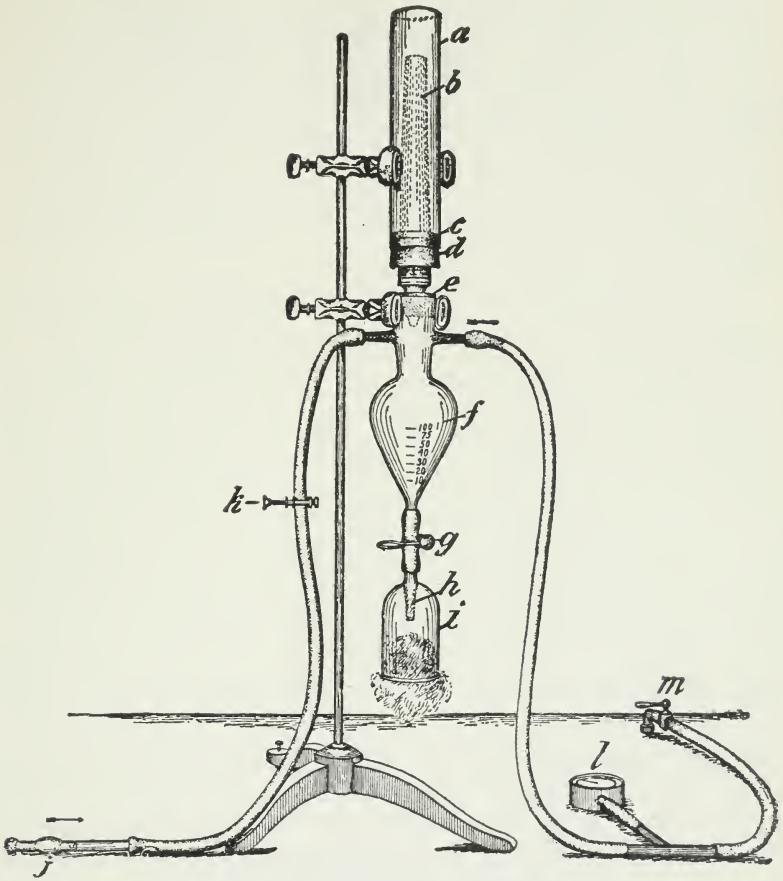
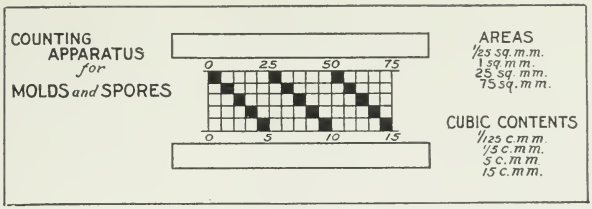


FIG. 4.—Apparatus for fractional filtration, designed for use with Pasteur-Chamberland or Berkefeld filters. *a*, Glass mantle surrounding filter; *b*, Chamberland filter; *c*, paraffin joint; *d* and *e*, rubber stoppers; *f*, double side-arm suction flask; *g*, pinchcock controlling outlet from suction flask; *h*, outlet tube surrounded by glass shield and attached to lower end of suction flask by means of short rubber tubing; *i*, glass shield fused to and surrounding outlet tube as a protection against contamination when the filtrates are drawn off; *j*, glass inlet tube plugged with cotton, for admitting air into suction flask; *k*, pinchcock governing the admission of air into flask; *l*, vacuum gauge; *m*, stopcock connected with vacuum pump.—(*U. S Dept. of Agriculture, Bureau of Animal Industry, Bull.* 113.)

as mold hyphæ, mold spores, protozoa, diatoms, etc., besides dirt particles, sand particles, starch granules, body cells, pus cells, etc., etc., which would be lost or rather which would not appear in the plating method. Furthermore, the counts can be made with a great saving of time—in a few hours as against 24 to 48 hr., and longer, by the plate cultural method. It is true that in many instances the direct method must be supplemented by the cultural methods when, in the judgment of the analyst, this becomes desirable or necessary.

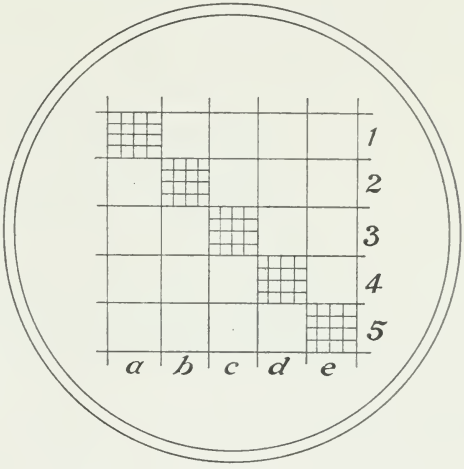
Concentrates may also be made by evaporation under reduced pressure. With a little ingenuity a suitable equipment may be constructed in the laboratory. The container of suitable capacity (1 liter and more) is connected with an exhaust pump which lowers the pressure sufficiently to cause boiling at a temperature not to exceed 37° C.; 24 hr. is usually sufficient time to evaporate the liquid to nearly dryness. After the evaporating process has continued for several hours, various enrichment media may be added to the liquid to be evaporated, which will of course aid the intended isolation and development of the desired bacteria. If the enrichment medium is added from the first, annoying bubbling and frothing may take place. This method is especially useful in isolating the typhoid bacillus, the paratyphoid group and the intestinal bacteria in general.

b. Substances Which do not Require Concentration.—Badly contaminated substances as sewage, milk from badly managed dairying establishments, badly contaminated liquids of all kinds, soups, broths, beer, wines and such products as tomato catsups, jams, jellies, canned oysters, etc., may be examined directly without the necessity of making concentrations, or of centrifugalization, in order to make quantitative and certain qualitative estimates. The substances of this class may be divided as follows, based upon the approximate number of organisms per cc. as determined by means of the spore and yeast counter described under Fig. 5, and the Thoma-Zeiss hemacytometer.



A

B



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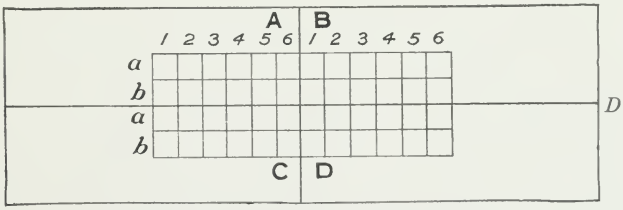

FIG. 5.—*A*, Counting apparatus for molds (hyphæ and spores) and yeasts. The rulings are 75 sq. mm., 25 sq. mm., 1 sq. mm. and $\frac{1}{25}$ sq. mm. On either side of the ruled area are glass slips 0.2 mm. thick, so that the entire capacity of the space within the ruled area is 15 c.mm., subdivided into 5 c.mm., $\frac{1}{5}$ c.mm. and $\frac{1}{125}$ c.mm. areas.

1. Substances in which the organisms are not too numerous to permit the use of the $\frac{1}{25}$ sq. mm. areas without making dilutions. That is, substances in which the number of organisms does not exceed 10,000,000 per cc., hence the number of yeast cells, spores, bacteria, etc., may not exceed forty in one of the $\frac{1}{250}$ c.mm. areas of the hemacytometer. The limit for the spore and yeast counter would be 5,000,000, before making the dilution is necessary.

2. Substances in which the number of organisms and spores are too numerous to permit the use of the $\frac{1}{25}$ sq. mm. areas of the hemacytometer, but permitting the use of the $\frac{1}{400}$ sq. mm. areas without making dilutions. The total number of spores, bacteria and other organisms may range from 10,000,000 to 100,000,000 per cc., numbers derived from finding on an average from 2.5 to 25 organisms in one of the $\frac{1}{4000}$ c.mm. areas of the hemacytometer.

The counter is used as follows: A bit of the thoroughly mixed substance, as jam, jelly, tomato paste, catsup, etc., is placed on the slide in the ruled areas and covered with a rectangular cover glass (No. 2). Slight pressure may be necessary to make the cover glass rest evenly on the two slips. The counting is done in areas entirely filled (from slide to cover glass) by the substance mounted. The larger areas may prove useful in estimating the amount of sand particles, dirt, etc., present. The $\frac{1}{125}$ c.mm. areas will be used in counting spores, yeast cells and mold hyphæ and similar contaminations. It is possible to make counts without dilutions as long as the number of organisms in the areas does not exceed forty. If more organisms are present in one area dilution becomes necessary, as already explained. Making dilutions of 1–10, 1–100 and 1–1000 makes the counting limits 50,000,000, 500,000,000 and 5,000,000,000 per cc. The $\frac{1}{125}$ c.mm. areas are also used in estimating the quantity of mold hyphæ present. Finding clusters of mold hyphæ in 25 per cent. of these smallest areas is presumptive proof that the substance is unfit for human consumption. Naturally the more finely divided the substance is the more numerous are the mold fragments. For making mold counts the material to be examined should be reduced to uniform fineness. This could be accomplished by rubbing a thoroughly mixed sample through a sieve of standard mesh, say $\frac{1}{4}$ mm.

B, a simplified modification of the counting apparatus just described, is made as follows: The two slips 0.2 mm. thick are placed in position, but the ruling is omitted and in place thereof an eye-piece scale *C* is used, the measuring value of which has been carefully determined by means of the stage micrometer. The rulings on the eye-piece must be delicate and the analyst must be careful not to move the eye or change the direction of his vision while making counts.

The ruled slide (*D*) will be found useful for making quantitative estimates of seeds, sand particles, dirt, larger parasites such as vinegar eels, ova of intestinal parasites, etc., in catsups, crushed berries (strawberries, raspberries, loganberries, etc.), jams and in other vegetable substances. Definite quantities of the substance to be examined are placed upon the ruled area of the slide by means of a small measuring spoon (0.25 gram, 0.5 gram, 1 gram), spread and covered with a suitable cover glass and the counts made in the entire amount placed on the slide, using the low power (80 diam.) of the compound microscope.

3. Substances in which the organisms are too numerous to permit ready counting by means of the 0.004 c.mm. areas of the Thoma-Zeiss hemacytometer. It now becomes necessary to use dilutions, which are made as follows.

Making the Dilutions.—The dilutions generally used are 1–10. Rarely will it be found necessary to use higher dilutions. Should this, however, become desirable, a dilution of 1–100 is to be made. The highest counts so far recorded were in the case of two tomato pastes which showed 2,400,000,000 and 4,000,000,000 bacilli per cc. In these instances dilutions of 1–10 were used and proved quite satisfactory, though it was evident that a greater number of

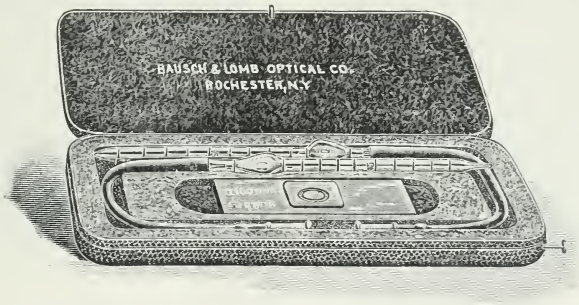

FIG. 6.—Thoma-Zeiss hemacytometer. Complete equipment for blood counting. This is very convenient for making bacterial counts in catsups, jams, jellies and other vegetable foods and also in animal food substances.

bacilli per cc. would have necessitated the use of a dilution of 1–100. However, a dilution of 1–10 is all that is required for practical purposes, as a bacterial count of 4,000,000,000 and more per cc. would indicate the decomposed condition of the food substance and its unfitness for human consumption.

In case of liquids and near liquids, 9 cc. of distilled water is added to 1 cc. of the substance, and in the case of pastes and similar products, 9 cc. of distilled water is added to 1 gram (or 1 cc. semiliquid) of the substance. The dilutions are made in 25 cc. graduated cylinders, which answer the purpose very well. Or 100 cc. graduates may be used for making the dilutions, adding

90 cc. of distilled water to 10 grams (or 10 cc.) of the substance. Place the thumb firmly over the opening of the graduate; the contents are thoroughly mixed by shaking vigorously for about 20 sec. By means of a slender glass rod slipped well into the mixture, take up a droplet of the mixed material and touch the end of the rod lightly and quickly upon the middle of the ruled area of the hemacytometer. All this must be done rapidly, before the organisms

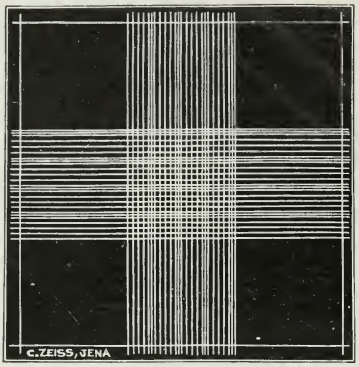

Fig. 7.

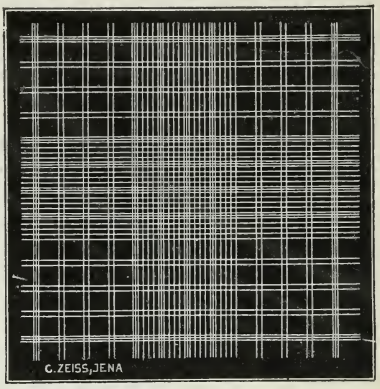

Fig. 8.

Fig. 7.—Zappert ruling of the Thoma-Zeiss hemacytometer. This form of ruling is especially convenient for making bacterial counts and counts of fat globules in milk.—(*Carl Zeiss.*)

Fig. 8.—Turck ruling of the Thoma-Zeiss hemacytometer. This is especially useful if it is desired to combine the bacterial count with the spore and yeast count. The smaller areas (1–400 sq. mm.) may be used for making the bacterial counts, while the larger areas (1–25 sq. mm.) may be used for making the spore and yeast counts.—(*Carl Zeiss.*)

have had time to settle to the bottom of the graduate, and before they have had time to accumulate at the end of the glass rod.

Making the Count.—After having cleaned the hemacytometer (do not use alcohol), it is sometimes desirable to rub a very soft, grit-free graphite pencil over the ruled area so as to render the lines more readily visible. Usually, however, this is not necessary. After placing the droplet of material as above described, cover with a No. 2 cover glass and orientate the ruled area by

means of the low power and make counts under the suitable high powers. From ten to twenty of the ruled areas should be counted and from these countings figure the average. It is desirable to make two or three mounts of each sample, thus giving the average of from twenty to thirty areas counted. The countings are to be made in areas free from pulp fragments and including all organisms lying within the ruled bounding lines and inclusive of half averages of those organisms which lie across the rulings. All countings which present characters of doubt are omitted from the final estimates.

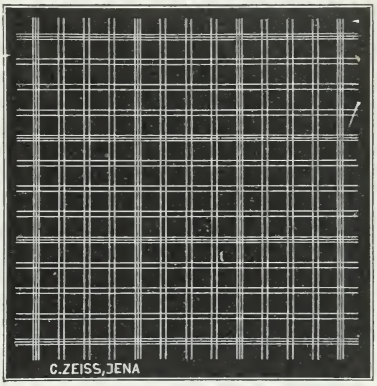

Fig. 9.—Bürker ruling, useful in making counts of milk fat globules, spores, and yeast cells. The average of many counts is taken.—(*Carl Zeiss.*)

Those organisms which occur within the cell-lumen of the vegetable tissues are not to be counted. To do so is practicably impossible and such countings, even if possible, would add nothing to the value of the findings. In case the cells contain numerous bacteria this should be noted in the report, as it certainly indicates decomposition of the material. The principal decomposition changes due to the invasion of bacteria and other organisms are, however, largely limited to the exterior of cells, especially by those organisms which develop during or after the factory processing. The numerical determinations are therefore limited to organisms which occur in the matrix and those which have been washed from the exterior of cells by the thorough mixing. The thorough mixing of the samples is a very important part of the procedure. In the case of liquids and semiliquids, mixing is done by thorough shaking, and in the case of pastes and similar materials, by means of a spatula or a small spoon.

In making counts of very small or comparatively short bacilli,

some difficulty is caused by those organisms which happen to be vertically suspended in the counting chamber, thus presenting an end view which gives the appearance of small granules or spherical particles which the comparatively inexperienced observer may not recognize, or which may be mistaken for inorganic particles or organic particles other than microbic. In case of doubt, allow the

FIG. 10.—Tomato pulp cells in normal catsup. The cells are large, thin-walled, containing granular particles. The coloring matter of the tomato frequently appears as deep scarlet-red crystalline particles usually arranged in groups within the cell.—(*Howard, Yearbook U. S. Dept. of Agriculture*, 1911.)

mount to remain at rest for 10 or 15 min., thus allowing the bacilli to settle to the bottom of the cell where they will assume the horizontal position, thus presenting the long axis to view and making counting easier.

In order that all of the cells (individuals) of the bacilli may be counted, it is necessary to use a high power (480 to 500 diam.). Lower powers are not satisfactory for counting bacteria. For

counting spores and yeast cells a magnification of 180 diam. would prove very satisfactory, especially with a well-corrected wide aperture objective. The counting of cocci is more confusing than the counting of bacilli, but fortunately the microbic contami-

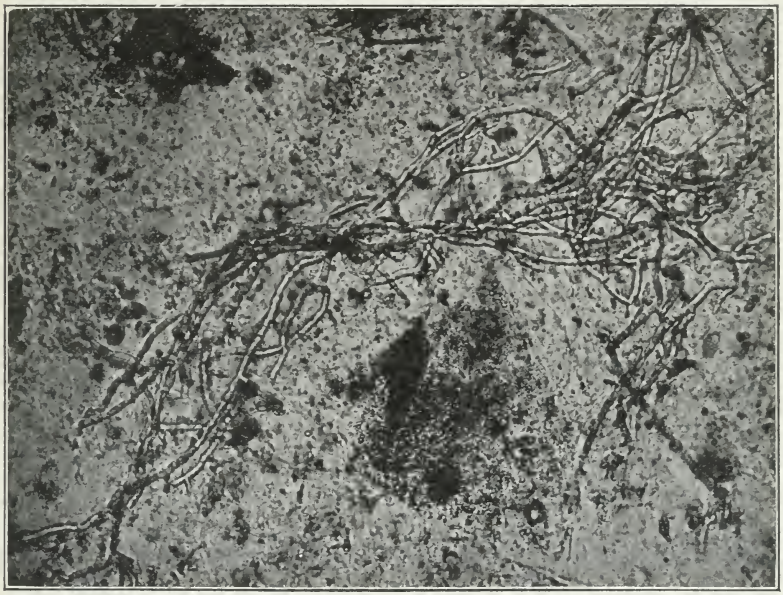

FIG. 11.—Cluster of mold hyphæ in granular (decomposed) tomato pulp. This type of mold is traceable to field-rotted tomatoes. The finding of hyphæ of this type in tomato catsup indicates the use of rotted tomatoes, therefore, indicates inadequate culling at the factory.—(*Howard, Yearbook U. S. Dept. of Agriculture*, 1911.)

nations of most vegetable substances are bacillar, though there are some notable exceptions.

Mold Counting.—Thus far no satisfactory method for making estimates of the amount of mold hyphæ present in fruit and in animal products has come into use. The method recommended by B. J. Howard, Chief of the Micro-chemical Laboratory of the U. S. Bureau of Chemistry, namely, determining the degree of

mold contamination from the number of microscopic fields of the compound microscope which show the presence of hyphal clusters, is far from satisfactory. It indicates the amount of contamination in a general way only. More reliable and more accurate estimates could be obtained through the use of a counting apparatus in which the number of hyphal clusters could be ascertained in definite quantities of the material under examination. The hemacytometer already mentioned does not serve the purpose because of the smallness of the counting areas. The special counter described in Fig. 5 would serve the purpose very well. It is furthermore necessary to reduce the material to a uniform and standard fineness by rubbing it through a sieve. A very small standard mesh sieve would answer the purpose. Take 1 gram of the thoroughly mixed material and by means of a small spatula rub all of it through the sieve and make the estimations from the pulp which has been passed through the meshes of the sieve.

Precautions.—The following are some of the factors which necessitate caution in making counts of microbes, yeast cells, spores and mold fragments.

a. Badly decomposed factory pulp which compels prolonged heating in order to render it suitable for canning, often presents such a granular appearance as to make accurate counting of the microbes rather difficult. In such materials many of the more or less disintegrated pulp cells are filled with bacteria and these cannot be included in the count. Commonly in such substances many of the mold fragments are also very much disintegrated through decomposition changes, probably initiated by enzymes formed by the bacteria and other organisms.

b. While it is quite easy to distinguish between living yeast cells, dead yeast cells and spores, it is not thought advisable to attempt such differentiation in routine laboratory practice, excepting in cases where identification is simple and where there is very little room for doubt. One of the first important problems for the food and drugs bacteriologist to solve is the identification

of those micro-organisms which commonly attack foods and drugs, more especially the molds and yeasts.

c. It is neither practicable nor necessary to differentiate between the different kinds of spores which may be present in a product, excepting as suggested under (*b*).

d. In many instances it would be desirable to resort to plating methods in order to determine the number of viable organisms present. This would be simple for bacteria and mold spores, but more difficult for yeasts.

Differentiating between Living and Dead Bacteria and other Low Forms of Organisms.—It would be most desirable to determine some practical working method for distinguishing between living and dead bacteria in foods and drugs. Often the question arises as to the time and place source of the contamination. Did the organisms present develop in the fruit, in the pulped material during the processing or in the containers after manufacture? Again, are the organisms estimated by the direct count dead or alive?

Several investigators have stated that dead and living bacteria react differently with certain stains. For example G. Broca, an Italian bacteriologist, declares that the use of the following mixed stain will serve this purpose. To 8 cc. of concentrated carbol-fuchsin add 100 cc. of Loeffler's methylene blue. Let the mixture stand for 24 hr. before using. Exposed to this stain, dead bacteria (killed by heat or by disinfectants) are colored red while living bacteria are colored blue. It is declared that other stains, as Giemsa's, will react in a similar manner.

More recent experiments would indicate that selenium and tellurium compounds will serve to differentiate living bacterial contaminations. It would appear that these substances are decomposed into metallic tellurium and selenium when brought in contact with living organisms. Much experimental work along this line has been done by Hansen, Gmelin, Gosio and others, and still more recently (1913) by King and Davis of the

Research Laboratory of Parke, Davis and Company. Potassium tellurite is said to be the most satisfactory reagent. In dilutions of 1 : 50,000 this substance forms characteristic black compounds with all of the more common micro-organisms when in the living state. The reaction does not take place in the presence of dead micro-organisms and the different organisms do not all react in the same degree or manner. Some are much more susceptible than others. The *Bacillus coli* appears to be the most sensitive to the reagent. With most species of bacteria the time required to produce the characteristic color and precipitation reaction ranges from 12 to 96 hr. at a temperature of 37° C., but with the colon bacillus a distinct coloration or color ring becomes visible several minutes after the reagent is added. King and Davis summarize the experimental results as follows:

1. Nearly all of the more common micro-organisms (bacteria and yeasts) react with potassium tellurite, forming characteristic, black compounds.

2. This capacity depends on an active stage of metabolism of the reacting organism, and the action is, in all probability, a reduction of the tellurite.

3. The "tellurite reaction" can be used as an indicator of microbial life, and is especially suitable for revealing microbic contamination.

4. A dilution of 1 : 50,000 of the salt seems to be most suitable for its action as a general microbic indicator. In this concentration, it produces no irritative action when introduced into test animals.

5. The bacteria of the "colon-typhoid group" show differences in resistance to the antiseptic action of potassium tellurite and in the appearance of their reaction with this salt. These variations are sufficient to suggest the experimental use of potassium tellurite for differential diagnosis in the group.

6. The intensity of bacterial action on potassium tellurite depends upon the individual resistance of the bacterium and the concentration of the salt present. The velocity of reduction of the tellurite is apparently a specific function of an organism, apart from its resistance to antiseptic action. With the colon bacillus, the "tellurite reaction" is almost instantaneous.

7. Treatment with potassium tellurite has practically no influence on the biological characteristics of an organism.

3. Numerical Limits of Micro-organisms in Foods and Drugs

What should be the maximum limit of the number of bacteria and other micro-organisms in food and drugs within the intent

of the U. S. Pure Food and Drugs Act? This is as yet an unsettled question and one that requires further careful consideration, even calling for some extensive investigation in order that certain disputed points may be finally settled. There are, however, certain results based upon extensive observation which

FIG. 12.—Type of mold development in the tomato pulp during and after the processing. According to tests made by B. J. Howard of the Bureau of Chemistry, mold will develop in tomato catsup containing 0.1 per cent. sodium benzoate. Compare the hyphæ with those shown in Fig. 11. They are much larger in transverse diameter and the walls of the cells are much thinner.—(*Bitting, Bull.* 119, *Bureau of Chemistry, U. S. Dept. of Agriculture.*)

may be set down as conclusive. The organisms of all kinds which may occur in and upon clean and uncontaminated ripe fruit, for example, are negligible quantitatively as well as qualitatively. Such organisms as do occur are limited to the exterior. Only under abnormal conditions do micro-organisms find their way

into the tissues beneath the epidermis and into the parenchymatous cells of whole fruits. It would be interesting to determine the average number of bacteria on the exterior of such fruits as the apple, the peach, the pear, the apricot, the tomato, the cucumber, etc., and from these figures to estimate the number of organisms per cc. of the fruit substance. The practical value of such information would, however, not be great, as may be understood from the statements already made. It must be admitted without question or doubt that fruit products of any kind, which contain only such organisms as normally occur on clean uncontaminated ripe fruit, will never come under the ban of the pure food and drugs act. This also applies to foods and drugs in general. The organisms which concern the analyst are those which occur in and upon contaminated and diseased fruits and those which are introduced or added or allowed to develop and multiply during the processing, and afterward. We may therefore make the following postulate: All fruit products from clean uncontaminated fruit (ripe or green), prepared under modern sanitary conditions, contain micro-organisms in negligible quantities only. It is true that the ideal conditions implied in this postulate may not always be attained in practice, yet we are warranted in making a second postulate, namely: that the number of organisms present in fruit products, over and above the negligible quantities mentioned, are in direct proportion to the carelessness in the various steps of the processing. Stating it conversely, as the manufacturers of food products attain the practically ideal conditions, the number of organisms in their products will become gradually negligible. That such conditions are attainable is clearly shown by the canned products of the careful housewife and of the careful manufacturer. What may be done by the careful housewife may be done even better by the careful manufacturer, because the latter can employ the most approved modern methods, aided by special machinery, which are not at the disposal of the housewife or even of the small manufacturer.

In a general way, the number of micro-organisms in food products and in liquids intended for internal use, not including the fermented products, is negligible when they do not exceed 250,000 per cc. (ranging from 5000 per cc. to the maximum). In fer-

FIG. 13.—Various stages in the germination of spores in catsups. Note transverse septation and branching of the hyphæ. Germinating spores may be traceable to the tomato from the field or they may be from spoiling factory pulp.—(*Bitting, Bull.* 119, *Bureau of Chemistry, U. S. Dept. oi Agriculture.*)

mented products, as cider, vinegar, wines, beer, etc., the number of organisms present may be much greater, but even here the quantitative estimates generally become negligible if the modern methods of purifying or clarifying (through sedimentation, the use of albumen, gelatin, casein, etc.), filtration, centrifugalization, and sterilization are carried out. Of course, in such products as

sour milk, ripened cream, ripened cheese, sauerkraut, pickles, etc., the processes of clarification are not applicable, and hence we always find a large number of certain predominating types or species of organisms present.

The microscopical examination of products which have undergone normal fermentation shows that the number of organisms present is quite variable, depending upon a variety of causes and conditions. This can readily be ascertained from the examination

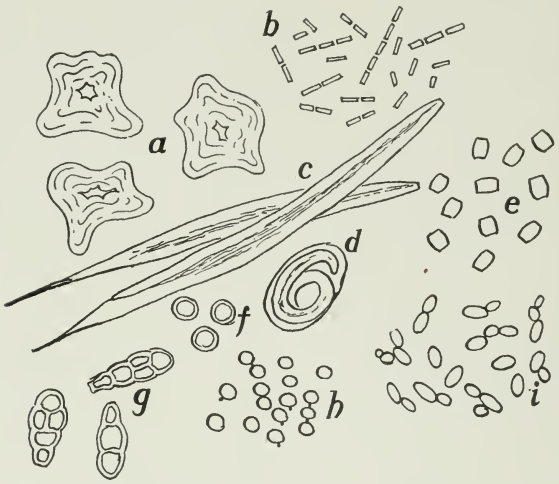

FIG. 14.—Substances frequently found in tomato catsup. *a*, Heat dextrinized corn starch. Starch is frequently used as a filler or stiffening agent. *b*, bacteria which frequently appear in great numbers. *c*, Vinegar eels derived from cider or wine vinegar. Soil nematodes may also be found, indicating gross soil contamination and inadequate washing at the cannery. *d*, Nematode larvæ derived from the soil. *e*, *f*, *g*, *h*, Spore types frequently met with in catsups. *i*, Yeast cells.

of such common household products as vinegar, sour cream, cider, apple butter, sour milk, etc. It would be most desirable to determine the exact identity of the organisms which produce the most favorable fermentation changes in fermented food products. This has been done in some cases and pure cultures of the specific organisms are used for manufacturing purposes, resulting in the production of superior food articles. When the fermentation

processes are left to nature the result is not by any means uniform and we have products which are often so vitiated by the development of undesirable associated organisms as to make the food unfit for use. There is a definite biological relationship between those organisms which initiate desirable fermentations and those which are objectionable; both kinds are generally present, but fortunately

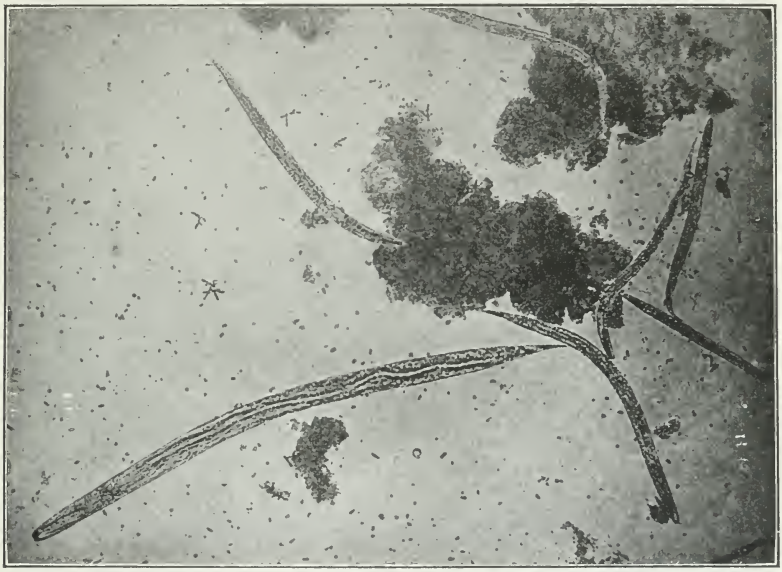

FIG. 15.—Vinegar eels from decomposed blackberry pulp. The small particles scattered through the field are yeast cells. Bacteria were also present but they do not show in the illustration.—(*Howard, Yearbook U. S. Dept. of Agriculture,* 1911.)

the desirable or beneficent forms overgrow the objectionable forms very rapidly, but not always. It should be one of the principal efforts of the food and drugs bacteriologist to isolate and identify the organisms which are desirable in the production of fermented food products and those which are unquestionably undesirable and objectionable, for in these products it is not a question so much of *quantity* as of *quality* of the organisms present.

This is by no means a simple problem. Much of this field of work is as yet untouched, and it is not likely that definite conclusions will be reached in the very near future. It means an investigation of those conditions which are recognized as diseases in industrial or manufactured products, characterized by unaccountable de-

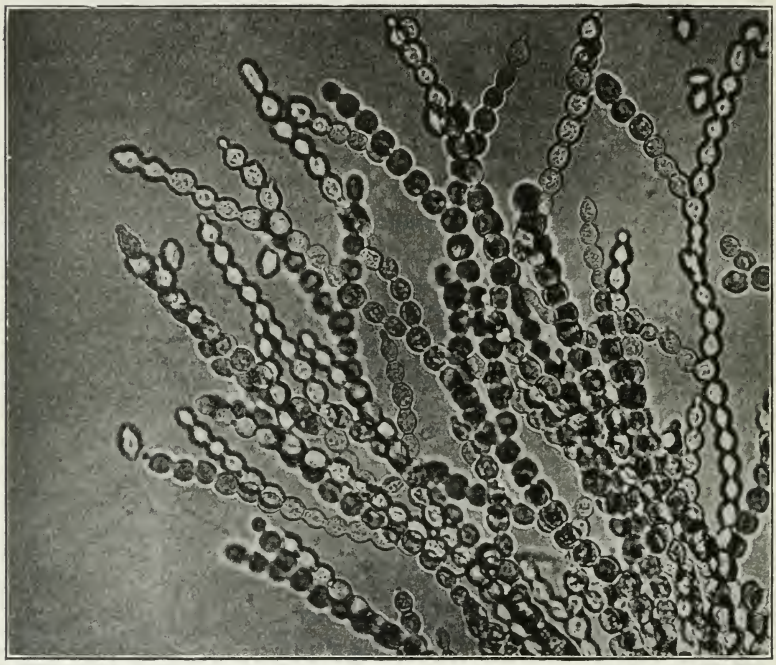

FIG. 16.—Mold from decomposing plum.—(*Howard, Yearbook U. S. Dept. of Agriculture*, 1911.)

teriorations in flavor, in taste, in color, in nutritive value, etc. It means a very careful study of organisms which are similar in morphology and yet quite different in specific functional activities, giving rise to objectionable fermentation products.

The following tables will give some idea of the number of organisms which occur in certain canned food products. Animal food

products are not included in Tables II and III because there are not sufficient data available on which to base suggestions. There appears to be no plausible reason why canned animal products should not be subjected to the same method of examination as vegetable substances, particularly sausage meats, canned meats, canned oysters and shellfish generally, canned eggs and canned soup stocks. Pickled herring which shows 8,000,000,000 bacteria per cc. in the liquor is certainly a questionable food article. In

FIG. 17.—Spores and hyphal fragments from decaying sweet pepper. "Dry rot" fungus.—(*Howard, Yearbook U. S. Dept. of Agriculture*, 1911.)

this particular instance there was no objectionable odor noticeable, but the meat of the herring was somewhat soft. Smoked meats and fish should be examined for mold in addition to bacteria. This subject should receive immediate careful consideration on the part of food bacteriologists.

Table I shows the number of organisms which may occur in some of the more common household food substances, fermented and unfermented. The figures are based upon direct counts. Table II is based upon the examination of factory products obtained in the open market. The numerical extremes in the micro-organisms given in Table II, are in direct ratio to the relative

TABLE I[1].

Name of Substance	Number of Organisms per Cc.			Hyphæ
	Bacteria	Yeasts	Spores	
Blackberry jam	500,000	.....	.....	Few.
Blackberry jelly	Few	.....	.....	Few.
Cheese, California	80,000,000	Few	.....	Entirely permeated.
Cider	50,000 to 500,000	50,000 to 30,000,000	.....	.....
Cider vinegar	1,000,000	Few	.....	.....
Currant jelly	Few	.....	.....	.....
Fruits, canned	Few	Few	.....	.....
Herring, pickled	8,000,000,000	.....	.....	.....
Jams	Few	Few	Few	Few.
Jellies	Few	Few	.....	.....
Meat, sausage	1,000,000 to 150,000,000	.....	.....	.....
Milk, ordinary	25,000 to 2,000,000	.....	.....	.....
Milk, certified	1,000 to 15,000	.....	.....	.....
Milk, sour	2,000,000,000 to 7,000,000,000	.....	.....	.....
Plum preserve	Few	Few	Few	Few.
Plum relish	100,000,000	Few	2,000,000	Some.
Water, drinking (San F.)	800 to 32,000,000	.....	.....	.....

unsanitary conditions in the factories. It is quite evident that the products of the manufacturers who employ modern methods are fully up to the quality of those prepared by the careful housewife.

[1] The counts recorded in Tables I, II, and III were made by the direct method using the hemacytometer. In the case of the sausage meat some of the counts were checked by the plating method and it was found that the count by the plating method was invariably higher than by the direct method. Other investigators have noted similar discrepancies. The direct examination of meats for bacteria is occasionally unsatisfactory because of the confusion due to granular fragments traceable to broken up blood corpuscles, fragments of coagulated albumen, etc.

TABLE II

Name of Substance	Number of Organisms per Cc.			Hyphæ
	Bacteria	Yeasts	Spores	
Apple jam	.....	3,750,000	Few	Some.
Apple jam	500,000	494,000	.....	.....
Apple jam	400,000	9,250,000	Some	Some.
Apricot jam	25,000	.....	None	None.
Blackberry with apple.	40,000	1,728,000	Numerous	Very abundant.
Catsup	5,000,000	.....	.....	Abundant.
Catsup	440,000,000	.....	5,000,000	Very abundant.
Catsup	560,000,000	.....	27,500,000	Very abundant.
Catsup	800,000,000	.....	20,000,000	Entirely permeated.
Catsup	200,000,000	12,000,000	1,500,000	Very abundant.
Catsup	5,000,000	.....	Few	Trace.
Catsup	80,000,000	.....	5,000,000	Very abundant.
Catsup	400,000,000	.....	7,500,000	Very abundant.
Cherry jam with apple.	5,000,000	1,000,000	200,000	Very abundant.
Currant jam	.....	40,000,000	.....	.....
Currant jam	.....	45,000	.....	.....
Fig jam	1,000,000	1,250,000	.....	.....
Loganberry jam	Few	6,250,000	.....	Some.
Loganberry jam	2,500,000	8,750,000	.....	Quite abundant.
Orange marmalade	.....	.....	.....	.....
Plum jam	500,000	500,000	10,000	Some.
Peach jam	1,250,000	750,000	.....	.....
Strawberry jam	.....	4,500,000	Few	Some.
Strawberry jam	.....	500,000	750,000	Abundant.
Tomatoes	12,000,000	.....	1,400,000	Very abundant.
Tomatoes	2,000,000,000	.....	4,000,000	Very abundant.
Tomato paste	2,000,000,000	.....	1,000,000	Very abundant.
Tomato paste	2,000,000,000	.....	1,200,000	Very abundant.
Tomato paste	1,400,000,000	.....	5,000,000	Very abundant.
Tomato paste	4,000,000,000	.....	6,000,000	Very abundant.
Tomato paste	1,000,000,000	.....	1,000,000	Quite abundant.
Tomato paste	2,000,000,000	80,000,000	100,000,000	Entirely permeated.

TABLE II.—(*Continued*)

Name of Substance	Number of Organisms per Cc.			Hyphæ
	Bacteria	Yeasts	Spores	
Tomato pulp[1]....	Less than 5,000,000	.....	Less than 500,000	Practically none (1–3 per cent. of fields).
Tomato pulp[1]....	1,900,000,000	.....	37,000,000	Entirely permeated (100 per cent.).
Imitation jam....	Few.....	30,000,000	Few.....	Few.

TABLE III

Name of Substance	Maximum No. of Organisms per Cc.			Hyphæ[2]
	Bacteria	Yeasts	Spores	
Apple butter....	5,000 to 1,000,000	1,000,000 to 10,000,000	.....	.....
Berries.....	Few.....	500,000	500,000	15 per cent.
Catsup.....	10,000,000 to 50,000,000	Few.....	500,000	18 per cent.
Cider.....	500,000 to 2,000,000	500,000 to 5,000,000	.....	.....
Fruits.....	Few.....	50,000 to 500,000	500,000 to 1,000,000	10 to 12 per cent.
Jams.....	1,000,000	1,000,000 to 10,000,000	500,000	10 per cent.
Jellies.....	Few.....	1,000,000	Few.....	1 to 5 per cent.
Marmalade[3].....	.....	.....	.....	.....
Tomato pastes...	500,000,000	Few.....	2,000,000	20 to 25 per cent.
Vinegars (fruit)..	5,000,000	Few.....	.....	.....

[1] Both samples were from large factories and represent the extremes in the factory conditions. The first sample is from a factory where the conditions are what they should be, the second from a factory where the conditions are just the reverse.

[2] Percentages given this column refer to the number of the 1/125 c.mm. areas of the mold counter described in Fig. 5 which contain hyphal clusters. As a rule abundant spores indicate the presence of abundant hyphal tissue, and *vice versa*.

[3] The organism in orange marmalade, under ordinary conditions of manufacture, are negligible in amount.

Other manufacturers, either through greed, ignorance or carelessness, or through all three causes combined, refuse to employ modern methods and as a result their products are very often in an undescribably filthy condition, wholly unfit for consumption. In addition to the bacteria, yeast cells, mold, sand and dirt particles present in the inferior grades of catsup, jams, jellies, etc., there are found insect remnants (flies, aphides, beetles), vinegar eels, larvæ of various nematodes (from soil), etc. The presence of numerous fly remnants is certainly an indication of highly unsanitary factory conditions. The presence of vinegar eels indicates the use of bad vinegar and the presence of soil nematodes and of sand and dirt particles indicates insufficient or no washing. Laboratory experience has demonstrated that there is a definite relationship between the number of bacteria and other organisms and the amount of dirt and other impurities present in factory products. Unsanitary factory conditions encourage a certain recklessness in such factories, inducing the laborers about the place to even go out of the way to add more filth. Thus shovelfuls of refuse are taken up from the filth-coated floors and thrown into the mixing vats, the idea evidently being that it will add to the bulk and that no one will know the difference. Vats are often not cleaned until the conditions are almost undescribable. Refuse is added, often of such a character as to be unfit as food even for animals. This criminal negligence, carelessness and indifference is too frequently engendered by ignorance which, gives heed to nothing else than a strict enforcement of the law.

The filthy condition of some of these products is very generally not apparent to the layman because of certain methods employed primarily intended to hide or mask such defects. The odors of decomposition are quite effectually dissipated by the steaming and cooking process. The vitiated taste is quite effectually masked by the heavy spicing. Any appreciable change in color is

restored by means of added coloring substances. Any change in consistency is corrected by adding fillers, such as starch, gelatin and agar. The unscrupulous manufacturer will work up a supply of spoilt canning tomatoes, including rejected "swells" and "leaks," making them into catsup or paste. Overripe and partially decomposed fruits (culls and rejects) are worked up into jams preserves and into combinations in which the objectionable character and appearance are hidden or lost sight of.

We are justified in the conclusion that the number of microorganisms in food products is a reliable guide to the wholesomeness and sanitary quality of such products and the very natural question arises, what are the maximum numbers of bacteria, yeast cells and mold spores (including mold hyphæ) permissible under reasonable and practicable sanitary conditions. While ideal factory conditions may not always be practicably attainable, yet it is wholly reasonable to expect the operation or methods which will bring the maximum quantitative counts per cc. within the numerical limits given in Table III. These proposed maximum numerical limits are tentative only. As the sanitary conditions in the canneries are improved, as they undoubtedly will be, the limits can be correspondingly decreased, finally reaching the negligible quantities as already explained. Where numbers are omitted in the tables it indicates that the quantity of organisms is negligible. "Few," indicates that the number of organisms is somewhat more than in negligible amounts, yet not sufficient to make counting necessary or to question the suitableness of the article for food purposes.

It is quite evident that different numerical limits must be adopted for different classes or kinds of food products. This can be seen from a study of the tables. Some fruits and fruit products are more susceptible to the attacks by bacteria, yeasts and molds, than others. Acid fruits, as the cherry, the plum, tomatoes, loganberries, blackberries, etc., are much more likely to be attacked

by molds than are apples, peaches, pears and apricots. Yeasts very rarely appear in the whole fruit, but they develop very rapidly in fruit pulps which contain sugar (natural or added). Yeasts require in addition to sugar, a high percentage of moisture for their active growth, including an ample supply of oxygen (air). The presence in canned fruit products of numerous yeast cells indicates fermentation during the processing. The presence of numerous bacteria in fruit products indicates the use of rotted (bacterially) fruit or bacterial contamination and development during the processing, or both.

It would appear that most of the bacteria which develop in fruit pulps, especially those from fruits which are quite acid, as for example tomato pulps, belong to the lactic acid group. Numerous tests in the laboratories of the Bureau of Chemistry show a parallelism between the number of bacteria and the amount or percentage of lactic acid present in tomato catsups. The usual rotting bacteria require more air (oxygen) then is present in the pulp mass and as a result these are soon overgrown by the lactic acid bacilli, if the pulp is allowed to stand for a time without sterilization. It is, however, very evident that the contamination of such products as catsups, tomato pastes and tomato purees is never wholly limited to lactic acid bacilli. The inclusion of field rotted tomatoes and the rotted pulp material from filthy mixing vats and other parts of the machinery of the unsanitary factories, adds a sufficient number of rotting bacteria to render the article dangerous to health, if consumed. Ravenel and other investigators have shown that when certain food products, as cream and milk, are kept in cold storage, particularly after pasteurization or incomplete sterilization, the development of lactic acid bacilli is checked and the growth of toxin forming bacteria is encouraged, resulting in occasional poisoning to the consumer. It is very likely that similar conditions may exist in some of the incompletely sterilized canned food products (vegetable as well as animal) which have been stored for some time at a comparatively low temperature.

The question is frequently asked, what percentage of rotten or moldy fruit must be present to render the product unfit for human consumption? This question cannot be answered definitely. In a general way, it may be stated that where there is not over 5 per cent. of rotted or moldy fruit used, the number of organisms in the finished products will not reach the maximum limits given in Table

FIG. 18.—A type of mold, Spicaria sp., very frequently found on decaying tomatoes. Some of the filaments and numbers of spores are shown.—(*Howard, Yearbook U. S. Dept. of Agriculture*, 1911.)

III, in fact the counts will in all probability be considerably less. A careful culling of spoilt fruit in the field and at the factory, coupled with reasonably sanitary factory methods and modern methods of sterilization, will furnish products which will meet all of the requirements of any pure food law.

The statement is frequently made by manufacturers that even

though bacteria, yeasts and mold are present in considerable numbers, they are harmless and do not produce toxic effects when introduced into the digestive tract. This statement is wholly without foundation in fact. On the contrary it is known that certain bacteria, yeasts and molds do cause disease and more or less severe intoxications and intestinal disturbances. The objectionable character of mold is universally recognized

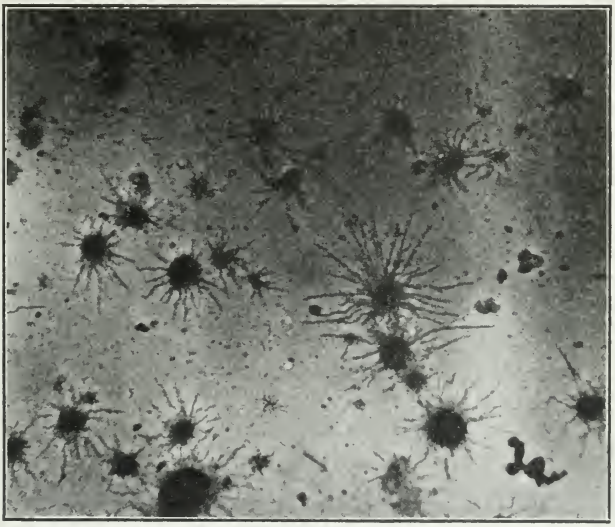

FIG. 19.—Mold colonies in gelatin seen under the low power of the microscope (× 80). This mold developed in the gelatin after it was spread on the screen to dry. This gelatin also contained numerous bacteria. Gelatin thus infected is not suitable for bacteriological purposes neither is it suitable for use as food.

and nearly all animals refuse to eat moldy and mold contaminated food materials. Various ulcerative diseases of the skin and of the digestive tract are caused by mold organisms. While many of the yeasts are entirely harmless and cause very important fermentative changes, some of them are pathogenic to man while others initiate objectionable fermentation changes in the food substances.

As already indicated the number of organisms in food substances is in direct ratio to the following conditions:

1. Insufficient culling of partially and wholly decomposed fruits.
2. Unsanitary factory conditions and unsuitable methods.

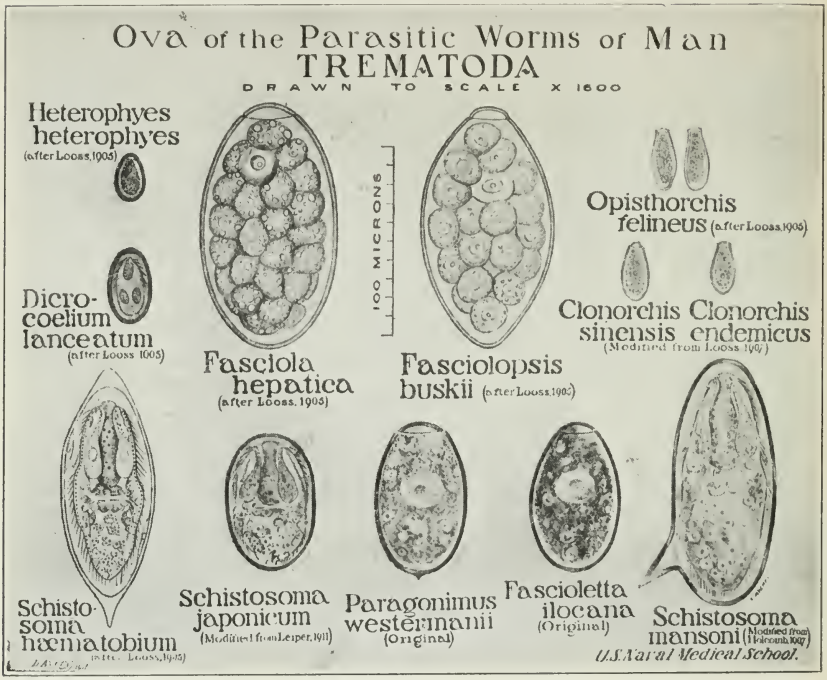

FIG. 20.—Intestinal ova. Trematodes. Ova of intestinal parasites may possibly occur in foods of vegetable origin contaminated by soil, sewage and fecal matter. Note comparative size and the actual measurements according to the scale. It may be mentioned that the extremely small seeds of *Vanilla planifolia* have been mistaken for ova of intestinal parasites.—(*Stitt.*)

We are warranted in establishing a maximum limit as to the number of organisms permissible in food substances. The method of estimating the quality of foods based upon the number of micro-organisms present has been tested out in different countries and has proven very reliable and satisfactory; and those who

are entrusted with the enforcement of the laws governing the physical well-being of the people are most emphatically in the right when they insist that the sanitation in and about our factories should be of a high order.

In addition to the purely quantitative estimates of micro-organisms based upon direct examination, the analyst is enabled

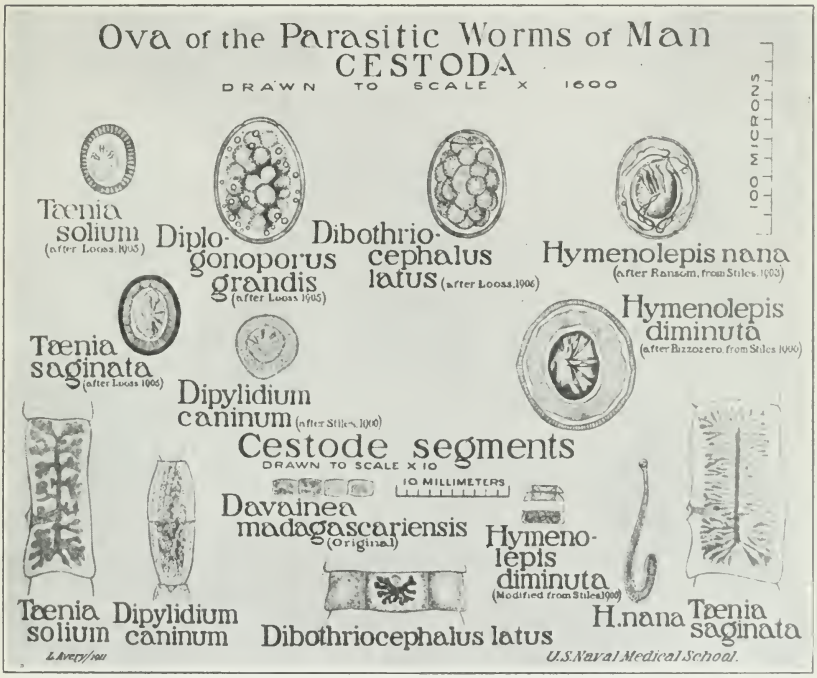

FIG. 21.—Intestinal ova. Cestodes.—(*Stilt.*)

to form certain opinions and conclusions regarding the source of the contamination. For example, the hyphal development in mold infested fruit is in marked contrast to the hyphal development in the fruit pulp, due to unsanitary factory conditions. This difference in hyphal structure is due to a difference in the amount of oxygen (air) supply, of moisture, of light and the

added ingredients (spices, sugar, vinegar, etc.), of the canning product. The analyst can thus determine approximately how much of the hyphal tissue present is derived from the use of moldy fruit and how much is traceable to unsanitary factory conditions. Again, the presence of one or more ova or larvæ of intestinal parasites, as the tape worm, would indicate sewage contamination or contamination with fecal matter. Sand and dirt particles indicate insufficient washing, etc. It is self evident that the value of the report by the analyst depends upon his knowledge of the subject and the range of his experience. Until the work is well under way and the methods are perfected, there is no place for inexperienced analysts in our food and drugs laboratories.

4. Quantitative Estimations by the Cultural Methods

Estimating the number of bacteria per cc. in foods and drugs, etc., by planting or plating definite amounts of the substances into plate (Petri dish) culture media, is a well-known and standard procedure. The general and special technique of the plating method is described in the various text-books and manuals on bacteriology. Some of the details of the method are standard, in so far as they are generally adopted by investigators, such as the preparation of certain culture media, making the dilutions, counting, etc.; in other regards there is anything but uniformity. It is generally admitted that the results of different investigators differ widely but there appears little unanimity of opinion as to the factors which are responsible for these variations in quantitative results.

Micro-organisms are sensitive to a degree and they respond readily to the slightest variations in moisture, temperature and food supply. A failure to recognize this fully in laboratory practice leads to confusion and erroneous results. The following are some of the more important factors which are responsible for errors and variations in results.

1. Culture Media.—Differences in the quality of the meat used in making the meat infusions has given some marked variations in the quantitative results. Meat extracts from younger animals give higher counts than do extracts from the meat of older animals. Again, the prepared extracts of the different packing houses give different results and the results obtained

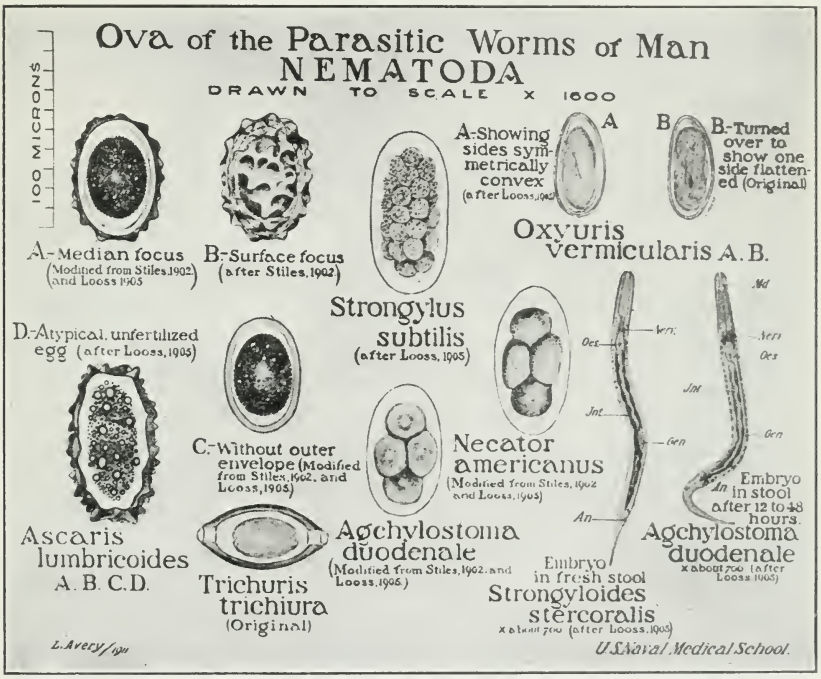

FIG. 22.—Intestinal ova. Nematodes.—(*Stitt.*)

from the use of media made with manufactured meat extracts differ from those obtained from the use of the laboratory made meat infusions. In fact most workers are opposed to the use of the manufactured meat extracts because of the fact that they are mixed products and also because of the uncertainty of the amount and number of the added ingredients any or all of which may

interfere with the growth and development of certain bacteria. Investigators have also noted great variations in results with different brands or makes of peptones used, the quantitative differences amounting to 50 per cent. in some cases. Equally remarkable are the differences due to the kinds of water used in the preparation of the culture media. For instance it is known that agar made up with sewage encourages the development of sewage organisms while the same medium made up with tap water encourages the growth of bacteria predominating in such tap water.

The gelatine used is yet another important factor in cultural results, depending upon the age of the gelatine, its purity, the degree of heating to which it has been exposed, its origin, possible contamination with arsenic, with bacteria and mold. Other ingredients used in the preparations of culture media cause more or less marked variation in comparable results. The above statements make it evident that it is absolutely necessary to adopt and to adhere to uniform methods in order that the comparable results may be approximately uniform.

2. Glassware.—Different investigators have found that the number of bacteria in and upon culture media varied with the composition of the glass containers used. The comparatively soluble glass, for example, yielded enough free alkali to inhibit the development of the more sensitive bacteria. The size of the containers and the thickness of the glass yielded differences in the results. It is therefore very desirable to adopt Petri dishes and test-tubes of standard form and thickness of standard cubic contents.

3. Other Factors.—The form and size of the incubating chamber, the degree of ventilation, degree of darkness, amount of oxygen present, etc., cause variations in the results.

Of even greater importance than any of the factors so far mentioned, is the personal equation in the laboratory technique. No two workers follow out the same details in the different steps

of the laboratory procedure and very frequently proper judgment is lacking in the application of certain details of the methods. For example, there is lack of uniformity in the degree of heat to be used in melting gelatin media preparatory to planting, in the amount of material to be planted in each Petri dish, manner of planting, time of incubating, etc.

We hereby submit the following technique in the preparation of culture media and in the methods of making cultures in plates as well as in test-tubes, following very generally the suggestions as given in the report of the Committee of the American Health Association.

5. Preparation of Standard Cultural Media, General Suggestions

1. Ingredients.—Distilled water is to be used in the preparation of all of the standard media. The distilled water must be comparatively free from bacteria and must be kept in clean sterilized containers and as free as possible from mineral and organic impurities. If other than distilled water is used, this is to be stated and the special reasons for using it indicated.

For making meat infusions, fresh lean meat is to be used, from comparatively young animals, free from disease. Meat extracts may be used in place of the meat infusion.

Unless otherwise specified, the peptone used should be made from fresh beef by pancreatic digestion. It should be dry and recently made. Workers should be sure to specify the kind of peptone desired. Egg albumen or fibrin peptone is not to be used in any of the standard media. The article should be secured from some reliable house.

The gelatin to be used in the preparation of the standard media should be of the best obtainable, the so-called French brand being, as a rule preferred. A 10 per cent. solution should not soften when kept at a temperature of 25° C. It should be entirely free from arsenic and as free as possible from acids, microorganisms, molds, and other impurities. A good grade of gelatin

should respond to the following test: Place 0.30 gram of the gelatin in a medium sized test-tube and add 15 cc. of distilled water, let stand for half an hour, warm gently until all of the gelatin has dissolved, then place the tube in water at a temperature of 15.5° C. and leave undisturbed for half an hour. The solution should remain in place when the tube is inverted.

The commercial gelatin is a variable product, being made from varying proportions of animal tissues as hides, ligaments, bone and bone cartilage. The purest and best gelatin is made from ligaments and this kind would no doubt give the most uniform results in bacteriological work, but it is apparently not possible to obtain such gelatin in the market. The next best grade (practically obtainable) would be that made from hides of comparatively young domestic cows free from all foreign additions as salt, arsenic and other hide preservatives.

Each lot of gelatin should be examined microscopically before making it into culture media. Old yellowed and brittle material should not be used. Examine from five to six sheets from each pound package, using the low power of the compound microscope. The examinations are made directly without mounting. If numerous mold colonies are found as shown in Fig. 19, or numerous mold filaments more or less scattered through the mass, it is unfit for use as a culture medium. Numerous formed mold colonies in the matrix indicate growth during the drying process after the gelatin was spread on the drying screens. More or less torn and disintegrated hyphal fragments unequally distributed through the mass indicate infection and growth before the gelatin was spread for drying. To examine for bacteria, mount small bits of the sheet on a slide in water covered with cover glass. If bacteria are numerous, approximating 10,000,000 per cc. and more, it should not be used. In order to make more accurate counts, take 1 gram of the gelatin and rub up in 9 cc. of boiled distilled water and make the counts of the thoroughly mixed sample by means of the hemacytometer. As a rule it is

not necessary to make plate or tube cultures to determine the fitness of the gelatin for bacteriological work. Incidentally it may be remarked that a gelatin which is unsuitable for bacteriological work is also unfit for use as human food.

The agar should be the highest grade obtainable, and if the shredded form is used it should always be washed in sterilized distilled water before making into culture media.

With regard to the other ingredients required in making culture media, such as dextrose, lactose, maltose, saccharose, glycerin, salt litmus, etc., etc., special efforts should be made to get these as pure as possible. The degree of purity should be determined by actual tests.

2. Sterilization.—Thorough sterilization of all culture media is absolutely necessary. It is, however, known that heating produces some marked changes in the molecular composition of the media, even inducing actual chemical decomposition. It is therefore desirable to make the time of heat exposure as brief as possible. Ordinarily it is therefore preferable to use the autoclave, bringing the temperature up to 120° C. (15 lb. pressure) for a period of 15 min. This temperature will sterilize all media. A shorter period does not insure complete sterilization and a longer exposure is apt to produce inversion of the sugars used and also permanently lower the melting point of the gelatin. Solid media as gelatin and agar should be liquefied before placing in the autoclave.

The following rules should be strictly observed in using the autoclave:

a. The sterilizer should be hot when the media are introduced. About 100° C. Let all air escape from chamber.

b. At the end of the period of sterilization (15 min.), remove the media and cool them as rapidly as possible.

Compliance with these rules will reduce to a minimum the tendency toward liquefaction of the gelatin and a tendency to decompose the various chemicals used, due to prolonged heating.

If streaming or live steam is to be used in place of the steam under pressure in the autoclave, intermittent sterilization is to be practised. Place the media in the steam sterilizer for 30 min. on each of 3 successive days. Wait until the temperature in the sterilizer has risen to approximately 100° C. before placing the media therein. Agar media should first be liquefied. At the end of each period, remove the media and cool as rapidly as possible for reasons already given.

When media are prepared under the proper laboratory conditions and sterilized as above suggested, they are as a rule free from all living germs. However, if practicable, the media should be watched for a period of 2 days, stored in a room at ordinary temperature, in order to note possible bacterial developments.

3. Adjustment of Reaction of the Media.—As a rule bacteria develop most actively in media which are slightly alkaline to litmus and since certain media are quite acid in reaction (gelatin in particular) it becomes necessary to reduce them to a standard reaction. The standard indicator to be used is phenolphthalein. When phenolphthalein is not obtainable, litmus paper (or a 1 per cent. aqueous solution of Kahlbaum's azolitmin) may be used. The reaction adjustments are to be made as follows:

Place 5 cc. of the medium to be tested in 45 cc. of distilled water (making a dilution of 1–10). Boil briskly for 1 min., with stirring or rotary shaking. Add 1 cc. of the phenolphthalein solution (made by dissolving 5 grams of the salt in 1 liter of 50 per cent. alcohol). Titrate while hot with N/20 caustic soda solution (in distilled water). A distinct pink coloration marks the proper reaction. To be more precise, the pink should correspond to a mixture or combination of 25 per cent. red and 75 per cent. white of the color top recommended by the Committee on Standard Methods of the American Health Association. The reactions of the media are stated in terms of the percentages of normal acid or alkaline solutions required to neutralize them. Alkalinity is indicated by the minus (—)

sign and acidity by the plus (+) sign. Thus, if the reaction of a medium is given as + 1.00 it indicates that it would be necessary to add 1 per cent. of normal sodic hydrate solution to the medium in order to bring it to the neutral point (to phenolphthalein). It will be observed that while the titrating is done with the N/20 caustic soda solution, the normal solution is added to bring the medium to the desired reaction, the stronger solution being preferred because it reduces the amount of liquid introduced. The Committee on Standard Methods specifies that the reaction of all standard culture media shall be + 1.0 per cent. and if it differs in reaction by more than 0.20 per cent. the medium shall be readjusted and when a reaction other than the standard is used it shall be indicated and the reasons for using a different reaction shall be fully stated.

Media are preferably made in large quantities as this will reduce to a minimum the discrepancies due to variation in the composition of the ingredients used. As soon as made and titrated, the media should be put into tubes and in other culture containers, after which media containers and all are to be sterilized according to the methods already described. To guard against the evaporation of moisture from the media, the tubes, flasks, etc., should be sealed by dipping the plugged ends into melted paraffin, or they may be capped with rubber coverings especially made for that purpose. In case media are to be used within a few days, sealing is not necessary but they should be kept in a moist place, preferably in the ice-box.

6. Preparation of Required Standard Culture Media

Culture media used in bacteriological work may be divided into those which are required for general purposes and those which have special uses. The former should by all means be prepared according to the standards suggested by the Committee of the A. H. A. If special media are used, their exact composition and mode of preparation should be fully and explicitly given.

Furthermore, the reasons why the special media are used should be clearly set forth, so that co-workers may judge of their special value and may try them out intelligently, should they care to do so.

As special media are adopted into general use by the majority of bacteriologists they are to be relegated into the group of general media. For example, a few years ago, lactose-litmus-agar, Endo medium, Hess' medium, lactose-bile medium, etc., were special media. They are now in general use and they should be prepared according to a standard method. We hereby give the methods of preparing some of the more important media used in general bacteriological work, following the directions of the Committee of the A. H. A.

1. Nutrient Broth.—Infuse 500 grams of chopped lean meat for 24 hr. in distilled water. Shake occasionally and keep in the refrigerator. Any loss by evaporation is to be restored. Strain the infusion through cotton or through cotton flannel. Add 1 per cent. of peptone and warm over water bath or steam until the peptone is entirely dissolved. Heat for 30 min. in rice cooker or in steam sterilizer and restore any loss by evaporation. Titrate with normal sodic hydrate (or normal hydrochloric acid) to a reaction of + 1.0 per cent. Boil for 2 min. over open flame, stirring constantly. Restore loss by evaporation. Filter through cotton (placing the cotton on cotton flannel or on perforated filter paper). Pass the medium through this filter until it comes out perfectly clear. Again titrate and record the final reaction. Pour into tubes (10 cc. in each tube) and sterilize in the manner as already directed.

This medium is much used for general cultural purposes. It is used in making the cultures of typhoid fever germs, for determining the phenol coefficient of disinfectants by the Anderson-McClintic method of rating the germ destroying power of disinfectants. It is also used in culturing motile bacteria, etc. Various indicators may be added.

2. Sugar Broths.—Broths to which sugars are to be added are prepared in the same manner as nutrient broth, adding 1 per cent. of dextrose, lactose, saccharose or other sugar. The sugar is to be added before sterilizing. Sterilizing in the autoclave is to be preferred because the longer steam sterilization is apt to cause inversion of the sugar. The reaction of the sugar broths shall be neutral to phenolphthalein.

These media are much used in testing for the presence of *Bacillus coli* (dextrose broth). The committee states that the removal of muscle sugar by inoculating with *B. coli* is not necessary if small amounts of gas formation are to be disregarded. In the routine work of testing water for the presence of the *B. coli* a sufficient volume of the water to be tested is added so that the resulting mixture will be one of normal strength. The committee also advises against the use of beef extracts in place of the laboratory made beef infusions.

3. Nutrient Gelatin.—Make the beef infusion in the manner already described. After the first filtering through cotton or cotton flannel, add 10 per cent. of gelatin (the per cent. being based on the weight of the beef infusion instead of volume and the weight of the gelatin to be on a basis of dry condition, and 1 per cent. of peptone) and warm over water bath with constant stirring until the peptone and gelatin are entirely dissolved. While dissolving the peptone and gelatin the temperature should not rise above 60° C. Boil for 2 min. and adjust the reaction to +1.00 per cent. Heat for 40 min. over water bath or in steam sterilizer and restore any loss by evaporation. Again adjust the reaction if necessary and boil over open flame for 5 min. with constant stirring. Restore loss, filter until clear, titrate and record this final reaction. Tube and sterilize as for beef broth and at once store in ice chest. Protect against evaporation as already explained.

4. Nutrient Agar.—Boil 15 grams (dry weight) of washed thread agar in 500 cc. of distilled water for half an hour and make

up weight to 500 grams. Infuse 500 grams of lean meat in 500 cc. of distilled water for 24 hr. in ice chest. Make up loss by evaporation, strain, weigh filtered infusion and add 2 per cent. of peptone. Warm on water bath with constant stirring until all of the peptone is dissolved. To 500 grams of the meat infusion add 500 cc. of the 3 per cent. agar solution, keeping the temperature below 60° C. Boil for 1 min. and titrate to +1.0. Sterilize in steam for 40 min. and restore any loss by evaporation. Readjust if necessary and then boil for 5 min. with constant stirring. Restore any loss due to evaporation and filter by passing it through the filtering material (cotton and cotton flannel or perforated filter paper) at least three times. Titrate and record the final reaction. Tube, sterilize and store as for gelatin media. It must be borne in mind that agar media are never as clear as broth or gelatin media.

5. Lactose Litmus Agar.—To make this medium add 1 per cent. of lactose to nutrient agar just before sterilizing and make the reaction neutral to phenolphthalein.

If this medium is to be used in tubes the sterilized azolitmin (1 per cent. aqueous solution) is added just before the final mass sterilization, that is, the sterilization before pouring into the tubes.

If the medium is to be used in Petri dishes, the azolitmin is not added until ready to pour into the dishes.

The azolitmin and the lactose should be sterilized separately before adding to the agar medium, though it is permissible to mix the lactose with the agar and sterilize together, preferably in the autoclave (120° C. for 15 min.).

It would appear that the azolitmin of the market varies considerably and many bacteriologists prefer the pure litmus. A 1 per cent. aqueous suspension of azolitmin should dissolve readily when boiled for 5 min.

This medium is much used in bacteriological work on presumptive sewage contaminations, as estimating the temperature

differential colonies (20° C. and 37° C.), red colonies and total colonies, etc.

6. Lactose Bile.—This medium is to be made in two ways: Add 1 per cent. of peptone and 1 per cent. of lactose to sterilized undiluted fresh ox gall; or add the peptone and lactose to a 10 per cent. aqueous solution of freshly made dry ox gall. It is used without titrating. Old dried ox gall should not be used. Obtain it from a reliable dealer. If possible, make arrangements to get the fresh undiluted ox gall from some abattoir.

This is the standard medium for making the quantitative as well as qualitative tests for the colon group of bacilli.

7. Liver Broth.—Chop 500 grams of fresh beef liver into small pieces and place in 1000 cc. of distilled water. Weigh infusion and container. Boil for 2 hr. in rice cooker, starting cold and stirring occasionally. Make up loss in weight and pass through wire or cloth strainer. Add 10 grams of peptone, 10 grams of dextrose and 1 gram of di-potassium phosphate (K_2HPO_4). Dissolve the added ingredients by warming in rice cooker with stirring and then titrate to the neutral point (to phenolphthalein). Boil for 30 min. in the rice cooker and for 5 min. over open flame with constant stirring to prevent the caramelization of the dextrose. Make up loss due to evaporation and filter. Tube, sterilize and store as for other media.

This is a much used enriching medium which gives asg formation with all of the species which ferment dextrose. It is also much used to rejuvenate pure cultures of bacteria and encourages the development of attenuated forms of bacteria.

8. Hiss Typhoid Bacillus Medium.—Two media are used. One for the isolation of the typhoid bacillus by the plating method, and the other for the differentiation of the typhoid germ from other forms in tube cultures. The former is designated as the plate medium and the second as the tube medium. They are prepared as follows:

a. Plate Medium

Agar	10 grams
Gelatin	25 grams
Salt	5 grams
Liebig's meat extract	10 grams
Dextrose	10 grams
Water (distilled)	1000 cc.

Add gelatin when the agar is melted, dissolve the gelatin, add the other ingredients, titrate to +2.0 per cent., filter, etc., as for other media. The medium is to be clarified by adding the whites of one or two eggs, well beaten in 25 cc. of distilled water, boil for 45 min. and filter through absorbent cotton. Do not add the dextrose until after clearing.

b. Tube Medium

Agar	5 grams
Gelatin	80 grams
Salt	5 grams
Liebig's meat extract	5 grams
Dextrose	10 grams
Water (distilled)	1000 cc.

The manner of preparation is the same as for the plate medium. However, the reaction is to be +1.5 instead of +2.0 per cent. Without the dextrose and less salt and titrated to +1.0 per cent., the plate medium constitutes the ordinary nutrient agar-gelatin medium which was formerly very much used because it possessed the solidifying properties of agar combined with the nourishing properties of gelatin.

9. Endo Medium.—This medium is much used in testing for the colon bacillus. It is variously modified by different workers and it is highly important that some standard method of preparing the medium should be adopted and adhered to. The following is the method of preparation and use recommended by the committee.

Add 30 grams of powdered agar to 1 liter of cold water by sifting slowly upon the surface of the water and allowing it to

settle. Add 10 grams of peptone and 5 grams of Liebig's meat extract. Heat in rice cooker until the ingredients are entirely dissolved. Neutralize with sodium carbonate, using litmus as an indicator, and then add 10 cc. of a 10 per cent. solution of sodium carbonate.

Store the medium in lots of 100 cc. using flasks large enough to permit the addition of the other ingredients. Sterilize for 2 hr. in streaming steam.

To use the Endo medium proceed as follows: Make a 10 per cent. aqueous solution of sodium sulphite and add 2 cc. of fuchsin solution (10 per cent. of basic fuchsin in 96 per cent. alcohol) to 10 cc. of the sulphite solution and steam this mixture for a few minutes in the steam sterilizer. Add 1 gram of chemically pure sterilized lactose to each 100 cc. of the Endo medium after the medium has been liquefied and while the temperature is not above 60° C. While the medium is still liquid, add 0.5 cc. of the fuchsin-sulphite solution and then pour into the Petri plates and allow to harden in the incubator. The sulphite solution must be prepared fresh as needed.

10. Milk.—The milk to be used for cultural purposes must be pure and recently drawn. In all cases the milk of the grade or quality known as "certified milk" is to be preferred. The recently drawn milk is to be placed in the refrigerator for 12 hr., so as to permit the cream to rise to the top and any suspended matter to sink to the bottom. Skim the milk and siphon off all but the bottom sedimentary portion. Adjust to +1.0 per cent. Tube and sterilize.

Litmus milk is made by adding 1 per cent. of sterilized azolitmin to the above. In using litmus milk always set aside a control tube with the inoculated tubes for purposes of color comparison.

Because of the difficulty of always getting a uniformly high quality of cow's milk, it has been suggested that an artificial substitute be employed. Hill and his pupils recommend a

medium in which prepared casein (nutrose) is the principle ingredient. Chemically, nutrose is a caseinate of sodium and is prepared as follows: Moist casein precipitated from skimmed milk is washed with water in a solution of sodium hydroxide, evaporating the solution to dryness *in vacuo*, powdering the residue and washing successively with alcohol and ether and then drying. It is a coarse, white, odorless and tasteless powder, forming a turbid adhesive solution with water, having an alkaline reaction toward litmus and an acid reaction toward phenolphthalein. It is a food product intended for the sick because of its easy digestion. It is made in Germany but may be secured through any of the larger American pharmaceutical houses (Victor Koechl & Co., New York City).

The formula for making the artificial milk is as follows:

Nutrose	24 grams
Lactose	10 grams
Distilled water	1000 cc.

Dissolve the nutrose and lactose in the water (cold) for 12 hr. with occasional thorough shaking and then filter through cotton. Tube and sterilize at 110° C. for 20 min., or in the steam sterilizer in the usual manner. No adjustment is required.

This medium contains all of the nutritive ingredients of cow's milk with the exception of fat which is not desired for the ordinary cultural work. It is of uniform quality and is said to give far more uniform results than cow's milk. It is furthermore more translucent than cow's milk and shows the reactions with indicators much better. It would be advisable to make the artificial milk the standard substitute for cow's milk.

11. **Peptone Medium.**—This is simply a 1 per cent. peptone solution in distilled water and is intended to be used for making the indol test. Beef broth from which muscle sugar has been removed by inoculating with *B. coli* is believed to be objection-

able because of the toxins present and which interfere with the growth of many species of bacteria.

Other media of a more or less special character will be described or referred to under the discussion of methods. Those described above are the more important ones required in the bacteriological examination of foods and drugs.

7. Technique for Making Quantitative and Qualitative Estimations by the Plating Methods

As has been explained, the plating method is intended to determine the number of *living* bacteria present in foods and drugs and the results supplement the results of the method of making the direct counts already described. From this statement it is evident that the quantitative results by the two methods are not the same. For example, the bacterial count of a catsup by the direct method may be very high while the plating method may give negative results, due to the fact that the heat sterilization employed at the cannery killed all of the bacteria present. This also shows why it is absolutely necessary to employ both methods in order to form a correct estimate of the total contamination of the substance.

The following suggestions on laboratory technique are given with a view to the unification of methods, thereby leading to greater uniformity in comparative results.

1. Apparatus.—Test-tubes to be used for the usual cultural purposes shall be of medium weight and thickness, 15 cm. long by 1.6 cm. diam. Petri dishes shall be 10 cm. in diam. Petri dishes with porous covers are preferred. All glass ware must be scrupulously clean and may be sterilized by exposing to a dry heat of about 150° C. for a period of 1 hr., after being cleaned, wiped dry and plugged with a good grade of commercial cotton. A browning of the free ends of the cotton plugs indicates that the right degree of heating has been attained. A standard wire

loop is made as follows: Bend the end of a No. 27 platinum wire, 10 cm. long, around a piece of No. 10 wire. The free portion of the straight platinum wire inoculating needle, shall be 10 cm. long (No. 27 wire). The standard fermentation tube shall be of the following proportions. The length of the closed end of the fermentation tube (diameter about 1.5 cm.) shall be about 14 cm., and the open end shall be of bulbous form (diameter of bulb about 3.8 cm.) large enough to hold all of the liquid in the closed end. Larger and smaller fermentation tubes than the standard just described may be used for special purposes. Standard and other fermentation tubes may or may not be graduated as the special purposes may require.

2. Amounts of Media to be Tubed.—The standard amount of culture medium to be placed in each test-tube of standard size is 5 and 10 cc., the media to be introduced by means of a suitable burette. Greater or lesser quantities may be used as occasion may require. Tubes containing just 10 cc. of culture media are required for the plating purposes. 5 cc. quantities (of gelatin, agar and other solid media) are required for making slants.

3. Amounts of Culture Media to be Plated.—For the usual quantitative determinations by the plating method, 10 cc. of the culture medium shall be poured in each standard Petri dish.

The required number of tubes each containing 10 cc. of agar or gelatin culture medium are placed in the steam sterilizer until the medium is entirely liquefied and then placed in a beaker or other suitable container with lukewarm water, with thermometer. Plate the gelatin medium when the thermometer registers between 25° and 30° C. The temperature of the medium must not be more than 30° C. If the temperature is less than 25° C. the gelatin will begin to coagulate and will not pour and spread properly. Agar media must be plated at a higher temperature than gelatin media, usually 40° to 42° C. The Petri dishes should be warm when the media are poured, the temperature being approximately the same as that of the medium when it is poured.

This will insure a more uniform spreading of the medium over the bottom of the dish.

To pour the liquefied agar or gelatin from the tubes, remove the cotton plug and flame the mouth of the tube so as to kill any bacteria or spores that may be present; raise one side of the cover just high enough to permit bringing the tube to the middle of the dish and pour contents into the dish over the material planted into the middle of the dish. Let cover of the dish sink into place and by very slight tilting of the Petri dish induce the culture medium to spread evenly over the bottom of the dish before the medium has had time to coagulate. As the medium spreads it also causes the spreading of the planted material.

Many workers use 5 cc. of the medium for plating, instead of 10 cc. as above recommended. The smaller amount is satisfactory when 1 cc. quantities are to be planted or inoculated. However, in order to make sure that the entire area of the bottom of the dish is well covered, 10 cc. quantities should be used. The larger amount also minimizes the influences which the changes in evaporation in the media may have upon the quantitative results.

4. Method of Making the Plate Cultures.—Absolutely clean sterilized (dry heat of 150° C. for 1 hr.) Petri dishes of the standard size (10 cm. diam.) are used. 0.1 cc. quantities of the substance to be cultured, or dilutions thereof, are planted or delivered into the middle of the dish, an absolutely clean and sterile 1 cc. pipette accurately divided into tenths. The cover of the dish is to be lifted just high enough to permit placing the pipette in position, and is to be replaced just as soon as possible.

In the usual water analysis work, 1 cc. quantities are generally planted, instead of 0.1 cc. quantities as above recommended. For purely quantitative results, the smaller amounts should be planted because the larger amounts may include enough of the inoculating liquid to interfere with the uniformity of results.

Formerly it was customary to mix the material to be planted with the medium in the tube before plating. This method has

some very objectionable features, chief of which is that the residue remaining in the tube after pouring retained a certain percentage of the organisms, thus interfering with the accuracy of the results. It must, however, be admitted that the method has some advantages, chief of which is the more uniform mixing of the bacteria with the medium and their more uniform distribution in the plate, making accurate counting of the colonies easier.

5. Making the Dilutions.—Whether or not making dilutions is necessary depends upon the number of organisms present in the substance to be analyzed. The number of colonies in a Petri dish must not exceed 200 in order to make counting fairly easy and accurate. In fact with the method of direct planting, as usually recommended, which generally results in a somewhat irregular distribution of the bacteria (hence also the colonies to be counted) it would be desirable to make the dilutions such that the number of colonies in each plate shall not exceed 100. If 0.1 cc. quantites are to be plated or planted, as above recommended, it would follow that dilution would not be necessary as long as the number of bacteria per cc. does not exceed 1000.

However, since most food and drugs contain more than that number of bacteria per cc., it becomes necessary to make dilutions. The standard dilutions are made by tens, as 1–10, 1–100, 1–1000, and 1–10,000. The dilutions are made by adding 1 cc. of the substance to be analyzed to 9, 99, 999 and 9999 cc. of sterile distilled water, or other desirable sterile diluent, and shaking thoroughly. In practice it is desirable to plate three of the graded dilutions, so that the second higher dilution will in all probability yield about 100 bacteria in the 0.1 cc. of the material plated. Thus with fairly pure drinking water, the plantings would be made from the undiluted water, the 1–10 and the 1–100 dilutions, presuming that there are about 10,000 bacteria per cc. present. In case of unusually pure drinking water, that is water in which the number of bacteria is probably not more

than 50 per cc., it would be desirable to use 1 cc. quantities for plating which would give about 50 colonies in the plate.

The thorough mixing of the sample before making the dilutions is of the greatest importance, likewise the thorough mixing of each dilution before taking out the quantity to be plated. Each

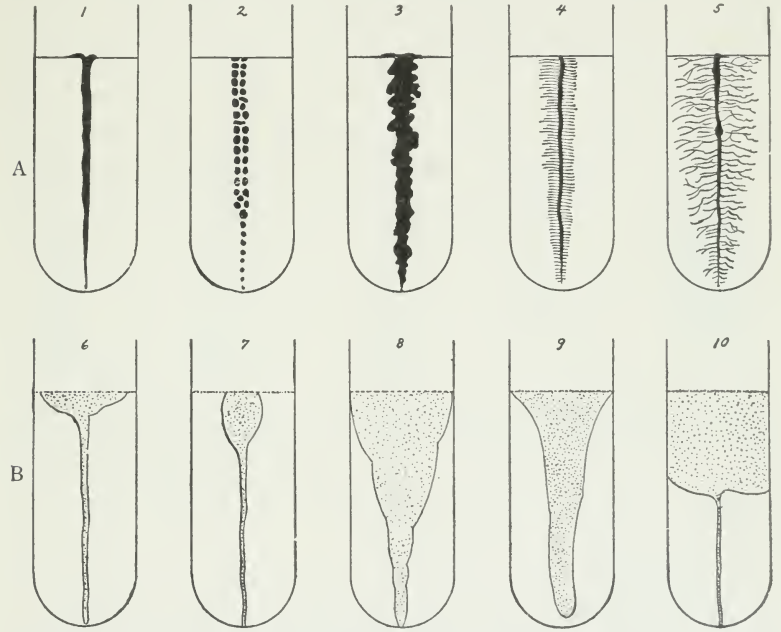

FIG. 23.—Types of growth in stab cultures. *A*, Non-liquefying. 1, Filiform (*Bacillus coli*); 2, beaded (*Streptococcus pyogenes*); 3, echinate (*Bacterium acidi lactici*); 4, villous (*Bacterium murisepticum*); 5, arborescent (*Bacillus mycoides*).

B, Gelatin liquefying. 6, Crateriform (*Bacillus vulgare*, 24 hr.; 7, napiform (*Bacillus subtilis*, 48 hr.); 8, infundibuliform (*Bacillus prodigiosus*); 9, saccate (*Microspira finkleri*); 10, stratiform (*Pseudomonas fluorescens*).—(*McFarland, after Frost.*)

test should be made in triplicate, taking up the 1 cc. amounts for making the dilutions with three different clean sterile pipettes. It is preferable to use a new pipette for each dilution. If it is not convenient to have on hand a sufficient number of clean sterilized pipettes, the pipette in use must be thoroughly

rinsed in sterilized distilled water, using a fresh supply of distilled water for each rinsing.

Draw the liquid to be plated into the pipette, place thumb over the upper end of pipette, let liquid run out until one of the 0.1 cc. marks is reached, then bring the lower end of the pipette close to the surface of the liquid in the diluting tube and let just 0.1 cc. run out, the finer degrees of accuracy are attained by using or not using the meniscus of the liquid projecting from the lower end of the pipette after the last drop has fallen. This correcting droplet is secured by touching the lower end of the pipette lightly against the inside of the diluting tube at a point near the surface of the liquid, without, however, actually touching the liquid. A similar adjustment may be made when taking up the 1 cc. amount to be diluted, only in this case the pipette is of course to be touched against the side of the tube or vessel containing the liquid of which the dilution is to be made.

Thorough mixing of the contents of the diluting tube is attained by vigorous shaking. Place the thumb over the opening of the tube, interposing a piece of sterilized rubber sheeting such as is used by dentists. Some workers mix the contents of the tube by rapidly rotating between the two hands and by tapping against the palm of one hand.

6. Incubation.—The regulation incubators are to be used. It is highly important that there should be ample ventilation, a matter to which amateurs and even experienced bacteriologists as a rule give little or no attention. All modern incubators are supplied with ventilating openings at the top which should be kept open most of the time. The air in the incubating chamber should be practically saturated with moisture, which may be accomplished by placing a flat dish containing water in the lower chamber.

Two standard incubating temperatures are employed, namely, 20° C. and 37° C., the first corresponding to the ordinary room temperature and the second to the body (human) temperature

or blood heat. The devices to regulate the temperature should be such that the variation from the two standards given shall not be more than 2°, that is, not more than 1° in either direction.

There is no standard time of incubation. For work in the study of water sanitation as carried out in Germany, England and also in the United States, gelatin plates are incubated for 2 days at a temperature of 20° C. It is suggested that the period be extended to 3 days in order to get more accurate results.

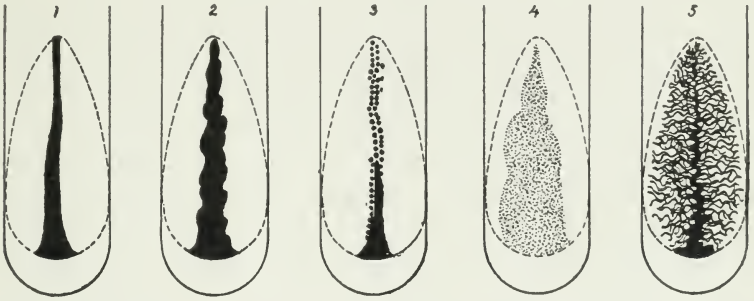

FIG. 24.—Types of streak culture. 1, Filiform (*B. coli*); 2, echinulate (*B. acidi lactici*); 3, beaded (*Str. pyogenes*); 4, effuse (*B. vulgaris*); 5, arborescent (*Bacillus mycoides*).—(*McFarland after Frost.*)

From 1 to 2 days is the usual time of exposure for the higher temperature (37° C).

8. Practical Application of the Quantitative Estimations by the Plating Methods

The relative importance of the quantitative bacteriological determinations by the method of direct counting and by the plating method has been explained. Both methods must be made standard in every food and drug laboratory. Quantitative estimations by the plating method should take precedence with all substances containing largely living organisms such as water supplies of all kinds, milk, raw meats, and shellfish, etc., and all substances in which infection is suspected, even though such sub-

stances may have been subjected to processes of sterilization during some phase of the processing or of manufacture.

In a general way the quantitative results by the plating method are to be interpreted in a manner similar to the results by the direct count. In some cases the question at issue may be relative to the presence or absence of viable bacteria in substances which presumably do not contain living organisms, such as canned foods generally. Manufacturers of canned products are of the opinion that the methods of heat sterilization employed will kill all of the bacteria which may have been present at the time of canning. This is undoubtedly true in many cases, but in other instances it is only too evident that retarded fermentation processes continue after the cans are sealed, which accounts for the high counts in canned food products which contained only small numbers of bacteria at the time the cans were sealed. These subdued fermentation processes as a rule do not result in the formation of sufficient gas to produce "swells" and hence the article is not suspected until the container is opened when a more or less disagreeable or peculiar odor is noticeable, which is, as a rule, not sufficiently pronounced to prevent the use of the article as food.

In addition to the purely quantitative results, the plating method indicates the general qualitative character of the organisms present and conveys some idea as to the course of the infection or contamination as shown by the characters of the colonies developed in the Petri dish or in the tubes.

9. Qualitative Determinations

The chief qualitative determinations in food and drugs laboratories pertain to sewage contamination. The recognition and determination of pathogenic bacteria as the typhoid bacillus, the cholera bacillus, diphtheria bacillus, etc., is an incidental possibility in the food laboratory routine and not a regular part of it. Of far greater significance is the recognition of the evidence

of the presence of intestinal parasites, such as the segments of tape worm, the larvæ and ova of such parasites, etc., as already stated under the discussion of the direct method of making counts. The bacteriologists in food and drugs laboratories should be qualified to recognize all of the possible disease germs and the smaller carriers of disease which may be associated with food substances and they should be able to demonstrate the presence of such contamination, if necessary. The thus far recognized routine in food and drugs laboratories and in public health laboratories is limited to making the so-called presumptive colon bacillus test, as indicative of sewage contamination or contamination with fecal matter. Sewage contamination means primary contamination with fecal matter. The reason why the colon bacillus test has been selected as giving satisfactory evidence of sewage contamination is because this bacillus is most abundant and is constantly present in fecal matter. Any considerable number of colon bacilli in water supplies or in food substances is evidence of gross negligence and defects as to sanitary requirements.

As far as the practical work in finding evidence of the sewage contamination of food substances is concerned, there is no effort made to isolate and identify a definite bacillus recognizable as *Bacillus coli*. It is rather the recognition of certain cultural characteristics which have come to be recognized as being peculiar to the bacilli, known as the *B. coli* group, all of which are traceable to intestinal origin. Furthermore this group of bacteria is very widely distributed in the animal kingdom, being in no wise limited to the intestinal tract of man. The *B. coli* group of the lower animals is in all probability different from that which inhabits man and certain workers have made attempts to differentiate them by means of special cultural methods, but thus far these methods are not sufficiently perfected to be used practically in food and drugs laboratories. These statements also apply to the Streptococci group of intestinal origin. However, some of the laboratory results thus far attained would indicate that in the

near future it will be possible to differentiate between pollutions traceable to human origin and such as are traceable to cow, horse or hog manure, for example. It cannot be denied that food materials intended for human consumption which are contaminated to any distinctly appreciable amount with the contents of the intestinal tract of any animal, are unsanitary and hence unfit for human consumption.

In-as-much as the intestinal bacteria (bacilli and streptococci) are very abundant and very widely distributed, it is quite evident that it would be impracticable to pronounce all foods unfit for use if only one or a few intestinal organisms were found to be present in a comparatively large quantity. Human feces contains about one-third bacteria (dry weight), the majority of which belong to the colon group, and the exterior of the human body carries bacteria derived from the intestinal tract, especially the hands and the deposits under the finger nails. Flies are carriers and distributors. of intestinal bacteria. The dust of the streets and street sweepings contain large numbers of bacteria derived from the intestinal tract of the horse, etc. It would be impracticable to enter into a fuller discussion of the distribution of bacteria traceable to intestinal origin. Suffice it to state that it is the work of the food bacteriologist to determine the presence, in articles intended as food, of those bacteria which indicate contamination with fecal matter, no matter what the source of such objectionable matter may be. The basis for the condemnation of contaminated foods is quantitative in the comparative sense. For example, the finding of a few colon bacilli in large quantities of water or their occasional presence in small quantities of water, does not indicate that the water is unsuitable for drinking purposes. If, however, the colon bacilli appear in a large proportion of many small samples (1 cc. or less) of water it is safe to conclude that there is considerable recent sewage contamination and that such water is dangerous to health. The intestinal bacteria are in themselves not seriously pathogenic to man even when taken in considerable numbers.

The real source of danger lies in the fact that the intestinal bacteria normal to man and the lower animals, may be and frequently are associated with pathogenic bacteria, such as the typhoid bacillus and the dysentery bacillus. Our long experience with the consumption of sewage contaminated water supplies, has shown that, as a rule, the first danger sign of the excessive contamination was usually a marked increase in the number of cases of dysentery, generally followed by sporadic cases and epidemics of typhoid fever.

The practical results of the quantitative bacterial determinations of food substances combined with the qualitative tests for the colon group, has proven of the highest value and it is considered entirely feasible to continue the application of the tests and to suggest ways and means of improving the laboratory technique covering such methods. The qualitative methods thus far worked out are based upon a knowledge of the life history of the bacteria concerned and may be briefly stated as follows: Normal intestinal bacteria and such other bacteria as may develop in the intestinal tract, such as the typhoid bacillus, the cholera bacillus, the dysentery bacilli, etc., are adapted to a temperature of about 37° C. and they feed upon the food materials found in the intestinal tract and have a somewhat reduced oxygen supply. Among the substances peculiar to the intestinal tract we find bile, pancreatin, and other enzymes and a certain water percentage and the various ingredients of food materials more or less digested and the various products elaborated by the different species and varieties of bacteria present. It is a study of the peculiarities of the intestinal bacteria which has suggested the technique for their isolation and their quantitative as well as qualitative estimation in food supplies, as can be seen readily from a study of the culture media and cultural methods recommended. To enter into any fuller discussion of the work done by American and European investigators on the bacteria which are normal to the intestinal tract or which may occur in the intestinal tract in

disease, is not practicable but we desire to give the following tabulation showing the relationship between the different species of the colon-typhoid group of intestinal bacteria in their behavior with dextrose and lactose media.

Bacteria of the Colon-typhoid Group

Species	Dextrose		Lactose	
	Gas Formation	Acid Formation	Gas Formation	Acid Formation
B. alcaligenes	none	none	none	none
B. typhi	none	slight	none	none
B. dysenteriæ	none	distinct	none	none
B. enteritidis	active	strong	none	slight
Paratyphoid group	active	strong	none	slight
Hog cholera bacillus	active	strong	none	slight
B. coli	active	strong	active	strong

There are numerous other distinguishing characteristics beside those indicated in the above tabulation, as agglutinating phenomena and behavior with other special culture media. It is simply desired to indicate somewhat more specifically the lines of research which were necessary to determine the identity of the related species and varieties of intestinal bacteria.

The *Bacillus coli* was isolated as early as 1884 from the feces of a cholera patient, at which time this organism was supposed to have some causal relationship to cholera. Later it was proven that this bacillus was a normal inhabitant of the intestinal tract of man and of other animals, being regularly present in their excreta, and this discovery proved of the highest importance to sanitarians as the presence of this germ in water supplies, in milk, in mineral waters, etc., is generally regarded as evidence of sewage contamination. The colon bacillus has been found in sewage contamination, river water, in spring water, ice, milk,

cream, butter, buttermilk, sour milk tablets, mineral waters, oysters, clams, flour, oatmeal, cornmeal, cereals, frozen eggs, dried nuts and fruit, etc. *B. coli* is not normally present in sea water and its occurrence in salt water shellfish is evidence of sewage contamination. The occurrence and general distribution of the colon bacilli is almost in direct proportion to the density of the population. Animals of all kinds are disseminators of colon bacilli, particularly the larger animals as the horse, cattle, the dog and domestic fowls. Within and about the home, the house-fly and the stable-fly are the chief distributors of colon bacilli. We may repeat that colon bacilli are found on the skin of persons, particularly on the hands and under the finger nails. The underclothing worn carries these bacilli and is the agent instrumental in distributing them over the exterior of the body, especially in those of uncleanly habits. Water in which the hands have been rinsed will generally yield positive colon bacillus tests. It is also apparent that the colon bacillus does not survive for a great length of time outside of its natural environment; thus sewage-contaminated waters purify themselves of colon bacilli after a time, the period varying with the temperature and the amount of organic matter present. Thus it sometimes happens that a water supply may show a high bacterial count and yet be quite free of colon bacilli. As a rule, however, water supplies and substances brought in contact with such water supplies which show a high general bacterial count, will also show a comparatively high count in colon bacilli. There may be notable exceptions to this rule. A water supply, or other liquid substance, may show a comparatively low bacterial count and yet yield numerous colon bacilli. Such an occurrence would indicate an unusual source of extensive sewage contamination.

From the foregoing it is evident that the sanitary examination of foods and drugs resolves itself into the making of quantitative bacterial counts, as already fully explained, and the presumptive colon bacillus test, with an occasional test for other specific

organisms, as will be explained later. It is also evident that the isolation or the identification of the colon bacillus in a mixed contamination as all sewage-contaminated substances are, is not as simple a matter as might appear on first consideration. However, the presumptive tests for the presence of the colon bacillus in definite quantites of the food materials or liquids used with, or associated with, certain food materials, is almost universally accepted as evidence of the dangerous contamination with sewage. It is, however, quite clear that health officers should not adopt hard and fast rules or standards for the condemnation of foods because of such evidence of sewage contamination. Very naturally, the standard for water supplies will not apply to oysters and shellfish generally and the standard for shellfish will not be practically applicable to mineral waters, etc. With substances of which the standard or quality is quite generally based upon a numerical count, as for example milk, the presumptive colon bacillus test need not be applied, unless it is to be carried out as giving corroborative evidence of the sewage contamination.

One of the first important duties of the food and drugs bacteriologists will be for them to get together and agree upon uniform methods and to decide upon the kinds of bacteriological examination under the pure food and drugs act to which the quantitative as well as the qualitative (presumptive colon bacillus test) determinations are applicable, in harmony with our present knowledge of food bacteriology. The working laboratory methods adopted must be practicable and must be carried out primarily as a better protection of the physical well-being of the consumer, incidentally also safeguarding the business interests of the conscientious manufacturers. The following suggestions are intended to indicate along what lines the practical qualitative work may be done and also to outline certain research work which should be carried on in order to develop the working methods to greater perfection and to add such new methods as may prove useful.

10. Evidence of Sewage Contamination. General Methods.

It may be assumed that the presence of any or all of the large group of colon bacilli in water or in food substances is indicative of sewage contamination or contamination with fecal matter. The colon bacilli are aerobic, nonsporeforming, motile, short and produce acid and gas in dextrose and lactose media and develop best at a comparatively high temperature (37° C.). A practical presumptive colon bacillus test depends upon the characteristics thus indicated and is carried out as follows:

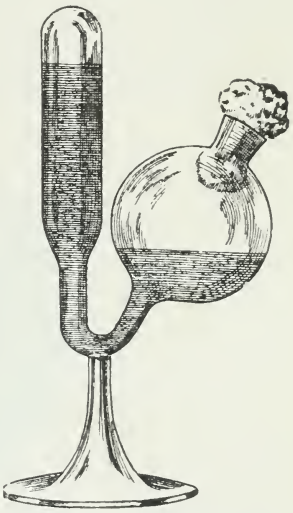

FIG. 25.—Fermentation tube. This type of fermentation tube is especially convenient for making the gas determination with the colon bacillus. Other forms of fermentation tube may be used.—(*Pittfield.*)

1. Presumptive Colon Bacillus Test.—Add the substances to be tested (water, sewage, mineral water, shellfish liquor, washings from vegetables, etc.) in 0.01 cc., 0.10 cc., 1 cc., 5 cc. and 10 cc. quantities (or these equivalents in dilutions) into fermentation tubes holding at least 40 cc. of lactose bile, incubate at 37° C. and look for the formation of gas. If gas formation is observed the presence of colon bacilli may be suspected. If, in the case of water supplies for example, the 0.10 cc. tubes show gas formation then it may be reasonably assumed that colon bacilli are present. If two out of five of such tubes give positive gas reactions, the test may be considered conclusive. To test the gas formed, fill the tubes showing gas formation with a 2-per cent. solution of sodic hydrate, hold thumb firmly over the opening of the fermentation tube and mix contents by tilting back and forth carefully. The volume of gas absorbed is CO_2 whereas the un-

absorbed portion is supposedly hydrogen. The colon bacillus shows a gas formation of ⅓ hydrogen. The standard time of incubation is 48 hr., but if colon bacilli are abundant, gas formation will be observed in the tubes carrying the larger amounts of the inoculated material at a much shorter time, occasionally within a few hours. Small numbers of attenuated colon bacilli may require 2 and 3 days before there is any gas formation noticeable. In this connection it may be mentioned that the attenuated colon bacilli indicate remote contamination, as all *B. coli* of recent contamination develop readily in lactose bile.

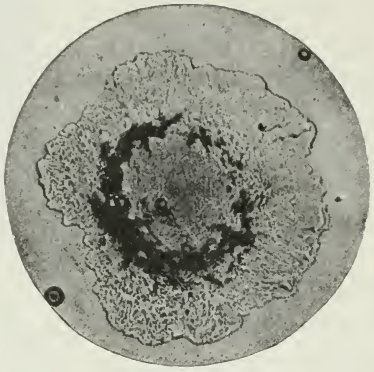

FIG. 26.—*Bacillus coli.* Superficial colony on a gelatin plate 2 days old (× 21). —(*McFarland after Heim.*)

This constitutes the usual presumptive test for the presence of sewage contamination. Some investigators, however, recommend that the test be supplemented as follows: Plant the suitable quantities or dilutions into liver broth (in test-tubes) and incubate at 37° C. for about 12 hr. and then transplant these cultures into the lactose bile as above explained. The liver broth enrichment medium is said to bring out the attenuated forms of colon bacilli. In routine procedures the liver broth culturing is usually omitted as the important point at issue is the determination of fairly recent contamination with sewage, or of sewage

contamination in large amount, and the lactose bile medium gives conclusive results regarding this.

The presumptive colon bacillus test is to be supplemented further as follows: Plate suitable dilutions of the substances to be tested for sewage contamination (0.001 cc., 0.01 cc., 0.10 cc., 1.00 cc.) into lactose litmus agar Petri dishes, making two sets. Incubate one set of these plate cultures at 20° C. and the other at 37° C. and note the following:

1. The relative number of colonies which develop at the two temperatures.
2. The number of acid-forming colonies.

The time of incubation at the lower temperature (20° C.) should be 3 days, although fairly conclusive results may be noted at the end of the second day. The standard time of incubation at the higher temperature (37° C.) is 48 hr., although certain results may be noted at the end of 24 and 36 hr. If the proportion of high temperature colonies is high, it is indicative of the presence of numerous bacteria derived from the intestinal tract. If the high temperature colonies approximate (numerically) the low temperature colonies, sewage contamination may be suspected. If in addition many of the high temperature colonies show pink or vermilion (on lactose-litmus agar), the sewage contamination is practically proven. Both the colon bacilli and the sewage streptococci show pink colonies on this medium, the latter being the brighter, more vermilion in coloration. This coloration is due to the formation of acid by the organisms named which reacts with the litmus. Examine the pink colonies under the microscope in order to determine which are the colon bacilli and which the streptococci. As a rule, high temperature colonies should not exceed 1 : 100 as compared with the low temperature colonies. It must be kept in mind that the pink colonies may turn blue within 24 hr. due to the liberation of ammonia and amines. Red colonies indicate lactose fermentation with formation of acid, but since bacteria other than the colon bacillus form acid (notably

the streptococci), it is desirable to examine such colonies microscopically and to inoculate into other media and perhaps to test for indol formation, in order to obtain satisfactory proof as to whether or not they are colon bacilli.

Neutral red (a safranine dye) reduction was at one time considered a very important check test for the colon group. Stokes, as early as 1904, recommended that neutral red be added to lactose broth in the fermentation tubes which contain the required dilu-

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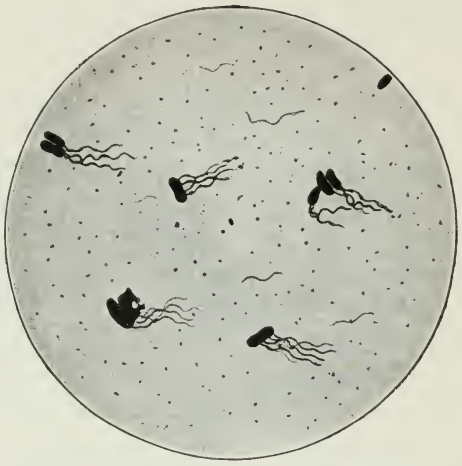


FIG. 27.—*B. coli* showing flagellæ stained by the van Ermengen method (× 1000).—(*MacNeal, from McFarland after Migula.*)

tions of the liquids to be examined. 30 to 50 per cent. gas formation in the closed arms of the tubes and the change of the neutral red to canary yellow, is said to be characteristic for the colon group. It would appear that the majority of bacteriologists are inclined to omit the neutral red test as being of little value.

The production of indol in peptone broth or solutions is another colon bacillus test much used in the United States. Boehmes' modification of the Ehrlich method is now generally employed, made as follows: Two solutions are required.

Solution No. I

Para-dimethyl-amido-benzaldehyde	4 parts
Absolute alcohol	380 parts
Concentrated HCl	80 parts

Solution No. II

Sat. sol. of potassium persulphate.

The indol test is performed as follows: Add 5 cc. of solution I to 10 cc. of a broth culture and then add 5 cc. of solution II, the

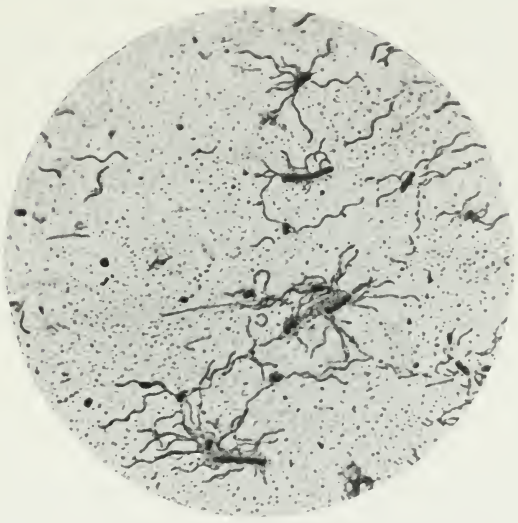


Fig. 28.—Bacillus of typhoid fever, stained by Loeffler's method to show flagella (× 1000).—(*Williams.*)

whole being then shaken. A red color indicates indol. Some of the leading bacteriologists consider this a very valuable test.

The so-called hog cholera group or the Gaertner group of bacilli is important from the standpoint of the food bacteriologist. The Gaertner group occupy a position intermediate between the chemically active coli group and the chemically inert typhoid group and includes the following important species or rather strains —the *Bacillus enteritidis* strain which includes many of the bacteria isolated in cases of food poisoning and some of the *B. typhi murium*

varieties, as *B. psittacosis*, and *B. suipestifer* and *B. paratyphosus B*. They differ from the typhoid group by gas formation in dextrose, and from the colon group by the production of an alkaline reaction in milk. They are concerned in the development of intestinal disturbances such as dysentery and diarrhea. No practical routine working method for the isolation of the Gaertner group has as yet been recommended. Some of the more important cultural characteristics are indicated in the table of Bacteria of the Colon-typhoid Group.

Another important group of bacteria from the standpoint of the food bacteriologist is the large group of sewage streptococci. They occur in the intestinal tracts of many animals. There are numerous strains of this group and they are somewhat less widely distributed than the colon group. The determination of sewage streptococci adds but little more than may be learned from the colon test and for this reason we shall not enter into any fuller discussion. This statement also applies to the host of other bacteria and related organisms which are more or less constantly associated with sewage and sewage contaminations.

For all practical purposes, the presumptive colon bacillus test supplemented, as the special cases may require, with certain special tests, combined with the quantitative counts by the plating method (gelatin media) will give all the information which is necessary to judge of the quality of certain foods, drinks and medicamenta, as far as the contamination with sewage is concerned. These points will be more fully discussed under special heads.

11. Possible Contamination of Foods with the Typhoid Bacillus

Testing food substances and medicamenta for the presence of the typhoid bacillus will never become a regular routine in the food laboratory. On occasion it will become an incidental procedure and must therefore receive some consideration. To understand the special significance and importance of this organism as a possible contaminator of foods, it is necessary to enter into

a brief statement of the typhoid fever and the organism which causes this disease. The primary cause of typhoid fever is the *Bacillus typhosus*, which in its general morphological characteristics resembles the colon bacillus, differing in that it is somewhat longer and more actively motile. When introduced into the intestinal tract of man it multiplies very actively and produces the symptoms of the disease known as typhoid fever. In disease, therefore, this organism grows in the same environment as the colon bacillus, excepting that the temperature (fever temperature) is higher. After recovery from the disease, the germs may remain

FIG. 29.—*Bacillus typhosus*, 72-hr. gelatin culture.—(*Stitt, after Kolle and Wassermann.*)

in the intestinal tract for long periods of time, for months and years. Furthermore, those who have never had the disease may become infected with the germs and carry them for long periods of time without developing the disease. Persons infected with the germs of typhoid fever without suffering from the disease are known as typhoid carriers, and it is self-evident that they may cause typhoid fever in those with whom they may come in contact. Numerous such carriers have been found and many sporadic cases of typhoid have been traced to such source. However, the majority of typhoid epidemics are traceable to foods and drinks contaminated

with the intestinal secretions of typhoid patients. The subject of typhoid contamination is therefore intimately associated with the general subject of sewage contamination or contamination with human fecal matter. Very naturally, the typhoid bacillus is far less common than the colon bacillus. In a general way it may be stated that the distribution of the typhoid bacillus is as wide as the distribution of typhoid contaminated sewage. As long as we adhere to the antiquated and highly unsanitary method

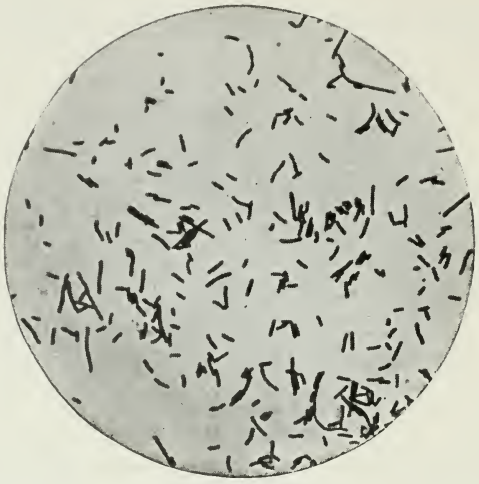


FIG. 30.—*B. typhosus* from gelatin smear preparation stained with fuchsin (× 1000).—(*MacNeal.*)

of emptying our sewage into the drinking-water supplies just so long will we continue to have epidemics of typhoid fever. Numerous statistical records show that the mortality rate from typhoid fever in our larger cities is directly proportional to the filthiness of the drinking-water supply. House-flies are known to be carriers of typhoid and the germs have been isolated from vegetable food materials, from oysters and other shellfish, from milk, etc.

The laboratory procedure in the examination of foods and liquids for the typhoid bacillus includes the isolation and identi-

fication of the germ. The proceedings are similar to those outlined for the colon bacillus, excepting that in this case the quantitative factor is not considered. The finding of a single typhoid fever germ in a mass of food materials is sufficient to condemn it. It may be assumed that where there is one typhoid bacillus there are more in the same vicinity and these may initiate an epidemic of typhoid fever.

The food bacteriologist may be called upon to examine food substances for the presence of typhoid contamination (from the feces of typhoid patients or of carriers) in instances where it is known that food has been exposed to typhoid infection or where such infection is merely suspected. The isolation from foods and the positive identification of the typhoid bacillus is by no means a simple matter. It is necessary to make use of special cultural methods, supplemented by the agglutination test, etc. The methods tried out by various bacteriologists are too numerous to even review and most of them have after a time been abandoned as unsatisfactory. The following tabulation from the work of Prescott and Wilson indicates some of the more practical laboratory procedures which have been tried with more or less success.

- Examination of water for typhoid bacilli
  - 1. Physical concentration
    - *a.* By filtration.
    - *b.* By agglutination
    - *c.* By chemical precipitation (Process: Schuder's, Fischer's, Wilson's, Müller's)
  - 2. Enrichment
    - *a.* Hoffman and Ficker's caffein process.
    - *b.* Jackson's lactose bile.
    - *c.* Parietti's carbol broth.
  - 3. Isolation
    - *a.* Elsner's gelatin medium.
    - *b.* Endo's medium.
    - *c.* Loeffler's malachite green medium.
    - *d.* Drigalski-Conradi agar.
    - *e.* Hiss's medium.
    - *f.* Hesse's medium.
  - 4. Identification
    - *a.* Morphological and cultural characteristics.
    - *b.* Agglutination.

Space will not permit discussing the methods thus outlined nor is this essential for the present purpose. Those interested are referred to the work by Prescott and Winslow, Elements of Water Bacteriology (1913), which contains a fairly complete digest of the methods. Furthermore, the methods adopted must be suited to the special cases in hand. The most suitable procedure for isolating the *Bacillus typhosus* from drinking water would not be practicably applicable in the examination of typhoid con-

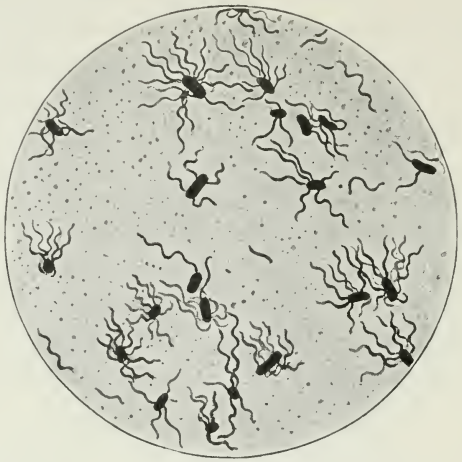


FIG. 31.—*B. typhosus* from an agar culture 6 hr. old. Highly magnified (× 1000), showing the flagellæ stained by the Loeffler method.—(*McFarland after MacNeal.*)

taminated sewage or milk, for example. For the time being there is no routine laboratory method for the isolation of the typhoid bacillus and we must content ourselves with a brief consideration of those methods which will in all probability give the best results.

It is of the highest importance that the food bacteriologist should search out typhoid contaminated foods *before* the occurrence of an epidemic. In fact, if such work is not undertaken until cases of typhoid have developed, the bacteriological find-

ings are often wholly negative, because of the long incubation period (14 days), so that the bacilli may all have disappeared from the sewage or water between the time of the infection and the manifestation of the symptoms of the disease. Under conditions favorable to the typhoid germs, as food supply, temperature, absence of sunlight, etc., they may survive for several months. It is generally conceded that the *Bacillus typhosus* is quite resistent and persistent. According to Ravenel, the germ survives for several months and longer in fecal matter deposited in snow which when carried into the stream supplying a city with drinking water by the early spring rains caused an outbreak of typhoid.

The highly objectionable method of using human excrement for fertilizing the soils of truck gardens, as practised by the Chinese and others, may lead to the typhoid contamination of the vegetables grown in such gardens. Washings of the soil and of the vegetables should be examined for typhoid germs.

FIG. 32.—Illustrating the Widal agglutination phenomenon. Upper half before the reaction. Lower half shows clumping of the motionless bacilli.—(*Pittfield.*)

The following general method for the isolation of the typhoid bacillus is suggested, subject to modification to suit special cases.

1. **Concentration.**—Run from 1 to 10, and more, liters of water (as from well, cistern, stream, water tank, etc.) through a clay filter. Just before all of the water has passed through the filter, shake it up and pour into a suitable centrifugal tube (the special tube already described will answer the purpose very well) and place in incubator for 30 min. at a temperature of 37° C. The incubating is done for the purpose of increasing the motility of the typhoid bacilli.

2. **Separation by Centrifugalization.**—Take tube from the incubator and centrifugalize for from 5 to 30 min. at a high speed. The non-motile bacteria will be thrown down first, while the

highly motile *Bacillus typhosus* will tend to remain near the middle and upper parts of the tube.

3. Cultural Separation on Basis of Motility.—By means of a sterile pipette take up the upper half or third of the contents of the centrifugalized tube (2) and place in the special loop tube with phenol-broth and incubate at 37° C. for 24 hr., or longer if necessary.

4. Plate Cultures.—Take up several platinum loopfuls from the loop tube (the opening opposite the inoculated end) and plant in lactose-litmus-agar (at 37° C.) and note the character of the colonies which form. Compare with the colon bacillus colonies. Examine colonies microscopically.

5. Other Cultural Tests.—Test for absence or presence of gas formation. Enrichment in liver broth may be tried, etc.

6. Agglutination Tests.—Two methods may be used. The microscopical and the macroscopical. The usual routine microscopical method is carried out as follows: By means of a clean sterile pipette place 0.1 cc. of the typhoid serum and 0.9 cc. of physiological salt solution (salt is necessary to bring about agglutination) in a clean sterile Syracuse watch crystal and mix thoroughly by means of a clean sterile glass rod. This gives a serum dilution of 1–10. Place one platinum loopful of a 24-hr. bouillon culture of the typhoid bacillus on a clean cover glass and add one loopful of the mixture from the Syracuse watch glass. This gives a dilution of 1–20. Two loopfuls of the culture and one of the serum mixture gives a dilution of 1–40. Three loopfuls of culture and one of serum mixture gives a dilution of 1–80. Make the dilutions one at a time and place the cover glass holding them (inverted) on a vaselined hollow or concave slide and examine at once under the high power, continuing the observation for 30 min. if necessary. The first change noticeable will be a gradual loss of motility, followed by a clumping of the now non-motile germs. This constitutes a positive agglutination reaction. Clumping with the lower

dilutions (1–20, 1–40) is not considered characteristic for the typhoid organism, since other bacteria may also produce agglutination with the typhoid serum. It is, however, not likely that sera will agglutinate other than the specific one in dilutions as high as 1–80. Higher dilutions should be tried on the principle that the positiveness of the test is in proportion to the serum dilution which will produce clumping. It should also be borne in mind that the agglutination phenomena are more marked at the body temperature (37° C.) and that in the case of the typhoid serum, the paratyphoid group will also give positive results. In reporting on the agglutinating phenomena always give the dilution and the time factors. The novice must frequently be reminded that all manner of solutions of salts, acids, etc., will produce agglutination with most bacteria. We would not recommend the use of the blood-counting pipette (which accompanies the hemacytometer) for making the dilutions and mixtures of the serum and the bacterial cultures, as is advised by some investigators, largely because of the danger of possible infection in sucking up the quantities of bacteria, and also because this method adds nothing to the value of the results.

For the so-called macroscopical method or precipitation method, as it is also called, small test-tubes are used in which the suitable dilutions of the serum (with normal salt solution) and the bacterial cultures are mixed. A positive reaction is indicated by flocculency and the deposition of a slight precipitate. Dead (formalized) typhoid cultures may be used. The method in general use in Germany is preferred, a description of which may be found in most text-books on bacteriology. Some of the American pharmaceutical houses (Parke, Davis & Co.) market a full equipment for making the macroscopic agglutination test with the typhoid germ. It contains full directions for using and according to reports is as reliable as this test can be made for practical purposes. It need hardly be stated that in all cases it is desirable to make a control test with normal salt solution.

The following is offered by way of fuller explanation of some of the details of the method above outlined for the isolation and identification of the *Bacillus typhosus*. The unusually active motility of the typhoid germ has been utilized by several investigators (Drigalski and Starkey) as a means for separating it from less highly motile forms. Drigalski allowed from 5 to 10 liters of the suspected water to stand in tall milk cans for 1 or 2 days at the room temperature, after which he plated definite amounts taken from the surface of the container into litmus-lactose-agar. By this method he was enabled to isolate typhoid bacilli from several contaminated springs. Starkey used glass tubes bent into four loops which after being filled with phenol broth were inoculated at one end and incubated anaerobically at 37° C. for 24 hr. The more actively motile typhoid bacilli found their way to the fourth loop from which they were isolated by plating. The centrifugal method above recommended is merely an adjunct to the methods employed by Drigalski and Starkey. The non-motile bacteria are thrown down first and in a very short period of time thus being an advantage over the Drigalski method in which gravity is the separating force. It is true that in time the motile forms would also be thrown down. It is therefore important not to prolong the centrifugalizing more than is necessary. In place of the four-loop Starkey tube we would suggest the use of four separate tubes; one a simple U-tube or single-loop, a W- or double-loop, a three-loop and a four-loop tube. These tubes, after being cleaned and sterilized are filled with phenol broth and inoculated at one end at the same time. Incubate at 37° C. or even at 40° C. and examine loopfuls taken from the ends opposite the ends inoculated as follows: The U-tube at the end of 6 hr., the double-loop tube and the three-loop tube at the end of 12 hr., the three-loop tube (reexamination) and the four-loop tube at the end of 24 hr., and the four-loop tube again at the end of 36 hr. if necessary. The phenol broth and the higher temperature hinders the growth of most bacteria without checking the growth of the

typhoid germs. These conditions will enable the highly motile *Bacillus typhosus* to reach the more remote loops first where they may be taken out by means of the platinum loop or the pipette.

In place of the loop tubes above described and which can be

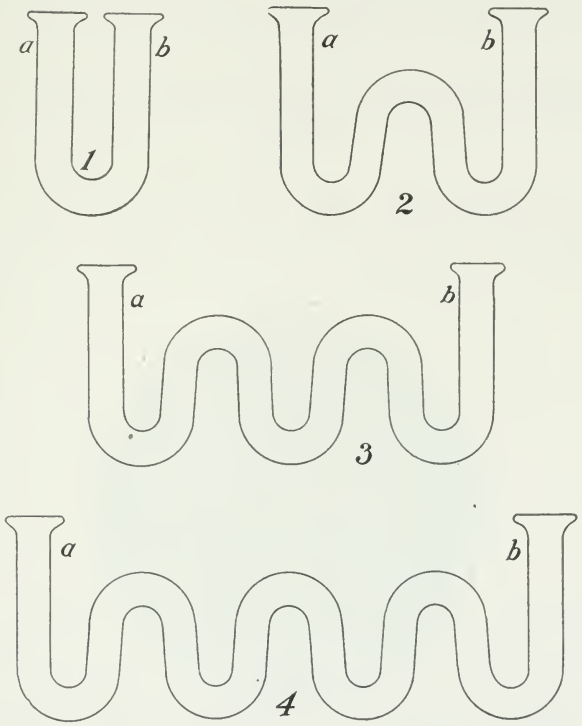


FIG. 33.—Loop tubes for culturing and isolating typhoid bacilli and other motile bacteria as explained in the text. 1, Single-loop or U-tube; 2, double-loop or W-tube; 3, three-loop tube; 4, four-loop tube. The tubes are filled with phenol broth or other desirable media and inoculated at the ends marked (*a*). Material for subculturing and for microscopical examination is taken from the opposite end (*b*), at varying intervals of time.

made in the laboratory, it would be preferable to use a single tube of four or five loops provided with openings at each of the upper turns of the loops, thus making five or six openings in all, from which the quantities to be examined and plated may be taken.

The tubes must be fastened to suitable stands or supports to prevent, as much as possible, the mechanical mixing of the contents after the inoculations are made. It is perhaps self-evident that concentrates or high contaminations are to be inoculated into the tubes. The tubes should be large enough to hold at least 50 to 100 cc. of medium and suspected water in equal parts.

12. Possible Contamination of Food Substances with the Cholera Bacillus

In the United States the contamination of foods with the cholera vibrio is far less likely than the contamination with the typhoid fever germ, yet it is a possibility to be reckoned with.

FIG. 34.—*Spirillum choleræ*, from broth culture, stained with fuchsin (× 1000).—(*Stitt, after Kolle and Wassermann.*)

The cholera germ is found in the feces (but not in the urine) of patients and in the feces of carriers, in which regards it resembles the typhoid bacillus. It is less resistent than the typhoid organism, disappearing rapidly from the stools, usually in 5 to 10 days. Under certain conditions (as in fresh water supplies) the infection may endure for longer periods, for several months and more. Like the typhoid germ, it shows some marked tend-

encies to locate in the bile duct or gall-gladder, where it may remain dormant for a long period of time. This observation has led to the use of bile as an enriching medium for both organisms.

The cholera vibrio work in the food and drug laboratory may resolve itself into the isolation of the germ from water supplies, from vegetables and possibly from feces and from sewage, and consists in the use of special culture media, special cultural methods, inoculation methods and agglutination tests. It is interesting to note that the method now in use for isolating the cholera vibrio from water supplies is the original Koch method, done as follows. Add 1 per cent. each of peptone and salt to 100 cc. of the suspected water and incubate at 38° C. Examine microscopically at intervals of 8, 12 and 18 hr. As soon as curved and comma-shaped organisms appear, plate on agar and make such additional tests as may be necessary to prove the presence of the cholera germ, such as the nitroso reaction, agglutination test, Pfeiffer's phenomenon, etc. It is not practical to enter into a fuller discussion of the subject. More complete details will be found in the works on bacteriology and in bulletins and reports on bacteriology and on hygiene. For example, the U. S. Public Health Service has worked out a quick routine method for isolating the cholera germ from feces, used in the U. S. Quarantine Service and at the quarantine station of New York, as reported in the Journ. of the Am. Pub. Health Association (Dec., 1911) and a condensed summary of the general methods may be found in the admirable little work by Stitt (Practical Bacteriology, Blood Work and Parasitology, 1913). Numerous special reports will be found in American and foreign bacteriological literature.

FIG. 35.—*S. cholerœ* showing involution forms (× 1000).—(*MacNeal, after Van Emengen.*)

13. Biological Water Analysis

The complete biological analysis of water supplies is, as a rule, not a regular routine of the food and drugs bacteriologist, yet he should be prepared to make such analysis when occasion makes it necessary. The food bacteriologist will have to do more with the analysis of sewage contaminated water supplies and with foods and other substances which have come in contact with such contamination.

The complete biological analysis of water supplies may be outlined as follows the fuller details of which may be found in special text-books, bacteriological journals and reports on water analysis.

Securing the sample.
Bacteriological examination.
  Quantitative.
  Qualitative; the presumptive colon bacillus test.
Algæ; significance of.
  Diatoms.
  Desmids.
  Nostoc and oscillaria.
  Other algæ.
Molds and spores; significance of.
Ova and larvæ of higher parasites; significance of.
Sand, dirt, etc.

The water supply of a city or community should be watched at all times, but perhaps more particularly in the early spring when the melting snows and the heavy rains bring in materials accumulated and held back during the winter months. Furthermore, the rise in temperature encourages the rapid multiplication of various organisms, such as algæ and bacteria. In late summer and early fall the drinking water often becomes vitiated, through a reduction in supply, perhaps as the result of lack of rainfall. In the early spring, after the first days of warm weather, the water supply often becomes murky due to dirt washed in, green in tint due to the enormous development of algæ and generally accompanied by a decidedly disagreeable odor which is traceable to the presence of

blue-green algæ of the Nostoc and Oscillaria groups. Various more or less futile attempts are made by the water companies to correct these conditions. In order to reduce the growth of algæ the reservoirs are roofed over (the algæ requiring sunlight for their development), forgetting that while one evil is thus in a measure corrected, another and perhaps greater, is encouraged by such procedure, namely, the growth and development of bacteria which thrive best in the absence of sunlight. Numerous desmids, di-

FIG. 36.—*S. choleræ* very highly magnified, showing flagellæ.—(*MacNeal, from Kolle and Schürmann.*)

atoms and blue-green algæ in drinking water, indicate the presence of dead and decaying organic matter in comparatively large amount. Diatoms are especially abundant in water supplies from old wooden tanks and wood-lined reservoirs. Nostoc and Oscillaria are especially abundant in water supplies fed from soil drainage. Bacteria are present in all soil and sewage contaminated waters. The well water of the farms may be contaminated with all manner of organisms, such as sewage organisms and disease germs, includ-

ing the larvæ of Nematodes and the spores of fungi, to say nothing of dead and decayed animals as mice, rats, rabbits, frogs, etc.

In many instances the contamination (by bacteria and algæ) of the water supplies of cities and towns is so extensive as to make direct counting easy. We hereby give the report of the microscopical examination of a sample of water from a Berkely (California) reservoir, analyzed in March, 1912.

Diatoms	1,500,000 per cc.
Desmids	860,000 per cc.
Oscillaria filaments	125,000 per cc.
Bacteria	16,500,000 per cc.
Paramecia	60,000 per cc.
Spores	5,000 per cc.
Hyphæ of fungi	460 per cc.

The water was at the time decidedly greenish in tint with a disagreeable odor, due to the numerous algæ present. Water showing such a high and varied biological count shows surface seepage and indicates sewage contamination and is not fit for drinking purposes, yet the biologist for the water company declared it good and harmless. The only interpretation that can be put upon a count such as the above is that the water supply is dangerously contaminated. Diatoms and desmids feed upon dead and decaying vegetable matter. Oscillarias occur in wet soils rich in humus. Paramecia feed upon decaying organic matter. The molds likewise are proof of the decay of organic matter, animal and vegetable. In all cases of evidence of surface seepage, sewage contamination may be suspected and all sewage contaminated drinking waters are a menace to the public health.

In no case should the examination of concentrated (1000 cc. reduced to 10 cc.) and centrifugalized sediment be omitted, as this will perhaps reveal contaminations which might be overlooked in the direct examination. Nor must the presumptive colon bacillus test be omitted when there is the least indication that sewage contamination exists. In case of slight but suspicious contaminations

the colon bacillus test should be supplemented by the plate count and the examination of the centrifugalized sediment.

Although the bacteriological examination of water supplies is the work of the sanitarians, the food bacteriologists are frequently called upon to pass judgment on the potability of water supplies. There is no definite numerical standard for drinking waters. In the United States the presence of the colon bacillus is almost wholly the basis for condemnation, it being assumed that if bacteria are present in great numbers the colon bacillus is also generally present. This is, however, very frequently not the case. Distilled water may contain numerous bacteria without any colon organisms. Stagnant waters may contain bacteria in great numbers without colon bacilli. It is not practicable to adopt an arbitrary numerical limit as has been suggested by various investigators. Miquel (1891) suggested the following standards:

10 bacteria per cc. or less	Excessively pure
10–100 bacteria per cc.	Very pure
100–1000 bacteria per cc.	Pure
1000–10,000 bacteria per cc	Mediocre
10,000–100,000 bacteria per cc.	Impure
100,000 and more bacteria per cc.	Very impure

German sanitarians generally recognize a limit of 50 to 300 for drinking water. Dr. Sternberg of the Public Health Service (1892) suggested that less than 100 bacteria per cc. indicated a deep source of the water supply and uncontaminated by surface drainage and that a water supply with 500 bacteria per cc. was open to suspicion and that 1000 and over is presumptive indication of sewage contamination or of surface drainage. It is quite evident that there is very little excuse for the use of city and other communal drinking water supplies with a count higher than 5–10,000 per cc., and it is suggested that this be made the numerical limit for drinking water in the absence of or irrespective of the presence of the colon bacillus.

The general routine for making the tests for the presence of the

colon bacillus has already been explained. It is suggested that 1 cc., 0.10 cc. and 0.01 cc. quantities of the water be run into fermentation tubes with lactose-bile medium, making five sets of these tube cultures, and incubate at 37° C. for 48 hr., noting possible gas formation. Gas formation indicates sewage contamination. If the gas is formed quickly, in 6 to 12 hr., the contamination is probably recent, if more slowly, 24 to 36 hr., the contamination is probably older. Gas in the 0.01 cc. quantities or less, indicates very high sewage contamination, gas in the 0.01 to 0.10 cc. quantities indicates serious contamination, and condemnation of the water supply for drinking purposes may be based on the presence of gas formation in two out of three tubes containing 0.10 cc. quantities, or three out of five of the 1 cc. quantities, also taking into consideration the rate of gas formation and the numerical plate count as well as the findings based on the direct microscopical examination. In brief, condemnation of water supplies intended for drinking purposes must be based upon the judgment of a competent sanitarian, one who comprehends the significance of the findings in relation to the source of the water supply and the sources of the contaminations. It is not practicable to lay down hard and fast rules. Each case must be considered by itself. In one instance the gas formation may develop in 0.3 cc. quantities (three out of five tubes containing 0.10 cc. quantities) or even in 0.10 cc. quantities and yet the water may be considered potable, as might be the case in deep well water into which street and road dust is carried, or which might contain surface drainage from field or garden. Again the water may be quite unfit for drinking purposes with colon bacilli in 10 cc. or in 100 cc. quantities, as might be the case in wells or springs highly contaminated with old or much weathered sewage contamination.

14. Bacteriological Examination of Mineral Waters

The bacteriological analysis of bottled waters is very important because it is an efficient means of ascertaining the conditions at the

bottling establishments. A general opinion prevails that mineral waters are free from germs, due to the germ-destroying properties of the mineral salts present. This is not the case. Many mineral waters from contaminated sources or from unsanitary bottling establishments contain bacteria in large numbers, 300,000,000 per cc. and more. Even a medicinal water composed of concentrated ocean water (Magpotine) gave a count of 10,000 bacteria per cc. The Bureau of Chemistry has found mineral waters contaminated with sewage. Often the contamination is traceable to the inadequate cleansing and sterilizing of used bottles and to the dirty hands of those employed in the factory.

The bacteriological examination of mineral waters consists in making the presumptive colon bacillus test and in making bacterial counts by the plating method. It is, however, also desirable to make direct microscopical examinations, including quantitative cytometric counts of concentrates (1 liter quantities reduced to 10 cc.) and of centrifugalized samples, as already explained. This will give information regarding factory conditions which could not be ascertained by the usual plating methods.

In the case of bottled mineral waters, the securing, handling and shipping of samples is a very simple matter as no extra precautions and care are necessary. In the case of water from mineral springs or artificial waters in bulk, the securing of samples for examination must be done carefully to guard against outside contamination. Containers for samples must be clean and sterile and as soon as the sample is taken the container must be closed with a sterilized cork or other suitable stopper, sealed and taken to the laboratory by the shortest route for immediate examination. If the samples are to be transported long distances or if for any other reason, the examinations must be postponed for from 6 hr. to several days, the sample must be kept on ice during the entire period.

Mineral waters are or should be quite free from bacteria and other contaminating organisms. As yet no standards have been adopted as to the maximum number of bacteria and other organ-

isms permissible. The only quality test made by the Bureau of Chemistry is for the colon bacillus.

15. The Microscopical and Bacteriological Examination of Milk

It is not practicable to enter into a discussion of the dairying industry or the multitudinous factors which cause modification of the quality of cow's milk. These are matters which concern the food bacteriologist but little. Bovine diseases, inclusive of tuberculosis, must be left to the veterinarian and the making of dairy products concern the manufacturer primarily. By this it is, however, not intended to imply that the food bacteriologist need not have intimate knowledge of cattle diseases and of dairying methods. Not only should he be well informed regarding these things but he should be qualified to examine cattle for diseases, tuberculosis in particular, and should be prepared to examine and report upon the sanitary conditions, equipment and the moderness of dairying establishments. However, the chief efforts of the food bacteriologist are devoted to the examination of the milk and dairying products as they appear upon the market.

For the present purpose it will suffice to give a mere outline of the methods of examining and testing milk microscopically and bacteriologically. The report of the analysis should comprise the following.

Securing the sample.
Sealing the sample.
Keeping sample on ice until ready for examination.
Examining the sample.
  Direct examination.
    Determining the fat content by the microscopical method.
    Quantitative determination of
      Bacteria.
      Epithelial cells.
      Blood corpuscles.
      Pus cells and leucocytes.
  Plate cultures.
    Presumptive colon bacillus test.
    Numerical count.

Milk may be described as a uniform suspension of fat globules in an aqueous solution of milk-sugar and casein. The fat globules represent the so-called butter fat of the milk. They are fairly uniform in size, very uniformly distributed and under ordinary conditions do not tend to coalesce or clump. Pasteurization and boiling the milk does cause some of the globules to unite or rather to form aggregates but even in such cases it is possible to recognize the individual globules.

On mounting a droplet of diluted milk (1–150 to 1–200) on the hemacytometer it will be found that the fat globules soon rise to

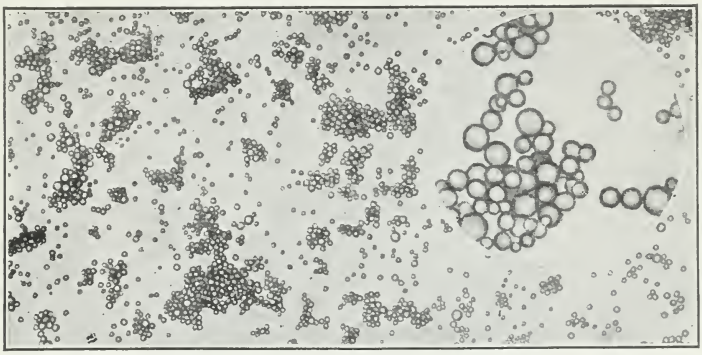


FIG. 37.—Milk fat globules. Larger field as they appear under the low power of the compound microscope (× 80), globules in the corner circle as they appear under the high power (× 500).—(*Hunter, after S. M. Babcock.*)

the top while the heavier particles, such as bacteria and body cells, sink to the bottom of the cell, thus separating these elements automatically, and making the counting of globules and bacteria possible in the same mount by simply focusing sharply upon the fat globules or upon the bacteria as may be desired. Some difficulty in making the counts is caused by the fact that the oil globules are out of focus when the rulings are in focus, making a constant shifting of focus from fat globule to lines and *vice versa* from lines to fat globule, necessary. Not only is this annoying but it makes accurate counting difficult. This difficulty can be overcome by com-

bining the use of an eye-piece micrometer scale with that of the hemacytometer, and it is suggested that such a combination be used, not only for milk examination, but also for making many of the cytometric counts of food products.

A practical method for determining the fat content of milk by means of the compound microscope was worked out in the bacteriological laboratory of the California College of Pharmacy. The procedure is as follows: Make dilutions of the milk from 1–150 to 1–200, using distilled water or normal salt solution (0.6 per cent.) and count the fat globules by means of the hemacytometer or the special counter above suggested. Numerous counts made have shown that 578,100,000 fat globules in 1 cc. of milk corresponds to 1 per cent. of butter fat. This number was obtained by comparing the fat globule count with the fat determination by the standard chemical method (combined with the use of the centrifugal machine). The following are a few comparisons as they were obtained in the laboratories of the California College of Pharmacy.

1,383,000,000 fat globules per cc. corresponded to 2.30 per cent. of butter fat.
933,000,000 fat globules per cc. corresponded to 1.60 per cent. of butter fat.
566,000,000 fat globules per cc. corresponded to 1.10 per cent. of butter fat.
470,000,000 fat globules per cc. corresponded to 0.80 per cent. of butter fat.

Dividing the sum total of the several counts of fat globules made by the sum total of the corresponding percentages of butter fat, gives 578,100,000 the average number of globules in 1 cc. of milk corresponding to 1 per cent. of butter fat. From this it will be seen that in round numbers, 2,000,000,000 fat globules per cc. represent a fair quality of milk, that is, milk having somewhat over 3.50 per cent. of butter fat. According to comparative tests made, the microscopical method is fully as accurate and reliable as the chemical methods. The microscopical method is not recommended for routine procedure in dairying establishments but it is certainly a most valuable adjunct to the food laboratory methods. It could at all times be employed as a substitute for the chemical fat determination if for any reason the latter method

is not applicable. Thus, it can be ascertained microscopically whether or not water has been added to the milk or if it is full milk, half milk or skimmed milk.

The bacteriological standardization of milk has received much attention within recent years and all civilized countries have adopted certain numerical limits of bacteria permissible in wholesome milk. Unfortunately, however, there is very little uniformity regarding these numerical limits in different countries or in different parts of the same country. In some cities and communities

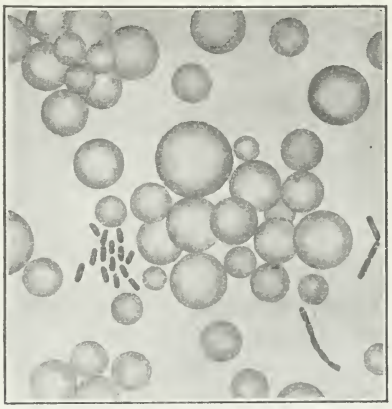


FIG. 38.—Milk fat globules very highly magnified (× 1000). A group of lactic acid bacteria at the left.—(*Hunter.*)

there are two standards, a summer or low (numerical limit higher) standard and a winter or high (numerical limit lower) standard. The terms summer and winter are, however, misleading in certain areas of the United States and, for regulatory purposes, it would be better to base the standards on a temperature differential, irrespective of season, combining this with a sliding scale of bacterial count. Under such a plan the Southern States, including the immediate Pacific Coast region, would be under a single standard, namely, the lower or so-called summer standard. The rest of the United States would have both standards.

The following is a tentative standard based upon the temperature differential as above suggested.

Standards	Number of Bacteria per Cc.			
	Ordinary Milk	Certified Milk	Inspected Milk	Cream (Fresh or Unripened)[1]
Temp. from lowest to 60° F. Winter standard	30,000 to 50,000	3,000 to 8,000	12,000 to 15,000	30,000 to 5,000,000
Temp. from 60°F. to highest. Summer standard	50,000 to 100,000	8,000 to 15,000	15,000 to 30,000	5,000,000 to 150,000,000

It is not practicable to fix a numerical bacterial limit for creams. Tests made show that the count varies within wide limits, even in cream from milk which has been kept under the most favorable sanitary conditions and surroundings. Fresh creams, that is, the cream removed from the milk as soon as formed, usually within 24 hr. after the milk is drawn, contains comparatively fewer bacteria than the cream which has been set aside to ripen. The ripening process is far from objectionable, in fact it is encouraged and regulated in the well-conducted dairying establishments in order to develop the desirable butter flavor. Most of these flavoring lactic acid bacteria are removed in the process of butter making, being drawn away and worked out with the buttermilk, only comparatively few remaining in the butter itself.

Taking milk samples is not unlike water sampling. Milk should be examined not later than 6 hr. after being drawn. If it cannot be examined within that time it must be kept on ice but in no case should the examination be made later than 12 hr. after the milk was drawn.

Body cell counts should not be omitted and proper judgment should be exercised in interpreting the findings. Body cell counts

[1] Ripened cream contains numerous lactic acid bacilli, 300,000,000 per cc., and even more.

give most valuable information regarding the health condition of the cows and will serve to indicate the danger point as to the usability of the milk. It is not practicable to give exact numerical limits at the present time. Further investigation is necessary to this end. However, the following suggestions will be of great value to the analyst in arriving at a better estimate of the quality of the milk under examination.

Epithelial cells few (1000 per cc.), of no significance.

Epithelial cells many (5,000,000 per cc. and more), indicates some irritation or serious inflammatory condition of udder or in milk ducts.

Epithelial cells many with some pus cells, danger. The diseased animal should be found and removed from the herd.

Pus cells few, indicates some slight abscess formation which should be treated if possible.

Pus cells many (5,000,000 per cc. or more) indicates danger. The diseased animal should be removed from the herd.

Blood corpuscles few, no special significance. Probably due to some slight injury resulting in capillary hemorrhage.

Blood corpuscles many. Indicates some mechanical injury which requires attention.

For practical purposes it is not advisable to attempt to distinguish between leucocytes and pus corpuscles. Numerous leucocytes indicate some serious inflammatory condition while numerous pus cells indicates abscess formation perhaps following a more severe inflammation.

Various methods have been submitted for making the body cell counts. That by Prescott and Breed is perhaps the simplest and also the most practical. It is carried out as follows. Spread 0.01 cc. of the milk on a glass slide[1] over an area of 1 sq. cm., evaporating the milk to dryness using moderate heat. Next dissolve out the butter fat by means of xylol, fix with alcohol, again dry, and stain with methylene blue. Decolorize partially with alcohol and examine under the compound microscope. The body cells in the entire area of the mount are counted and the

[1] The ruled slide elsewhere described (*D*, Fig. 5) will be found very useful for counting body cells in definite quantities of the milk.

entire number found multiplied by 100 gives the number of body cells per cc. of the milk. Prescott and Breed have examined numerous milk samples and declare that the average number of body cells is 1,500,000 per cc. and that a count as low as 100,000 per cc. is uncommon.

Little can be said regarding the microscopical and bacteriological examination of butter, cheese, cream and other factory products.

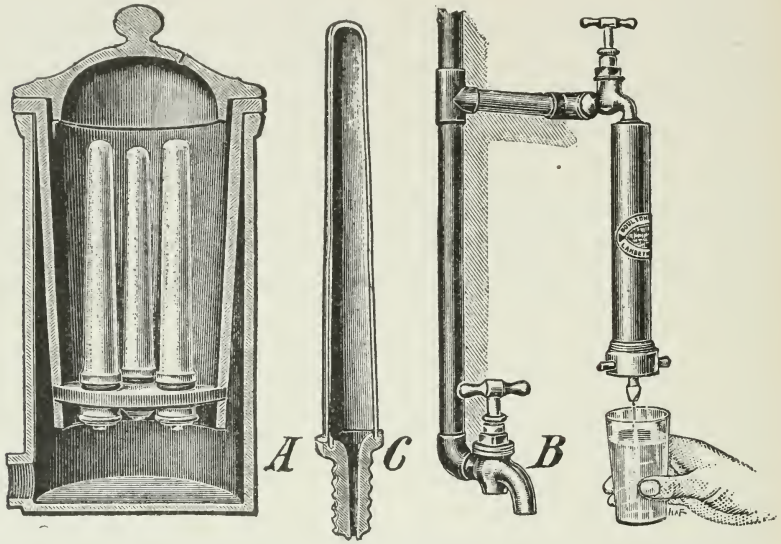


FIG. 39.—Unglazed porcelain filters. Chamberland system; *A*, without pressure; *B*, fitted to main water supply; *C*, section of a porous porcelain filter.

There are no bacterial standards and the laboratory work is very largely limited to the detection of adulterants such as excess of salt, of water and the presence of lard and oleomargarine in butter, fillers in cream and in ice cream, etc.

The following simple tests will be found useful in the laboratory:

1. **Spoon Test for Oleomargarine and Renovated Butter.**—Melt a small piece of the suspected butter in a tablespoon or small dish, using a small flame. Stir the melting substance with a small piece of wood such as a tooth-pick or match. At a

brisk boil, oleomargarine and renovated butter will sputter very briskly and noisily without foaming. Genuine butter boils less noisily and with abundant foam formation.

2. **Fat Cohesion Test.**—Fill a medium beaker about half full of sweet milk (preferably skimmed) and heat to within near the boiling point. Add about 5 grams of the sample and stir until completely melted. Remove from the fire and place beaker in ice water. When the fat begins to congeal, stir with a small piece of stick. Fat or oleomargarine will collect in one mass or lump at the end of the stick, whereas pure butter granulates and will not adhere to the stick. This test is not applicable to renovated butter which behaves like unrenovated or fresh butter.

As is generally known, milk is an excellent culture medium for a great variety of bacteria. For a time after the milk is drawn, bacterial development is checked by the bacterolytic properties which all fresh milk is said to possess. These lysins, however, gradually grow less and less until there is no longer any evidence of their existence.

Milk bacteria may be grouped into the acid formers, digesting bacteria and those which appear to have but little effect on the appearance of the milk. The acid-forming group is a large one and includes the true lactic acid bacteria which are carried into the milk from stable dust and other dirt in and about the stables and elsewhere. The initial bacterial changes in the milk are, however, not produced by the acid formers, but rather by those bacteria which decompose proteids, to which belong the *B. subtilis* and its aerobic allies. *Streptococcus acidi lactici* ferments both proteids and lactose as does also *B. coli communis* and some of its allies. In a short time, however, the true lactic acid bacteria multiply in such large numbers as to crowd out or almost completely check the development of the other species. They transform the lactose into lactic acid. On longer exposure, *Oidium lactis* enters from the atmosphere which fungus begins to decompose the lactic acid and some of the remaining proteids, having the effect of lowering the acidity which again encourages the renewed multiplication of the lactic acid group. This alternating preponderance of lactic acid bacteria and higher fungi continues until the proteids and the milk sugar are almost completely used up. Butyric acid

bacteria may enter the milk causing the very characteristic fermentation changes resulting in the formation and liberation of butyric and propionic acids from the splitting of lactose. Butyric acid milk has a very disagreeable odor. Various bacteria cause diseases of milk as blue milk and ropy milk.

In some American cities the routine examination of milk for *B. coli* is regularly adopted. The results in Baltimore have shown the presence of this bacillus in 25 per cent. of 0.001 cc. quantities of the milk in the winter time and 75 per cent. during the summer. It would appear that three positive tests out of a total of five from 0.001 cc. quantities of milk, would indicate the danger point as to quality. For making plate counts of milk bacteria, lactose-litmus-agar should be used in order to differentiate between acid formers and non-acid formers.

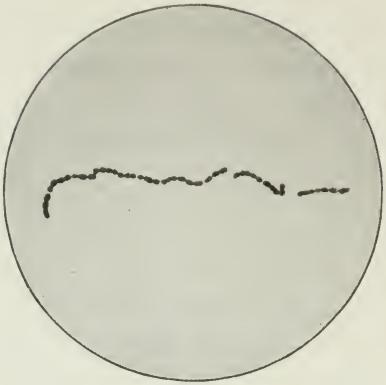
In most communities the milk streptococci are considered objectionable, as they belong to the group of pus-forming organisms. It is frequently found that a high streptococcus count goes with a high leucocyte count and the two are corroborative of the existence of some severe inflammatory condition of the udder or milk ducts. There is fairly conclusive evidence that the hemolytic milk streptococci are frequently causative of more or less severe and even fatal intestinal diseases among children, especially during the hot summer weather. It is also fairly well proven that some of the throat and mouth infections of children are traceable to the staphylococci and streptococci of milk. The problem of tuberculous milk is of lesser importance to the food bacteriologist because the health authorities of the land have this matter under jurisdiction. It is criminally unlawful to market milk from tubercular cows. Ravenel states that approximately 20 per cent. of the clinical cases of tuberculosis are of the bovine type and milk from tuberculous cows continues to be a very serious menace to the public health. It would be of the greatest value if some simple and practical micro-chemical laboratory test for tuberculous milk could be worked out. We would suggest this as one of the very

important problems to be undertaken. It is evident that the control exercised by the health authorities, while it has accomplished much, is not sufficiently stringent or far-reaching to stamp out tuberculosis in cows.

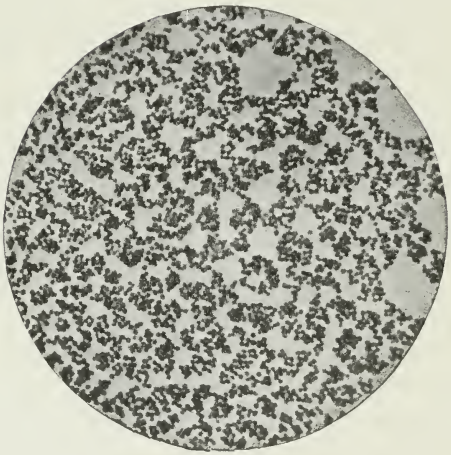
A milk test much used in Holland and other European countries is to ascertain the amount of gas formation in a unit of time, in a fermentation tube containing a mixture of definite quantities of milk and hydrogen dioxide. The amount of gas liberated is directly proportional to the amount of organic matter (bacteria, body cells and other organic impurities) present. Tests made in the laboratories of the California College of Pharmacy and in the laboratories of the San Francisco Board of Health would indicate that the method gives uniform results and that such a method would prove a most valuable addition to the routine milk examination, serving as a check and confirmation of the bacterial and body cell counts. In order that the test may yield uniform results in all laboratories, a uniform method of procedure must be adopted. The following tentative method is suggested.

A standard 10 per cent. volume (of available oxygen) solution of hydrogen dioxide should be used. The peroxide should be standardized to the specified quality. For determining the valuation of the peroxide we would recommend the Planés colorimetric test, made as follows. Dilute the dioxide to be tested with nine parts distilled. water. To 5 cc. of this solution (1–10) add 3 cc. of a 10 per cent solution of potassium iodide and 1 cc. of 8 per cent. sulphuric acid, in a standard test-tube. The color produced is matched against a n/10 iodine solution in a second standard test-tube. 1.8 cc. of the standard solution is equivalent to 1 cc. of oxygen.

Into graduated fermentation tubes with slender arms, having a capacity of 25 cc., run 10 cc. of milk and 10 cc. of the standard hydrogen dioxide, mix well in the bulb and at once run into the arm, excluding all air from the upper end of tube. Set aside in the incubator for 1 hr. at a temperature of 20° C. and record the amount of gas formed at the end of this period.



A



B

FIG. 40.—*Streptococcus* (*Staphylococcus*) *pyogenes* and *S. aureus*. There are three principal species of Streptococci (*S. pyogenes albus*, *S. pyogenes aureus* and *S. pyogenes citreus*), similar in form and appearance, concerned in pus formation, as in wound infection. These organisms are very widely distributed in soil and air.

Note the chain form arrangement of the cocci in *A*. *B* is a smear preparation.—(*Stitt* (*A*) *and Pittfield* (*B*).)

The following quick and simple test is recommended to distinguish between raw and boiled milk!

REAGENT

Methylene blue (alcoholic)	5 cc.
Formaldehyde (40 per cent.)	5 cc.
Water (distilled)	190 cc.

Add 1 cc. of this reagent to 20 cc. of the milk and heat for 10 min. at 40°–45° C. Raw milk is decolorized while boiled milk retains the blue coloration. This test should in all cases be checked by the microscopical examination. Boiling the milk causes the fat globules to unite and adhere more or less, a characteristic which is also noticeable in pasteurized milk. The flavor and odor of boiled milk is in itself quite characteristic.

FIG. 41.—Gelatin culture of *Staphylococcus aureus* 1 week old.—(*MacNeal.*)

Knapp recommends the following test for determining the addition of water to milk. 10 cc. of the suspected milk are run into a test-tube and curdled by adding one drop of rennet and placing the tube in the water bath for about 2 min. at a temperature of 35°–40° C. The whole is then poured upon a very fine wire sieve and the liquid allowed to drain off into a tube graduate, pressing the curd with a glass rod so as to remove the liquid as completely as possible. The amount of liquid remaining in the curd is fairly constant in the tests and therefore practically negligible for comparative purposes. If the amount of liquid drained off exceeds 8 cc., water has been added. This test should be checked by the chemical butter fat tests and also by the microscopical method for determining the fat content, as already explained.

Among the micro-organisms which cause the coagulation of milk and which are often found in sour milk, particularly in old sour

milk, is the *Streptococcus lacticus* of Kruse. The *Bacillus* (*lactis*) *aërogenes* which is very closely similar to *Bacillus coli*, also sours milk and is likely to be present at the beginning of the fermentation. The common pus streptococci and staphylococci are often found in milk in large numbers, traceable to dirt and filth and to diseased udders and less commonly to the hands of the milkers. The colon bacillus when present is traceable to stable dust and manure and to the unclean hands of the milkers.

The following are some of the organisms which cause diseases of milk:

1. *Bacillus cyanogenes*—Blue milk.
2. *Bacillus prodigiosus*—Red milk.
3. *Bacillus erythrogenes*—Red milk.
4. *Bacillus synxanthus*—Yellow milk.
5. *Torula amara*—Bitter milk.
6. *Streptococcus hollandicus*—Ropy milk.

Naturally the bacilli normally present in the milk which is stored for cream formation are also present in the cream after the skimming and cause the so-called ripening of the cream. In order that the ripening process may proceed in a desirable manner, the objectionable butyric acid formers must be excluded. The butyric acid formers are more generally associated with filth, hence, a careful compliance with sanitary rules and regulations in the dairying establishment will generally encourage the invasion and development of the desirable lactic acid organisms to the exclusion of the undesirable microbes, though this is by no means always the case. Occasionally, even with the most scrupulous adherence to sanitation, cream will not ripen properly and these occasional failures have prompted the more progressive dairymen to inoculate the milk and cream with pure cultures of the desirable cream ripening bacilli. Others use natural cream starters, that is, small quantities of old cream which has ripened with a desirable flavor.

Cream should not show colon bacilli in less than 0.10 cc. quantities and fresh unripened cream should not contain more than 5,000,-

000 bacteria per cc. Ripened cream should not contain more than 150,000,000 bacteria per cc. and most of which bacteria should be of the lactic acid group. Pathogenic bacteria which may be present in milk may also be present in the cream. Tubercle bacilli, diphtheria bacilli and typhoid bacilli are the most likely to occur. In the case of doubtful cream, the colon bacillus test should not be omitted and in the case of suspected contamination with pathogenic organisms, the cream, as well as the milk from the same source, should be examined, resorting to the usual animal inoculation tests.

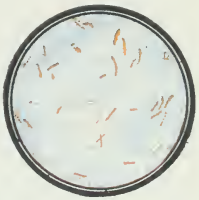


FIG. 42.—Tubercle bacilli in sputum. Stained with carbol-fuchsin and methylene blue.—(*Pittfield.*)

The tests for the presence of tubercle bacilli in milk, cream, meats, etc., comprises the microscopic examination of stained (Ziehl-Neelsen method of staining) sediments or concentrates as may be required, and animal inoculations. For the animal inoculation test, guinea-pigs are used. Centrifugalize (in a powerful machine) about 250 cc. of the milk in order to throw down the tubercle bacilli (with the other inclusions), and from this make the desired cover-slip preparations and inoculate (in the region of the left knee-joint of hind leg) the remainder of the sediment into three healthy guinea-pigs. Place the inoculated guinea-pigs in individual cages and keep them under observation for from 2 to 4 weeks. The reasons why several pigs should be inoculated are as follows. Some of the pigs may be killed by bacteria other than the tubercle bacilli and it is always desirable to duplicate the tests. At the end of the second week, one of the guinea-pigs should be dissected and the glands of the sublumbar region as well as the glands of the superficial tissues and of the popliteal region examined. If tubercular infection has taken place, these glands will be found much enlarged containing foci of tubercle bacilli. The enlarged glands are dissected and suitable cover-glass preparations made therefrom. If the evidence of tubercular infection

FIG. 43.—Tubercle bacillus slant culture on glycerin-agar, several months old.—(*Stitt, after Curtis.*)

is not conclusive, the other two inoculated guinea-pigs should be kept 2 weeks longer then dissected and examined like the first. Occasionally there is abscess formation at the point of inoculation but this need not necessarily interfere with the tubercular development in the glands and in the deeper tissues.

It is frequently possible to isolate the bacillus of tuberculosis (from sputum, glandular tissues, meat pulp, centrifugalized sediments of milk, cream, etc.) by special manipulation and the use of special culture media. The following method is suggested. Spread two or three drops of the material (concentrate, sediment, crushed, suspected tuberculous meat extract, etc.) evenly over the surface of two or three glass slips and place the smear preparations in the drying oven at 100° C. for 15 min., however, not before the material on the slips is well dried at the room temperature. Tubercle bacilli are quite resistant to dry heat and will withstand the temperature of 100° C. for from 30 min. to 1 hr. The exposure to that temperature for 15 min. will kill most of the bacteria associated with the tubercle germs and will in fact kill some of these. At the end of 15 min. take the glass slips from the drying oven and by means of a small sterile spatula or scalpel, scrape the dried suspected material over the surface

of the special medium in Petri dishes. The medium used (Hesse's agar) is made as follows:

Nutrose (sodium caseinate)	5 grams.
Sodium chloride	30 grams.
Glycerin	30 grams.
Agar	10 grams.
Na_2CO_2(crystalline) solution (28.6 per cent.)	5 cc.
Distilled water	1000 cc.

Mix ingredients. Heat until agar is dissolved. Filter through cotton. Pour into Petri dishes. Sterilize fractionally.

After inoculating two or three Petri dishes in the manner indicated, incubate at 37.5° C. in a moisture-saturated atmosphere for several days. If tubercle bacilli are present young colonies will appear which may be identified with a low power by the resemblance to broken wavy lines.

Instead of making the glass-slip smears as above suggested, good results may be obtained through the use of the cotton throat swabs such as are used by physicians for taking throat cultures in diphtheria cases. Dip or roll the cotton ends of three swabs in the suspected tuberculous material, suspend in air until perfectly dry and then place in drying over (100° C.) for 15 min., then rub the cotton over the surface of the special culture medium in Petri dishes. Make some six or seven parallel streaks over the surface of the medium. Incubate and examine as before. Should the glass-slip or cotton-swab preparations be placed in the drying oven before air drying them, all or nearly all of the tubercle bacilli would be killed in the drying oven.

Several investigators have recommended a direct method of examination for ascertaining the presence of tubercle bacilli in milk, and in other materials, through the use of agents which will completely dissolve all bacterial bodies excepting the acid-fast group of organisms to which the tubercle bacillus belongs. For this purpose antiformin (really a mixture of chlorinated sodium hypochlorite and Labarraque's solution) has been highly recom-

mended. This proprietary article is a strongly alkaline solution of sodium hypochlorite. In each cc. it contains approximately 5.68 grams of sodium hypochlorite, sodium hydroxide 7.8 grams and sodium carbonate 0.32 grams. The available chlorine amounts to about 5.68 grams. It dissolves all organic matter, such as that con-

FIG. 44.—*Bacillus tuberculosis* in the sputum of a consumptive; stained by Ziehl method (× 2100).—(*After Kossel.*)

tained in sputum and feces, excepting the tubercle bacilli. It is in itself an active antiseptic having a phenol coefficient of 3.

For bacteriological work, a 50 per cent. solution of the antiformin will be found satisfactory. Mix equal parts of the antiformin solution (50 per cent.) and milk or sputum or other mate-

rial supposed to contain the tubercle bacilli, in a suitable glass container and bring to a boil over the Bunsen burner. When the material is cool, add 1.5 cc. of a mixture of chloroform and alcohol (chloroform one part and alcohol nine parts) to each 10 cc. of the material and shake vigorously. The tubercle bacilli absorb some of the chloroform and become heavier than the rest of the organic matter. Next centrifugalize at a high speed for 15 min. which separates the material into three layers; the antiformin at the top, the sediment in the middle, and the chloroform with the tubercle bacilli at the bottom. Pipette off the layer of chloroform and examine for tubercle bacilli by resorting to the usual staining methods. The smear preparations can be made to stick to the cover or slide by mixing with serum or egg albumen solution. This method may also be tried in the examination of creams, cheese, buttermilk and butter. The strength of the antiformin solution should be graded according to the amount or percentage of organic matter to be dissolved, taking the strength required for sputum work as the highest. For milk work the 15 per cent. solution will be satisfactory. For cheese a 50 per cent. solution should be used, likewise for feces.

Stitt recommends the following antiformin method for culturing the tubercle bacilli. Mix 20 cc. of sputum, 65 cc. of sterile water and 15 cc. of antiformin. Stir with a glass rod. After a period ranging from 30 min. to 2 hr., the mixture should be homogeneous. Centrifugalize for 15 min. or longer, decant, and wash the sediment twice in sterile normal salt solution and smear out the well-washed sediment over serum or glycerin egg albumen or nutrose slants. It must be remembered that the tubercle bacillus will not grow in sunlight and that the colonies form on the surface of the culture media only.

Stitt also states that it is not wise to use the antiformin in solutions stronger than is necessary to dissolve the organic matter and bacteria other than the tubercle bacillus. For example for sputum, it is suggested that 20 or 25 per cent. of antiformin be

used. If stronger solutions are used, many of the tubercle bacilli are also disintegrated or considerably changed in form and in the behavior with the acid-fast stains.

In addition to the routine examination of ice creams for the presence of fillers and ingredients which do not properly belong to ice creams, the food bacteriologist will have occasion to make bacteriological and toxicological tests. According to Vaughan, the toxic changes in ice cream are due to the presence of a poison designated *tyrotoxicon*, presumably identical with the toxin occasionally found in milk and cheese. Ice-cream poisoning depends upon the development of the toxin-forming bacteria in the milk and cream before it is frozen. It is not at all likely that ice cream made from clean wholesome cream and milk will contain toxins, provided it is kept well frozen and is not stored too long. There is good evidence that slightly infected ice cream which is kept for several days and longer, may show sufficient toxic bacterial development to produce symptoms of poisoning. The virulency of the toxins produced by the bacteria appears to increase with the lowering of the temperature.

The danger from ice cream is directly proportional to the unsanitary conditions of the milk and cream used and ice-cream poisoning is far more likely to manifest itself during the hot summer weather. All suspicious ice creams should be examined bacterially, making numerical plate cultures and also the presumptive colon bacillus test and tests for streptococci and staphylococci. The toxicological test as recommended for meats is, however, far more important and should not be omitted. Ice cream should not contain more than 1,000,000 bacteria per cc. and should not develop colon bacilli in less than 0.10 cc. quantities by the standard presumptive colon bacillus test.

Of the more common ice-cream fillers we may mention starch and tragacanth. Vegetable mucilages other than tragacanth may be suspected. Gelatin is also used. Eggs are frequently added. A filler to which a small amount of rennet had been added has

been extensively advertised as an ice-cream producer which did not require the use of cream or of ice.

Occasionally it may become necessary to examine sour milk and buttermilk for the presence of toxins and objectionable bacteria and other undesirable organisms. Because of the careless and more or less promiscuous handling of buttermilk before it reaches the consumer, it is especially liable to the invasion of foreign organisms. The routine examination of buttermilk is largely limited to a direct microscopical inspection. Mold spores and yeast cells should be sparingly present and the predominating bacilli should be small[1] (lactic acid formers) and of irregular and rather indefinite outline. Mold and cocci should be very sparingly present.

To examine butter for the presence of bacteria (direct microscopical method) and other contaminations, place 1 gram of the butter in 10 cc. of ether and shake until all of the butter fat is dissolved. Pour the solution into the special centrifugal tube and centrifugalize for 5 min. Wash the contents of the 1 cc. end tube into 10 cc. of ether and again shake and centrifugalize. Pour off the ether and add 2 cc. of a 2 per cent. sodic hydrate solution and shake until the casein is dissolved. The sodic hydrate solution emulsifies the small amount of fat present. Examine the emulsion for bacteria, counting the bacteria and body cells by means of the hemacytometer.

Butter and cheese made from the milk of animals suffering from foot-and-mouth disease have transmitted this disease to humans. The bovine type of tuberculosis has resulted from the consumption of milk, cream and butter. Tubercle bacilli have been found in the more quickly ripened cheeses. Tubercle bacilli do, however, not survive long in soured cream or milk, perhaps not over 2 or 3 days.

The following are some of the more important organisms concerned in the ripening of cheese.

[1] The *Bacillus bulgarius* is comparatively large ($1 \times 6\mu$).

1. Lactic acid bacteria.—These are the chief agents concerned in the ripening of Cheddar, American and Edam cheese. Pure cultures of the *Bacillus acidi lactici* are often used as a starter. In the manufacture of the Edam cheese, slimy whey is used as a starter (*Streptococcus hollandicus*).

2. *Penicillium glaucum* the common green mold is the principle organism concerned in the ripening of Roquefort, Gorganzola and Brie cheeses. In some countries the green mold is scraped from molded bread and added to the curd.

3. A great variety of other bacteria, yeasts and mold are concerned in the development of the more specific flavors and aromas. Further investigation is necessary to ascertain the special function performed by each and the mutualistic relationship that may exist between them.

4. Gas generating bacteria are concerned in the formation of holes in the interior of the ripening cheese. These gas formers also modify the aroma or flavor of the cheese and in some instances constitute the chief ripening agents.

Spoiling of cheese is not uncommon, due to the invasion of a variety of undesirable organisms. The cheese "hopper" or "skipper" found in and upon old and overripened cheese and in cheeses which have not been properly screened, is the larva of the black two-winged fly *Piophila casei*. The insect deposits its eggs in the surface cracks and crevices of the cheese upon which the developing larva feeds. The name skipper or hopper is derived from the fact that the larvæ are capable of projecting themselves some distance by coiling and suddenly uncoiling.

This fly is a common pest in the dairying establishments. A less common but even more annoying pest is the larva of the "bacon beetle." Cheeses which are comparatively hard and smooth externally are not so likely to be infested by the skipper or bacon beetle larva as are the cheeses which are rough externally. It is customary to wipe the cheese in order to remove the parasites. If the cheeses which are stored for ripening are properly screened, the fly and beetle cannot get access to them to deposit the eggs. A small mite (*Trioglyphis siro*) also occurs on cheese upon which it feeds.

Inadequately screened cheeses also permit flies and other pests to deposit possible infections, thus typhoid contamination and also pus streptococci and staphylococci may be found upon this food substance.

Bitter cheese is due to a variety of bacteria, as *Tyrothrix geniculatus* (the bitter soft cheese bacillus), *Micrococcus casei amari* (bitter cheese coccus), Weigmann's bitter milk bacillus, Conn's bitter milk coccus, and others. Red coloration of cheese may be caused by yeasts (*Saccharomyces ruber*) or by cocci. Black cheese

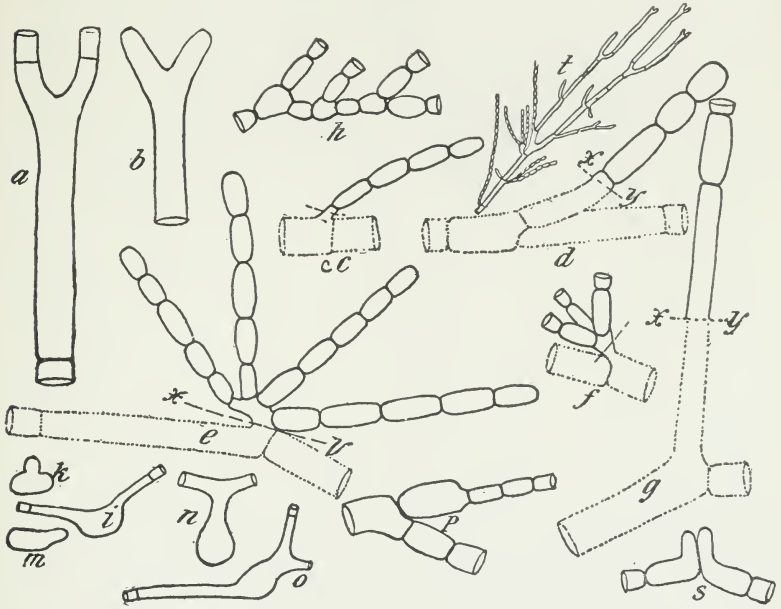


FIG. 45.—*Oidium lactis*. *a*, *b*, Dichotomous branching of growing hyphæ; *c*, *d*, *g*, simple chains of oidia breaking through substratum at dotted line *x*–*y*, dotted portions submerged; *e*, *f*, chains of oidia from a branching outgrowth of a submerged cell; *h*, branching chain of oidia; *k*, *l*, *m*, *n*, *o*, *p*, *s*, types of germination of oidia under varying conditions; *t*, diagram of a portion of a colony showing habit of *Oidium lactis* as seen in culture media.—(*From Bull.* 82, *Bur. Animal Industry, U. S. Dept. Agr.*)

may be due to the presence of iron in milk, perhaps traceable to the action of slightly soured milk in rusty buckets. Some yeasts and molds may produce dark to black decomposition changes. Blue cheese is the result of the action of a bacillus. Putrid cheese is the result of the invasion of saprophytic bacteria and other micro-

organisms. Cheese poisoning is not uncommon, due to the presence of bacteria which give rise to toxins (tyro-toxicon). In a general way it may be stated that cheese diseases are due to filthy and unsanitary conditions in the dairying establishment re-

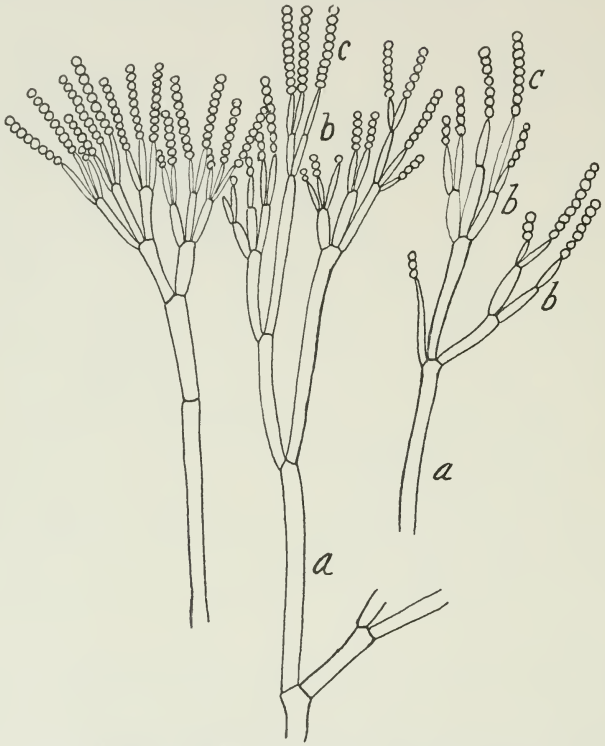


FIG. 46.—*Penicillium glaucum* showing the characteristic spore formation. This fungus is a true saprophyte, the common green mold, occurring on a great variety of organic substances.

sulting in infected milk, or to filthy and unsanitary conditions in the cheese factory, or the infection may be traceable to the improper and careless storing and handling of the cheese. Ripened cheese being in itself a decomposition product resulting from the invasion of certain desirable micro-organisms usually entering

from the air, it is but reasonable to expect irregularities in the final result unless the invasion of undesirable micro-organisms, which are also present in the air, is carefully guarded against.

Condensed milk is prepared by concentrating full or skimmed milk. It may be sweetened by adding cane sugar (40 per cent.). While condensed milk contains relatively fewer bacteria than does ordinary milk, due to the process of manufacture, yet none is entirely sterile. The number of bacteria usually present ranges from about 500 or even less to as high as 250,000 per cc. Colon bacilli, dysentery bacilli and streptococci are generally absent. Tubercle bacilli have been found. The method for examining condensed milk is much as for ordinary milk, with suitable modifications in making the dilutions.

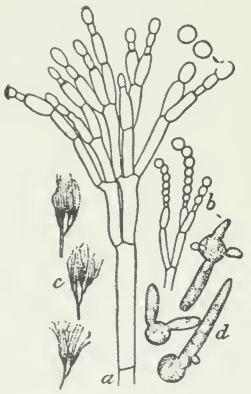


FIG. 47.—Penicillium of Camembert and Roquefart cheese. This mold grows at a very low temperature. It is closely similar to, if not identical, with *P. glaucum*.—(*Jordan after Thom.*)

Canned condensed milk occasionally spoils, due to the development of bacteria and yeast organisms. Yeast organisms are not likely to appear unless the milk is sweetened with sugar. Spoiling may become apparent through the "swelling" of the can. Organoleptic testing is occasionally a guide to the condition or quality of the milk. A numerical bacterial limit should be adopted for condensed milk. If more than 1,000,000 bacteria per cc. are present it is not suitable for human consumption. Tubercle bacilli should be absent. According to the limited reports on the subject we may assume that the process of condensing the milk kills all pathogenic bacteria which may be present, including even the more resistant tubercle bacilli. The contaminating bacteria may produce toxins and in marked bacterial invasion it would be well to make inoculation tests with white mice or guinea-pigs, as for toxins in meat and in ice cream. An examination of the centrifugalized

sediment must not be omitted as this will convey information regarding the sanitary conditions of the factory as well as of the dairying establishments which supplied the milk to the factory.

Dried or powdered milk is prepared by spraying milk (usually skimmed) into a partial vacuum or by spraying it on a revolving drum or on a moving belt in a partial vacuum. The dried material is then placed in suitable containers. The dried milk contains all of the ingredients of the milk excepting the water, the lysins and certain enzymes. The fat globules are altered physically but not chemically. Mixing dried milk with the required amounts of water makes a liquid resembling ordinary milk. The microscopical and bacteriological examination of dried milk is as for condensed milk. Like the condensed milk it is quite free from disease germs of all kinds but bacterial invasion is not excluded from material which has been carelessly prepared or canned. Toxins and ptomaines should be absent. The absence of moisture in powdered milk prevents the ready growth of micro-organisms and it may be kept in good condition for a period of 5 or 6 months and even longer, in dry sterile containers stored in a cool dry place.

Attempts have been made to commercialize frozen milk but so far without success. It is rather difficult to handle frozen milk and the article furthermore loses the milk flavor on thawing.

16. The Bacteriological Examination of Shellfish

The term shellfish includes oysters, mussels and clams. Only those species and varieties which serve as food for man are of interest to the food bacteriologist. Since it has been conclusively proven that shellfish, oysters in particular, have been responsible for typhoid epidemics, much attention has been given to the bacteriology of this class of food. In tracing such epidemics it was discovered that the causative oysters had been floated or grown in heavily polluted waters. In several instances contamination of

the water supply washing the oyster beds, was traceable to the discharges from typhoid fever patients.

All shellfish are easily adaptable to filthy habits and surroundings. They appear to thrive in proportion to the amount of organic contamination of the water supply constituting the food beds. It must, however, not be supposed that sewage and other highly objectionable (to man) contamination is normal to the life of the shellfish. We know that the domestic hog is fond of the highly contaminated refuse materials from the kitchen known as swill but we also know that hogs thrive better on sanitary food. Thus the filth feeding oyster grows equally well, if not better, in clean sea water, that is, water free from sewage contamination and decayed animal matter.

The danger from shellfish (to man) is due to the fact that these animals are often from highly contaminated water supplies and that they are generally eaten raw or only partially cooked. The possible diseases traceable to the eating of shellfish are Asiatic cholera (in countries where this disease prevails), typhoid fever and a variety of less severe intestinal diseases such as dysentery, colitis and intestinal ulcerations. The work of the food bacteriologist is, however, not the finding of the specific germs causing an epidemic, but rather an endeavor to ascertain the danger point in the quality of the food as represented by the positive colon bacillus tests. The prime object of the pure food laws is the maintenance of health rather than finding the cause of disease. This most important fact is sometimes not understood as is clearly indicated by a supreme court decision permitting the bleaching of flour. It is the intent of the pure food law to clearly mark the danger points in our food supplies so that the consumer may maintain his physical well-being through the avoidance of such dangers. He who advises against the heeding of the proper and timely warnings set up by those entrusted with this duty, either through ignorance or indifference, is a menace to the public welfare. The danger sign, "avoid bleached flour" and not the actual physical

disturbances which results from the eating of such flour, is the proper warning. The presence of a limited number of colon bacilli in foods and drinks is the danger mark and not the actual occurrence of cholera, of typhoid, of dysentery, due to the eating of more highly contaminated foods. The danger signal must be within the zone of safety and not beyond it.

The methods for the bacteriological examination of shellfish are but modifications of the methods used in the examination of water supplies. As in the case of drinking water, the chief index to the pollution of shellfish is the colon bacillus test. The examination of the water source above the oyster beds very frequently gives inferential information as to the possibility of the contamination of the shellfish which obtain their food supply from beds flooded by such waters. The following is the method for the bacteriological examination of shellfish adopted by the American Health Association at the 1912 meeting.

1. **Selection of Sample.**—Twelve oysters of average size of the lot to be examined, having deep bowls, short lips and shell tightly closed, are picked out by hand or by means of a sterilized long-handled spoon and prepared for immediate transportation to the laboratory.

2. **Making a Record of the Sample.**—This record should cover the following points. The exact location of the bed from which the sample was taken. The depth of the water at the time the oysters were gathered. Weather conditions, direction and velocity of wind, state of tide, day and hour when the stock was taken from the water, the conditions under which the stock had been kept since removal from the water and up to the time when the sample was taken, presence of abnormal odors, temperature of stock, and the day and hour of taking the sample.

3. **Transportation of the Sample.**—The sample oysters are to be packed in a suitable metal or pasteboard container of the size and shape convenient for shipping. The important points to bear in mind are: the prevention of the mixing of the oyster liquors of

the different samples and avoiding the mixing of the oysters with the ice water of the packing ice. The samples must in all cases be placed on ice or packed in ice if they cannot be examined inside of 36 hr. or if the outside temperature is above 50° F. It is, however, not necessary to place the oysters in absolutely tight containers provided the above conditions are maintained.

4. Laboratory Procedure.—Record the date of receiving the sample, condition of seals, of the sample oysters and the temperature of the interior of the container at the time of opening. The bacteriological examination should in all cases be started as soon as possible after the receipt of the sample.

Before beginning operations the hands must be thoroughly scrubbed and all vessels to be used must be sterilized. The shell of the oyster may be opened by means of a sterilized oyster knife or by drilling a hole through the shell near the hinge. The drill must be sterilized and the area of the shell to be operated upon must be cleaned, flamed before drilling and flamed at least once more during the drilling process.

The simplest and quickest method for opening the oyster shells is that employed by Stiles of the Bureau of Chemistry. By means of a pair of sterilized wire nippers crush and break off enough of the two valves so as to make the use of the oyster knife easy.

Before opening the oysters see that they are thoroughly scrubbed and then rinsed in boiled (sterile) water, and each oyster is wiped quite dry and flamed before it is opened.

5. Bacterial Counts.—Bacterial counts are made of the composite sample of each lot obtained by mixing the shell liquor of five oysters. Agar shall be used for the culture medium and in general the procedure shall be in accordance with the method recommended for the examination of water. The water used for making the dilutions shall contain 1 per cent. of sodium chloride, in order to approximate the natural salinity of the oyster liquor. The agar plate cultures shall be incubated at 20° C. for 3 days and the colonies counted in the usual manner.

6. Determining Bacteria of the Colon Bacillus Group.—Measured quantities of the shell liquor of each of five oysters selected from the dozen shall be placed in fermentation tubes containing lactose-peptone-bile. The measured quantities shall be 1 cc., 0.10 cc., and 0.01 cc., or such other quantities or corresponding dilutions as may be desired. The fermentation tube inoculations thus prepared shall be incubated for 3 days at a temperature of 37° C., and the presence of gas noted daily. From 10 to 85 per cent. of gas during this period shall be considered a positive test indicating a presumption of the presence of at least one bacterium of the colon bacillus group in the quantity of the water used in the test. But no final colon bacillus rating shall be made unless confirmatory tests for the presence of organisms of the colon bacillus group shall have been obtained from the tube of highest or next highest dilution from each oyster showing the presence of gas. These confirmatory tests shall be begun immediately upon noting the formation of gas and shall be carried out in conformity with the procedure recommended by the Committee on Standard Methods of Water Analysis.

7. Statement of Results.—The results of the bacterial counts shall be expressed as the number of bacteria per cc. The results of the colon bacillus test shall be expressed either in the form of an arbitrary numerical system or in estimated number of colon bacilli per cc. of the sample.

It is suggested that the arbitrary numerical method proposed by the American Health Association be given the preference. The following are the rating valuations according to this method.

Colon bacillus in 1.00 cc. but not in 0.10 cc., a value of 1
Colon bacillus in 0.10 cc. but not in 0.01 cc., a value of 10
Colon bacillus in 0.01 cc. but not in 0.001 cc., a value of 100, etc.

The sum of these values for five oysters gives the total value of the sample examined and this figure indicates the rating for *Bacillus coli*. According to this system the highest (best) rating

is indicated by 0 and the lowest (worst) by 500, represented in tabular form by the following possible results of two analyses:

EXAMPLE A

Oysters	1.00 cc.	0.10 cc.	0.01 cc.	Numerical Value
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
	Total rating for *B. coli* =			0

EXAMPLE B

Oysters	1.00 cc.	0.10 cc.	0.01 cc.	Numerical Value
1	+	+	+	100
2	+	+	+	100
3	+	+	+	100
4	+	+	+	100
5	+	+	+	100
	Total rating for *B. coli* =			500

The (+) mark means that gas formation in the lactose bile tubes took place, indicating contamination with the colon bacillus.

The (0) mark indicates that no gas formation took place in the lactose bile tubes.

The results above indicated are, however, not generally obtained in practice. The important question is at what rating shall the shellfish be pronounced unfit for human use, or rather what rating shall be the danger signal as to the quality of this food? There seems to be no uniformity of opinion as regards this point. Thus far, the Bureau of Chemistry has barred oysters from interstate shipment which gave three positive tests out of five in 0.10 cc. quantities of oyster liquor, which standard is also adopted by

the Rhode Island Shellfish Commission. This standard may be graphically represented as follows (Example C):

EXAMPLE C

Oysters	1.00 cc.	0.10 cc.	0.01 cc.	Numerical Value
1	+	+	o	10
2	+	+	o	10
3	+	+	o	10
4	+	o	o	1
5	+	o	o	1
	Total rating for *B. coli* =			32

It sometimes happens in laboratory practice that the smaller quantities of shell water from a number of oysters show positive results, whereas larger amounts of liquor from an equal number of oysters show negative results. In such cases it is customary to give the next lower numerical value to the positive results in the high dilutions, and such positive results shall be considered as being transferred to a lower dilution giving negative results in another oyster. This recession of assigned values shall, however, not be carried beyond the point where the number of such recessions is greater than the number of instances where other oysters in the series of five failed to give positive results. This may be illustrated as follows (Examples D and E):

EXAMPLE D

Oysters	1.00 cc.	0.10 cc.	0.01 cc.	Numerical Value
1	+	+	o	10
2	+	+	o	10
3	+	+	o	10
4	+	o	o	10 (not 1)
5	+	+	+	10 (not 100)
	Total rating for *B. coli* =			50

EXAMPLE E

Oysters	1.00 cc.	0.10 cc.	0.01 cc.	Numerical Value
1	+	+	+	10 (not 100)
2	+	+	+	10 (not 100)
3	+	o	o	1
4	o	o	o	1 (not o)
5	o	o	o	1 (not o)
	Total rating for *B. coli* =			23

The bacteriological examination of oysters from opened or shucked stock very naturally must be somewhat modified from the method as outlined for oysters in the shell. The stock in the container from which the sample is to be taken must be thoroughly mixed. The containers (wide-mouthed glass jars) must be sterilized and should have a capacity of 1 quart. By means of a suitable sterilized ladle (may be flamed with alcohol on the spot), half fill the containers with the oysters and seal containers in such manner as to exclude all outside contamination. Unless the examination can be made within 3 hr. after taking the sample, said sample must be placed on ice. It is very desirable to make the bacteriological examination shortly after the sample is taken. The laboratory technique is much as for oysters in the shell, though it must be borne in mind that dilutions higher than 0.01 cc. are usually required. The results of the bacteriological examination of the opened or shucked stock shall be expressed in the same way as that specified for oysters in the shell, except that in the calculation for *B. coli* rating the values for the results of the positive fermentation tests, after confirmation, shall be recorded for each of the inoculations of each and every dilution. All tests are to be made in triplicate, that is, three fermentation tubes are to be inoculated for each dilution used.

Clams, mussels and other shellfish are to be examined in the same manner as oysters, in so far as this is possible. In opening

soft-shelled clams it will be found that if two incisions are made through the mantle the shell water may be poured out without opening the shell. It is stated that hard-shell clams may be opened by striking the shell over the dorsal muscle with a hammer. An opening is formed which will permit the insertion of a knife with which to cut the muscle. In case any one shellfish does not contain enough shell water to make a test, the water from several individuals may be mixed.

The examination of shellfish for sewage pollution is of the utmost importance, as dangerously contaminated oysters are very common. In fact it would be advisable to discontinue the oyster as an article of diet. At its very best it is a filthy article. It is unquestionably a dangerous article of food, in this regard comparable to the mushrooms in the vegetable kingdom. However, there is not the least likelihood that the oyster will be left from our dining tables as long as there are any available. It is therefore most desirable that the supervising of this food on the part of those who are entrusted with the safeguarding of the health of the people should be carefully and consistently done.

Some authorities (English) recommend that the liquor and oysters be mixed, the latter finely chopped, for the purpose of making the colon bacillus test. There appears to be no gain from this procedure and the method cannot be recommended.

17. The Bacteriological and Toxicological Examination of Meat and Meat Products

Remarkable as it may seem, food bacteriologists have given but little attention to the examination of meats and meat products, despite the fact that fatal poisoning from eating infected meats is very common. Intoxications ranging from mild to very severe, resulting from the ingestion of more or less highly contaminated meats are of daily occurrence in every community. At each instance of a death or deaths resulting from the eating of bad meat,

the health authorities get busy and almost invariably find the true source of the trouble, and there the matter usually rests. No rational attempt is made to prevent a repetition of the occurrence.

Meats of all kinds when left exposed to the air soon show signs of decomposition. The aerobic forms of bacteria are first to develop, causing the decomposition of proteids and sugars. Inasmuch as sugar is usually present in small amounts only, the sugar decomposers are soon crowded out by the proteid-splitting forms. The small amount of acid formed by the sugar decomposers is neutralized by the ammonia which is formed during proteid decomposition. The aerobes very naturally act on the outside of the meat particles, using up the oxygen in the air on and within the immediate surface tissues of the meat. This reduction in oxygen gradually permits the anaerobes to get a start, especially *B. perfringens* and *B. bifermentens sporogenes*. These use up proteids as well as sugar, and the complete removal of sugar encourages the more active development of pure aerobes which act upon proteids only. The following tabulation from the work by Ellis

Organisms	Action on	Products Formed
Proteus vulgaris	Gluten and fibrin	Phenol, indol, amines, fatty acids.
Proteus vulgaris	Casein	Albumoses, peptones and amino-acids.
Streptococcus longus	Fibrin	Tyrosin, leucin, amines and fatty acids.
B. coli communis	Casein	Albumoses.
B. coli communis	Peptone	Ammonia and indol.
B. coli communis	Mixture of eggs and meat	Skatol, phenol, leucin and oxy-acids.
Micrococcus pyogenes	Gluten	Phenol, indol, amines and fatty acids.
Aerobic peptonizing lactic acid bacteria	Casein	Leucin, tyrosin, fatty acids, aromatic fatty acids and tryptophan.
B. subtilis and *B. prodigiosus*	Albumoses	Leucin, tyrosin and tryptophan.
Cholera vibrio	Albumen	Leucin, tyrosin, indol, amines and fatty acids.

indicates the activities of putrefactive bacteria in culture, which correspond with the putrefactive changes produced in nature:

The rotting bacteria produce the familiar changes in meat usually designated as spoiling, rotting and tainting, and such meat is universally recognized as unfit for food because of the deleterious effects following the ingestion of such meats. Tainted meats may appear entirely normal to the naked eye and slight decay of the inner tissues may not be appreciable to the sense of smell. The decomposition changes resulting in the liberation of indol, skatol and related substances having disagreeable odors usually begin near the bones and joints, and such decomposition may not become apparent until the bony structure is exposed by cutting or until the odors are dissipated more actively by boiling. This odor is very persistent; boiling for several hours will not cause it to disappear entirely. It must not be supposed that meats free from bad odors are necessarily free from ptomaines and toxins. For example, perfectly fresh meat may absorb these poisonous substances when placed in contact with badly tainted meats and, again, some toxin-forming bacteria do not produce odoriferous gases.

The bacteriology and toxicology of canned meats and soup stocks containing meat has not received the attention that it should. It is these substances which are so largely responsible for the multitudinous lesser intestinal disturbances following their use as food. The present methods of canning meats should be thoroughly investigated and ways and means devised to improve them in accord with modern advance in the manufacture of food products. It is generally believed that the eating of canned meats and soups is fraught with danger to life and health, and this is not far from the truth. The proper canning of meats requires infinitely more care than the canning of vegetable substances. The careful supervision of the marketing of meats and meat products is vastly more important than the supervision of vegetable foods. It is comparatively rare for toxins and ptomaines to be formed in vegetable substances, whereas this is the rule in the decomposition of meats.

Furthermore, meats decompose much more readily than vegetable substances, which makes it necessary to observe greater care in the preparation of this class of food for the market. Some meats decompose much more readily than others. Meats of higher animals resist decomposition longer than do meats of lower animals. Fish meats decompose quickly when exposed to the air. The story is current among fishermen that certain kinds of fish

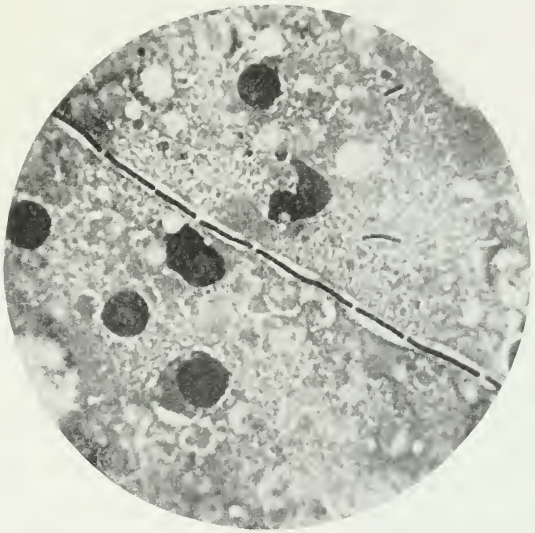


FIG. 48.—*Bacillus welchii*, also known as *B. aerogenes capsulatus* and *B. phlegmones emphysematosæ* in smear preparation. This is the common "gas bacillus," because of the abundant gas formation in the tissues invaded and in culture media. It is a plump large nonmotile, anaerobic, capsulated, Gram positive, spore-bearing bacillus and is very widely distributed in nature.—(*Williams.*)

begin to decompose before they can be removed from the hook. This is of course exaggeration, but the statements made indicate in a way the comparative resisting power of different kinds of meat to rotting bacteria. It is highly probable that the difference in the resisting power to decomposition is due in part at least to the presence of bacteriolysins. Little is known regarding the changes which take place in cold storage meats. Cold storage

FIG. 49.—Typical cultural characteristics of *Bacillus aerogenes capsulatus* (*B. welchii*) in agar. Culture 48 hr. old. The agar mass is separated by the gas which is formed.—(*MacNeal.*)

does check the growth of all kinds of bacteria and of higher fungi, but not in the same ratio. For example, the freezing temperature inhibits the development of the usual rotting bacteria very effectually, whereas many of the toxin formers multiply slowly, in time forming enough of the poison to produce marked symptoms of poisoning when meat thus affected is eaten. Little is known of the changes which take place in incompletely sterilized canned meats, and no attempt has so far been made to ascertain the degree of decomposition which usually takes place in the meats before they are placed in the cans and sterilized. This is a matter of the utmost importance and should receive the immediate attention of the food bacteriologists.

What shall be the routine method in the examination of meats? It is quite evident that the methods which are applicable in the examination of vegetable substances are not suitable in the examination of meats. We hereby suggest the following outline of methods applicable in the food laboratory:

1. Direct microscopical examination of meats.
   - *a.* Bacteria on surface of meats.
   - *b.* Mold and spores present, as in moldy bacon, pork, etc.
   - *c.* Presence of bladder worms, larvæ of parasites, etc.

d. Trichinæ in pork.
e. Cereal fillers and starches in sausage meats. Tragacanth fillers.
f. Coloring substances and preservatives in sausage meats.

2. Plate cultures. (Lactose-litmus-agar and gelatin media.)
   a. Numerical counts of bacteria.
   b. Number of gas formers and acid formers.
   c. *B. botulinus* in pork meats.
3. Toxicological tests.
   a. Inoculation tests (guinea-pigs) to prove the absence or presence of ptomaines or toxins.
   b. Tests for tuberculous and other diseased meats.
4. Determining the source of the meat.
   a. By the precsipitin test.
   b. Sugar test for horse meat.
   c. Microscopical identification based on differences in the size and structure of the muscular fibers and the differences in the size and form of the fat crystals derived from different animals.

Of the above tests the numerical bacterial count and the toxicological tests are of the greatest importance and should be carried out in the examination of suspected raw meats, sausage meats and of canned meats and soup stocks. There certainly should be a limit to the number of bacteria in all raw meats, whether ground into sausage or not, as this would be the means of regulating the sanitary requirements in the proper handling of meats. The only practical method for determining the quality of canned meats is to make inoculation tests on guinea-pigs or white mice, using filtered aqueous extracts of the suspected meat products. If ptomaines or toxins are present the tests will show it. It would be very desirable to work out a micro-chemical test for determining the presence of toxins and ptomaines in meats. As above indicated, there are some very important differences between toxins and ptomaines. The former are destroyed by the boiling temperature, whereas the latter are not. For example, the thorough cooking of sausage meats prevents botulism but it does not prevent the ill effects resulting from the eating of meats with ptomaine poison.

Dried and smoked meats should be examined for the presence of bacteria and molds. Dried fish in particular is very fre-

quently highly contaminated with molds. It is very evident that the present method of pickling fish of all kinds must be changed. The method of pickling herring, for example, in wooden vats or casks must be abandoned, as the containers are wholly unsuitable from a sanitary standpoint. The liquor from canned fish (in tin cans) is frequently very highly contaminated with bacteria in spite of the high salt content. The gelatin of the market requires careful examination, as much of the sheet variety is not infrequently

FIG. 50.

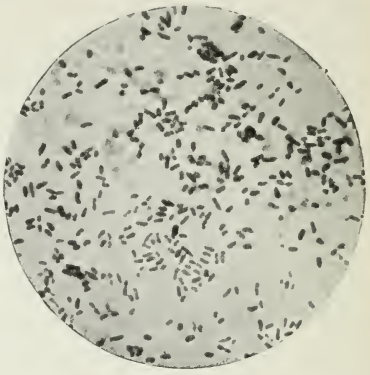


FIG. 51.

FIG. 50.—*Bacillus botulinus* from a sugar-gelatin culture.—(*Pittfield, after Kolle and Wassermann.*)

FIG. 51.—*Bacillus enteritidis.* Under this name is included a number of organisms of the Gaertner group which play a very important part in meat decomposition and meat poisoning. It is also known as the dysentery group. The organisms are actively motile, non-sporogenous, aerobic, non-liquefying and Gram negative.—(*Jordan after Kolle and Wassermann.*)

entirely permeated by mold and bacteria, rendering it not only unfit for food for man but also unsuitable for bacteriological work.

The entire subject of meat poisoning is as yet not very well understood. Dr. Savage states that the bacteria concerned in meat poisoning may be classed under three groups: (*a*) the Gaertner group of bacilli, (*b*) aerobic bacilli not belonging to the Gaertner group, such as *B. proteus* and *B. coli*, and (*c*) *Bacillus botulinus*. In the majority of cases of outbreaks of fatal food poisoning, some

form of the Gaertner group of bacilli has been the infecting organism. The Gaertner bacilli are large coli-typhoid types which occupy a position intermediate between the chemically active colon group and the chemically inert typhoid group, and includes *B. enteritidis, B. typhi murium, B. suipestifer* and *B. paratyphosus B.* Sausages and ham are the commonest sources of botulism. From the anaerobic character of the bacillus it follows that poisoning is rarely due to the eating of fresh sausage and pork. Invasion of meats by *B. botulinus* can take place only when the necessary anaerobic conditions exist, as for example when a ham is stored at the bottom of the pickling vat and entirely covered by the pickling solution, and in the interior of insufficiently cooked sausages and in stored masses of sausage meats.

The use of the compound microscope in the examination of meats and meat preparations is still in its infancy. The work done shows very clearly that with more experience very valuable information can be obtained from the microscopical examination regarding the quality of meats of all kinds. It is highly probable that the microscope will show diagnostic differences in the musculature of different animals, thus making it possible to determine the source of the meat.

Considerable attention has already been given to the microscopical study of fat crystals derived from the fats of different species of animals. So-called rancid fats or fats which have aged considerably, even though they may not yet give evidence of rancidity to the unaided senses, will show more or less abundant crystalline structure, arranged in clusters, which may be readily seen under the low power of the compound microscope. These crystals are not apparent in fresh fats, but are generally more or less abundantly present in canned meats and soup stocks containing animal products, in meat extracts and in other meat derivatives having fat admixtures. The presence of crystal clusters indicates fat decomposition and these are therefore an indication of the quality of the meat product containing them. The degree of ran-

cidity or, to state it more accurately, the quality of the product dependent upon age is in direct proportion to the quantity of crystalline clusters present. It would appear that the quantity of crystals present is not proportional to the amount of bacterial contamination and decomposition. The indications are that it is possible to determine the source of the fat from the color, size and arrangement of the fat crystal aggregates. For example, the crystal clusters of lard are smaller than those of the fat of the domestic fowl. The fat crystal aggregates of the hen are comparatively large and the individual crystals are long and slender. The inexperienced analyst is apt to mistake the crystal clusters for mold colonies (Leptothrix). This mistake can very readily be avoided by applying heat which causes the prompt melting of the fat crystals whereas the mold hyphæ are not greatly disturbed or changed. The differential characteristics which would be concerned in the microscopical examination of fat crystals may be given as follows:

1. Differences in the size of the aggregates.
2. Differences in the length of the individual crystals.
3. Differences in the diameter of the individual crystals.
4. Differences in the form of the ends of the individual crystal. Ends may be rounded or pointed.
5. Differences in color. These will in all probability pertain to different races or families of the animal kingdom. For example, lard crystals are colorless whereas those of the domestic fowl are yellowish.

The use of certain chemicals will aid in the microscopical findings. For example, sulphuric acid produces characteristic color reactions with certain fats. If two drops of concentrated sulphuric acid are added to twenty drops of goose fat, a greenish-yellow color is produced which changes to reddish brown on stirring. Under the same conditions cod-liver oil turns a violet color whereas turtle oil turns brown. Castor oil turns yellowish to yellowish brown and finally wine red with a very distinct zone. A similar reaction is observed with neats foot oil. Raw linseed oil turns a deep reddish brown to very dark brown. Lard oil shows a distinct

brown zone which deepens to wine red. The reaction for sperm oil is much as for cod-liver oil. These color reactions with sulphuric acid are perhaps of little value in the detection of fat adulterations and admixtures but they will prove helpful aids in the examination of these substances as to identity. Pure concentrated acid should be used. It must also be kept in mind that the fat impurities which may be present modify the color reactions. Pure samples of fats should be kept on hand for purposes of making check and comparative tests. The Bureau of Animal Industry has suggested a method for distinguishing between fats and oils derived from the animal and the vegetable kingdoms based upon differences in the appearance of the crystals (phytosterol and cholesterol).

In addition to the study of fat crystals which are formed spontaneously in more or less decomposed and aged meat products as above set forth, certain methods for testing fat crystals isolated in the pure state by chemical methods are now generally carried out in meat inspection and food laboratories. These tests combine the use of the compound microscope and should therefore be carried out by the micro-analyst or bacteriologist and for that reason are hereby included. R. H. Kerr of the Biochemic Division of the Bureau of Animal Industry has worked out a method for detecting vegetable fats in mixtures of animal and vegetable fats and *vice versa*, the method will also serve to demonstrate the presence of animal fats in supposedly pure vegetable fats. The method is a slight modification of several methods which have been in use for some time and which are described in various text-books, and it is hereby given in full as it appears in Circular No. 212 (May 10, 1913) of the Bureau of Animal Industry.

The Detection of Phytosterol in Mixtures of Animal and Vegetable Fats

Sample.—The amount of sample used depends on the amount of material available. From 200 to 300 grams is the amount usually taken. The test is seldom

attempted if less than 100 grams are available, and an amount greater than 500 grams is never taken.

Extraction with Alcohol.—The sample is melted and poured into a flat-bottomed flask of 1-liter capacity which is closed with a rubber stopper perforated with three holes. This flask is set on the top of the steam bath and connected to a reflux condenser and to a 700 cc. round-bottomed flask containing 500 cc. of 95 per cent. alcohol. A glass tube which is adjusted so that its lower end is about one-fourth of an inch above the surface of the fat and whose upper end is bent at a right angle and closed by means of a short piece of rubber tubing and a pinchcock fills the third hole in the stopper. The distilling flask is set down in the steam so that the alcohol boils briskly. The outlet tube reaches down to the bottom of the flask containing the sample so that the alcohol vapor as it distills over bubbles up through the fat and keeps it in a state of vigorous agitation. The alcohol vapor is condensed in the reflux condenser and returned to the flask containing the fat. The distillation is continued until all of the alcohol has collected in the flask containing the fat. The distilling flask is now disconnected. The alcohol in the flask immediately ceases to boil and soon separates from the fat. The empty distilling flask is next connected to the bent tube by a piece of glass tubing of sufficient length, the pinchcock opened, and the alcohol layer siphoned off into the distilling flask. This is then connected as before and the distillation continued until the alcohol has again collected in the first flask. It is then siphoned into the distilling flask as before, and a third extraction made. After the third extraction the alcohol layer is again siphoned off into the distilling flask and the fat is discarded. The alcohol now contains practically all of the cholesterol and phytosterol originally present in the fat.

Saponification and Extraction with Ether.—The alcohol in the distilling flask is next concentrated by boiling to about 250 cc., and 20 cc. of a concentrated potassium-hydrate solution (100 grams KOH dissolved in 100 cc. water) added to the boiling liquid. It is boiled for 10 min. to insure complete saponification of all the fat and is then removed from the steam bath and allowed to cool almost to room temperature. After it has cooled sufficiently it is poured into a large separatory funnel containing 500 cc. of warm ether and shaken to insure thorough mixing. The mixture may be clear, but is more often opalescent. There is now poured in 500 cc. of distilled water, and the funnel is rotated gently. Shaking must be avoided, as it leads to the formation of extremely stubborn emulsions, but the water should be mixed with the alcohol-ether-soap solution. Separation takes place at once and is clear and sharp. The soap solution is drawn off and the ether layer washed with 300 cc. of distilled water, shaking being still avoided. After this washing it is washed repeatedly with small quantities of water until all soap is removed. The ether layer is then transferred to a flask and the ether distilled off. Distillation is stopped when the contents of the flask have been reduced to about 25 cc., and the concentrated ether solution containing the cholesterol, phytosterol, and all other unsaponifiable matter is transferred to a tall 50 cc. beaker. The evaporation is continued until all ether is driven off and the residue is perfectly dry. If desired, a tared beaker may be used and the weight of the unsaponifiable matter determined at this point.

Preparation of the Acetates.—A small amount (3 to 5 cc.) of acetic anhydrid is added to the dry residue in the beaker and heated to boiling over a free flame, the beaker being covered with a watch glass during the process. After a brief boiling—a few seconds is sufficient—the flame is removed and the beaker transferred to the steam bath and left there until the acetic anhydrid is driven off.

Purification of the Acetates.—Thirty-five cc. of hot 80 per cent. alcohol are added to the acetylated residue in the beaker and heated to boiling with vigorous stirring. The liquid is then filtered quickly through a folded filter and the insoluble residue washed well with boiling 80 per cent. alcohol. The acetates of cholesterol and phytosterol are dissolved, while the greater portion of the impurities present are not dissolved by the alcohol and remain on the filter. Paraffin and paraf-

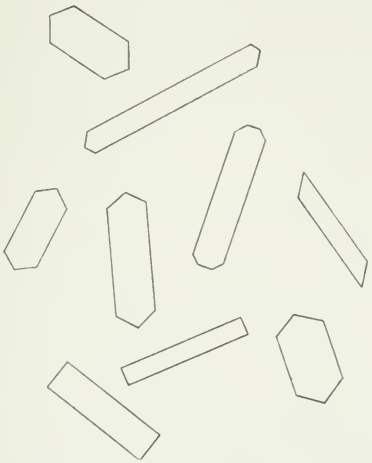


Fig. 52.—Phytosterol crystals.

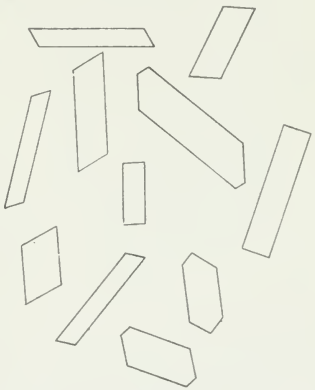


Fig. 53.—Cholesterol crystals.

fin oil, if present, are likewise separated by this treatment. The combined filtrate and washings are next cooled to a temperature of 10° to 12° C. and allowed to stand at that temperature for 2 to 3 hr. During this time the acetates of cholesterol and phytosterol crystallize from the solution. They are removed by filtration, washed with cold 80 per cent. alcohol, and then dissolved on the filter with a stream of hot absolute alcohol from a wash bottle, as little alcohol as possible being used. The alcoholic solution of the acetates is caught in a small glass evaporating dish, two or three drops of distilled water being added to the solution and heat applied if it is not perfectly clear. The dish is then set out on a desk in the laboratory and the alcohol allowed to evaporate spontaneously. The contents are stirred occasionally and the deposit of crystals which forms around the edges of the liquid and on the sides of the dish rubbed down into the solution with the stirring rod. As soon as a good deposit of crystals has formed they are removed by filtering through a hardened

filter, washed twice with cold 90 per cent. alcohol, and dried by suction. After drying by suction they are dried at 100° C. for half an hour and the melting point determined.

Determination of the Melting Point.—A tube of about 1 mm. diam., sealed at one end and having a slight flare at the other, is filled to a depth of about 5 mm. with the dried crystals, which are packed somewhat firmly in the lower end by tapping on a hard surface. This is attached to the bulb of a suitable thermometer and the melting point determined. A thermometer graduated from 95° to 200° C. in one-fifth degrees is used in this laboratory. The determination is made in an Anschutz apparatus, the outer bulb being filled with concentrated sulphuric acid and the inner tube with glycerin. The apparatus is so adjusted that no correction of the observed temperature is required. The melting point of the first crop of crystals usually gives definite information as to the presence or absence of phytosterol, but the conclusion indicated is confirmed by recrystallizing from absolute alcohol and again determining the melting point. If the crystals are pure cholesterol acetate, the melting point of the second crop should agree closely with that of the first. If phytosterol acetate is present, however, a higher melting point should be noted, as phytosterol acetate is less soluble than cholesterol acetate.

The Emery Method for the Detection of Beef Fat in Lard

James A. Emery of the Biochemic Division of the Bureau of Animal Industry recommends the following method[1] for detecting beef fat in lard. It is given here because of its value in isolating the crystals of fats for microscopical examination.

Technique of Method.—Five grams of the warm filtered fat is weighed (on a balance sensitive to 0.1 gram) in a glass-stoppered graduated cylinder of 25 cc. capacity, 150 to 175 mm. in height, with an internal diameter of about 18 mm., and warm ether is added until the 25 cc. graduation is reached. The glass stopper is securely replaced and the cylinder is shaken vigorously until complete solution of the fat takes place. The cylinder with its contents is then allowed to stand in a suitable place where a constant temperature, at which it is desired to have the crystallization proceed, may be maintained. (An apparatus described by Rogers proved efficient for the maintenance of this constant temperature.)[2] After 18 hr. the cylinder is re-

[1] Circular 132, May 23, 1908. Bureau of Animal Industry, U. S. Dept. of Agriculture.

[2] It is necessary to observe great caution in the use of this form of apparatus, as the sparking of the thermo-regulator is a source of danger if the solutions are carelessly handled. A better form for this work would be one in which the temperature is controlled by a circulating hot-water system heated by a small lamp outside of the box, the regulation of which could be adjusted by using one of the many forms of gas regulators on the market.

moved and the supernatant ether solution carefully decanted from the crystallized glycerids, which are usually found in a firm mass at the bottom of the vessel. Cold ether is then added in three portions of 5 cc. each from a small wash bottle, care being taken not to break up the deposit while washing and decanting the first two portions. The third portion is, however, actively agitated in the cylinder with a sharp rotary motion and by a quick movement transferred, with the crystals, to a small filter paper. The crystals are then washed with successive small portions of the cold ether, with the use of the wash bottle, until 10 to 15 cc. has been used, dependent on the amount of crystals. Then by means of a slight exhaust the small amount of remaining ether is rapidly removed. The paper with its contents is then transferred to a suitable place, where it should be spread out and any large lumps of the glycerids broken up by gentle pressure. When dry the mass is thoroughly comminuted and the melting point of the crystals determined.

As the difference between the melting points of the glycerids obtained in this manner from beef fat and lard is not very great, being only about 3.5 degrees, and as the writer has mentioned a standard melting-point temperature for the glycerids of pure lard obtained under certain conditions, a description of the apparatus used in determining the melting points, together with its manipulation, is essential and may be of some assistance.

FIG. 54.—Beef fat crystals. *a*, Clusters of crystals as seen under the low power of the compound microscope; *b*, crystals highly magnified.

Determination of the Melting Point.—A large test tube approximately 150 by 25 mm., containing water (free from air) into which the bulb of a thermometer[1] with the melting-point tube attached is immersed, is placed in a beaker of water and so adjusted that the surface of the liquid contained in the two vessels is at the same level. The water in the beaker should be heated rapidly to about 55° C. and that temperature maintained until the thermometer carrying the melting-point tube registers between 50° and 55° C., then heat is again applied and the temperature of the outer bath carried somewhat rapidly to 67° C., when the lamp is removed. The melting point of the crystals is regarded as that point when the fused substance becomes perfectly clear and transparent. The use of a dark background placed about 4 in. from the apparatus will prove of advantage.

The melting-point tube should be of about 1 mm. internal diam., sealed at one end and with a slight flare at the other extremity, in order that the loading may be expedited. The amount of the substance taken for each determination should be

[1] The thermometer used was one graduated in one-fifth degrees and extending from 0° to 100° C.

approximately the same and should occupy a space about 9 mm. in length, being somewhat firmly packed in the lower end of the tube by tapping it sharply on a hard surface. The water in the outer bath should be agitated frequently during the determination.

Possible Sources of Error.—In applying the foregoing method too great care cannot be exercised with the preparation of the sample. The presence of water, the incomplete solution of the fat in the ether, or the presence of small particles of extraneous matter may interfere with the process of crystallization, frequently caus-

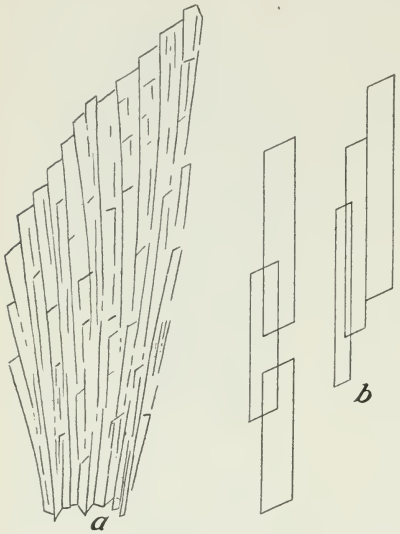


FIG. 55.—Lard crystals. *a*, Clusters of crystals as seen under the low-power of the compound microscope; *b*, crystals highly magnified.

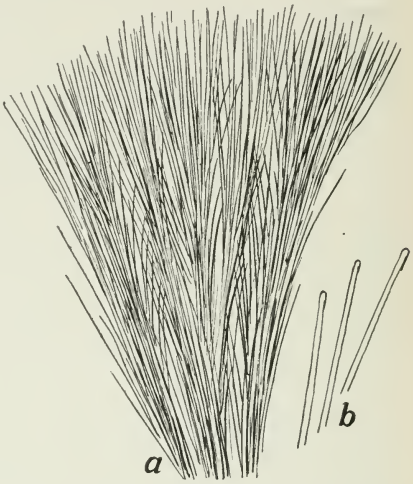


FIG. 56.—Duck fat crystals. *a*, Clusters of crystals as seen under the low power of the compound microscope; *b*, crystals highly magnified.

ing it to proceed too rapidly and resulting in the formation of a large mass of small fluffy crystals instead of the compact mass of larger crystals desired. These fine crystals render the preliminary washing by decantation with ether difficult, and they also persistently hold the unsaturated glycerids in larger amount than is desirable. The temperature at which the crystallization should be allowed to proceed should not be less than 15° C. nor more than 20° C., with the best results obtainable in the neighborhood of an average between the two. Although larger crystals are formed at the higher temperature (20° C.), only lards of high grade afford crystalline deposits in working quantity, and in many cases where lards of inferior grades are tested the amount of solid glycerids entering into their composition is so reduced as not to yield any deposit at all.

Pure fresh butter shows no crystalline structure. Salted butter will of course show the characteristic salt crystals. Melted butter which is allowed to cool slowly shows a marked crystalline structure under polarized light, even under the low powers of the compound microscope, but this is not a diagnostic character inasmuch as other fats show a similar behavior with polarized light.

Horse meat has been used as food for man for many ages and is at the present time a regularly marketed food article in many countries. During the siege of Paris (1870) when food became very scarce, experiments with the meats of various animals were made, as that of rats, mice, cats, dogs, mules and horses. Horse meat especially met with general favor and since that time has become quite common in the French meat markets. It is stated that it is a frequent substitute for beef in our restaurants (the cheaper eating places in our larger cities). Horse meat differs from beef in that it is somwhat coarser grained, darker in color and that it contains a higher percentage of glycogen. As a rule the meats from cattle contain little or no glycogen, although it is stated that fresh meat from well-nourished cattle may contain as much glycogen as does the meat of the horse. It must also be borne in mind that the meat from dogs, cats, starved calves and fetuses contains considerable glycogen. Should such meats be added to sausages the admixture might be recognized by the color, the meat of fetuses and starved calves being much lighter than that of the horse or of mature cattle.

In time the glycogen of horse meat is changed into grape sugar and will respond to the Fehling's solution reaction for sugar. For this purpose use a cold aqueous extract of the suspected meat. In the case of fresh horse meat the following tests are recommended. The Bräutigam and Edelmann test for the presence of horse meat is made as follows: Grind or chop (finely) 50 grams of the meat and boil for 1 hr. in 200 cc. of water. Add 1.5 grams (3 per cent. by weight of the meat) of caustic potash and heat over

water bath until the muscle fibers are disintegrated. Boil down to 50 grams and filter. When cool add an equal part of dilute nitric acid (10 per cent.) to precipitate the albuminoids, and again filter. Pour the filtrate into a test-tube and carefully pour iodine water down the inside of the tube. If horse meat is present a burgundy red zone appears at the point of contact of the two solutions. The width and intensity of the colored zone is in direct proportion to the amount of horse meat present.

If starch is present (as in sausages and sausage meats seasoned with starch-bearing spices or mixed with starch fillers), this must be precipitated from the boiled meat extract and removed by filtration. To the extract add two or three times the volume of concentrated acetic acid and let stand for 2 or 3 hr., and then filter through two or three thicknesses of filter paper. Test the filtrate with the iodine water as above suggested. However, before making the glycogen test the test for starch should be applied, for if it responds to this test the precipitation of starch must be repeated. Because of the dilution with the three or more volumes of acetic acid (to precipitate the starch) negative results may be obtained in cases where horse meat is present. It is therefore advisable to precipitate the glycogen by means of alcohol, using from ten to twelve times the volume of the acidulated meat filtrate. The cloudy alcoholic suspension is run through a small filter and the precipitated glycogen on and in the filter paper is washed out by means of hot acidulated (acetic acid) water, and this filtrate is then tested with the iodine water. This test is positive in the presence of 5 per cent. quantities of horse meat. The wine-red color reaction is temporary only and it must be kept in mind that dextrin interferes with the reaction.

Because of the fact that meats other than that derived from the horse may contain glycogen, it is sometimes necessary to supplement the above color reaction with the biological test or the precipitin test which has come into use within recent years. The general routine for making the test is as follows: Inject

(subcutaneously or intravenously) rabbits with 10 cc. of filtered defibrinated horse blood (or serum) every other day five or six times. At the end of this time draw blood from the rabbit, allow it to clot kept on ice, remove the serum and filter, whereupon the reagent is ready for use. Express and extract (in saline solution) the juice from the meat suspected to contain horse meat, filter and keep on ice until wanted for use. To the filtrate thus prepared add a few drops of the equinized rabbit serum. If cloudiness and slight whitish precipitate forms it constitutes a positive test, proving conclusively that the suspected meat is horse meat or contains horse meat. Only raw fresh meat responds to this test. Heating destroys the action of the reagent. Inoculating rabbits with the defibrinated and filtered blood serum of various animals, as of hog, domestic fowl, deer, dog, bear, etc., and testing in the manner outlined in the following method by Dr. Karl F. Meyer of the State University of California, the meat of the responding animal may be identified.

The Precipitin Test for the Detection of Horse and Deer Meat and for Meat Adulterations in General

The method can be used for fresh, dried, frozen, pickled, raw and smoked, but not for boiled, meat. The meat may not be heated above 60°–70° C. for the biologic test.

For the tests are needed:

a. Specific antisera (anti-horse or deer precipitin serum; precipitin).

b. Aqueous extract of the meat to be identified (precipitinogen).

1. Antisera.—The sera must be specific and highly active against the meat protein to be determined. Rabbits are injected subcutaneously, intravenously or intraperitoneally with serum, defibrinated blood or extract of the fat free meat. The *best results* are obtained by inoculating fresh serum intravenously. The sera for injection can readily be obtained from abattoirs

or from serum institutes or laboratories. Horse serum is not as toxic to rabbits as are some other sera. Meat extracts should always be filtered to avoid infection of the animals to be immunized, but extensive sloughing is likely to occur with any method of immunization and the mortality rate is high. The blood or serum used as antigen can be preserved by the addition of chloroform (1–2 per cent.), or by drying.

On account of the individual differences existing in rabbits in regard to the development of precipitins, it is advisable to treat at least six animals at the same time. The injections of 2–3 cc. of horse or deer serum are made at intervals of 5 days. Ten days after the last injection the blood is tested for precipitins. The further treatment of the animals differs individually, depending on the precipitin contents of the rabbits. Animals which show a high precipitin reaction are given subsequent inoculations subcutaneously or intraperitoneally, to avoid anaphylactic death which frequently results from intravenous inoculations. Some rabbits fail to produce precipitins, whatever the method used.

Fornet and Müller[1] recommend the intraperitoneal injection of 5, 10 and 15 cc., respectively, of protein material on the 1st, 2d and 3d day, respectively. The test for *antibodies* is carried out on the 12th day. *Gay and Fitzgerald*[1] inject on three consecutive days 1 cc. of the antigen, bleed, and test the serum on the 10th day. Both methods frequently give very good results. The precipitin content of an immune serum is occasionally titrated during the process of immunization by withdrawing a few cubic centimeters of blood from an ear vein. The hair over the marginal vein is removed and the skin rubbed with alcohol. A fine pipette is introduced into the vein and the blood collected by capillary attraction or by suction. It is, however, advisable for the beginner to cut the vein transversely and to collect the blood in a centrifugal tube. The hemorrhage is stopped by covering the wound with

[1] University of California publications, Pathology, Vol. II, 75, 1912.

cotton soaked in liq. ferri sesquichloridi (ferric chloride) or by placing a small hemostat for ½ to 1 hr. on the incision. The serum which has separated from the clot is centrifugalized and the titer is determined as follows:

Preliminary Titration.—Into each of a series of six test-tubes place 2.0 cc. of the following dilutions of serum (horse or deer) antigen, mixed *with* 0.85 *per cent. saline* 1:100, 1:500, 1:1000, 1:5000, 1:10,000 and 1:20,000. To each cubic centimeters of the dilution 0.1 cc. of antiserum is added. The solution of 1:1000 should become turbid instantaneously or within 1 to 2 min., the other dilutions in from 3 to 5 min. The serum should have a titer of 1:20,000; that means the serum should cause a turbidity in a dilution (of horse serum or extract of meat) of 1:20,000 in less than 5 min. The antiserum is either introduced by allowing it to run down the side of the tube (no shaking is permissible), or it is stratified on the diluted horse serum. In the first case the turbidity appears from the bottom, in the second case in form of a grayish ring; both reactions are positive. The coloration is best seen against a dark background. The pipettes and test-tubes must be perfectly clean and sterile. The equipment designed by Uhlenhuth is very satisfactory. The test-tubes are long and narrow, 10 cm. by 0.8 cm., and are suspended in beveled holes of the test-tube rack. Pipettes of 1 cc. capacity graduated into $\frac{1}{100}$ cc., and 5 and 10 cc. pipettes graduated into $\frac{1}{10}$ cc. will be found satisfactory.

Preservation of Serum.—In case the titer of the serum is satisfactory, the rabbit is bled to death (aseptically) from the carotids. For full details on technique, consult the text-books on Immunity. The centrifuged serum should be perfectly clear and sterile and should not be opalescent. Kept cool and in the dark (ice chest) it will remain potent for months, even years. To avoid opalescence the animal should be bled only after a period of fasting. On account of autoprecipitation, it will lose some of its potency. The precipitate formed can be removed by cen-

trifugalizing or by filtration, but the titer must again be tested. Preservatives such as carbolic acid, etc., should not be added to the sera. Sterile sera are obtained by filtration through Berkefeld filters. Drying of the sera on filter paper is the best method known for preserving them (*Jacobsthal und v. Eisler*).

2. The Preparation of the Meat Extract.—To make the biologic test for horse or deer meat, remove from the deeper parts of the specimen, by means of a flamed or boiled knife and through a fresh opening, a piece of muscle of about 30 grams weight. It should contain as little fat as possible. On a sterilized tile (best covered with unused writing paper) chop the meat carefully. The finely minced meat is placed in a sterilized 100 cc. Erlenmeyer flask and spread out with a sterile glass rod and covered with 50 cc. sterile saline solution. Salted meat is washed for 10 min. in a large flask with distilled water, renewing the water several times, without shaking the flask.

The mixture of saline and meat is kept for about 6 hr. at room temperature, or over night in the refrigerator. To obtain a clear solution the flask should not be shaken.

Since the presence of fat interferes with the reaction, it is advisable to remove it by means of ether and chloroform. To make the extraction, take 75–100 grams of the minced meat, place in a large Erlenmeyer flask and cover with equal parts of ether and chloroform. After 24 hr. the ether and chloroform are poured off, the meat is washed once or twice with saline solution and then extracted, as stated above.

To determine whether a sufficient quantity of protein substances has passed into solution, place 2 cc. of the extract in a test-tube and shake vigorously. If a fine foam develops and persists for some time, the extraction may be said to be sufficiently complete. The protein solution must be perfectly clear and must therefore be filtered. With extracts from fresh meat this is usually accomplished by filtering through a firm filter paper previously moistened with saline solution. If it is not crystal

clear, and especially if the meat to be examined was fat or salt, it is filtered through a sterile Berkefeld or through a layer of infusorial earth stratified in a Büchner funnel.

The filtrate is suitable for the test when a foam is developed by shaking and when it contains about 1 part of protein in 300 parts of salt solution. To determine this, 2 cc. of the clear filtrate are placed in a test-tube and heated, and a drop of dilute nitric acid (sp. gr. 1.153) is added; if a marked cloudiness and a flocculent precipitate forms, the extract is too highly concentrated and must be diluted with normal salt solution until the heat and acid test causes only a diffuse, opalescent cloudiness which settles to the bottom of the tube after 5 min. as a slight precipitate.

Before proceeding with the test, the reaction of the meat extract should be tested with litmus paper and if it is found to be acid it should be neutralized very carefully with 0.1 per cent. sodium hydroxide or magnesium oxide solution. Only slightly acid or alkaline solutions should be used. For the extraction of the meat, spigot, tap or distilled water should not be used. Fresh meat frequently produces a sufficiently strong protein solution in 1 hr. In boiled, preserved and decomposed meat, the extraction proceeds very slowly (24 hr.) and the solutions are difficult to clarify.

Technique of the Test.—If, for example, the object is to determine whether a piece of meat is horse flesh or, if sausage, contains the meat of this animal, the test is conducted as follows:

Tube 1.—2 cc. of unknown extract (1 : 300) + 0.1 cc. of anti-horse serum.
Tube 2.—2 cc. of unknown extract (1 : 300) + 0.1 cc. of normal rabbit serum.
Tube 3.—2 cc. of horse flesh extract (1 : 300) + 0.1 cc. of anti-horse serum.
Tube 4.—2 cc. of pork extract (1 : 300) + 0.1 cc. of anti-horse serum.
Tube 5.—2 cc. of beef extract (1 : 300) + 0.1 cc. of anti-horse serum.
Tube 6.—2 cc. of saline solution + 0.1 cc. of anti-horse serum.

The immune serum is added to each tube very carefully and run down the sides of the tube, or stratified. The tubes must not be shaken. The tubes are kept at room temperature. The

test must not be made with a mixture of the sera of different rabbits.

Interpretation of the Results.—If in tubes 1 and 3 a misty cloudiness should appear *within* 5 *min.*, and if a definite precipitate forms within 30 min., the other tubes remaining perfectly clear, the extract is very probably one of horse flesh or the flesh of some other single-toed animal. Precipitates which develop more slowly cannot be considered as positive. The protein of horses and donkeys cannot be differentiated by this test. In a similar manner, tests may be made for the meat of deer, dogs or any other animals, if the respective immune sera are used with the extract.

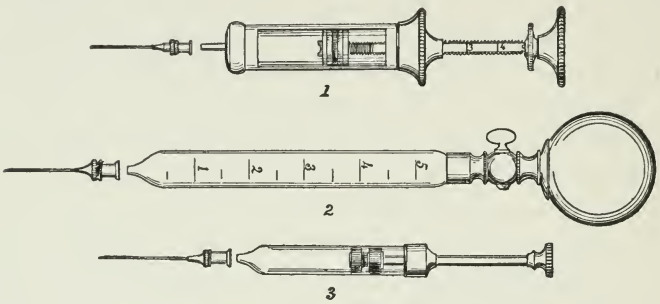


FIG. 57.—Types of syringes: 1, Roux's bacteriologic syringe; 2, Koch syringe; 3, Meyer's bacteriologic syringe. The Meyer syringe is the simplest and best for general purposes.—(*McFarland.*)

Heterologous precipitates, which occur when antisera are added to concentrated foreign protein solutions, rarely are disturbing factors of the tests when the above technique is used. The elective absorption (according to Kister and Weichardt) with the foreign protein is occasionally necessary for scientific tests.

The organoleptic tests are not always conclusive as to the quality of the meat. It is a well-known fact that the stinking or putrefactive odors are generally wholly absent in even highly decayed salted and brine-pickled fish and meats and in heavily seasoned sausage meats and in smoked meats. On the other

hand, it is advisable to reject or condemn all meats which emit offensive odors, provided such odors are not normal to the meat. Under normal offensive odors may be mentioned the fishy odor of meats from animals which feed upon fish, mussels and other aquatic animals; the sex odor which is often marked in the meats from older males; the various vegetable odors due to feeding, such as the turnip odor and taste in beef, fenugreek odor, etc., etc. Distinctively putrefactive odors in meats are a very reliable indication of their unfitness for consumption. Marked changes in consistency (sloppy, smeary and porous meats) and in color (grayish, yellowish, greenish) usually indicate advanced stages of decomposition. Some authorities have recommended that the presence of free ammonia should be the test for putrefactive changes in meats and should serve as the basis for condemnation procedures, but others point out the fact that toxins are formed even before there is any appreciable formation of ammonia. The safest guide to the quality of meats is undoubtedly the bacteriological test. As to the question on what bacteriological findings shall the quality estimates of meat be based, it is suggested that judgment be based upon the number of bacteria present and generally irrespective of kind. If exposed and comminuted meats do not contain more than 1,000,000 bacteria per gram, they may be presumed to be reasonably wholesome. The exceptions to this numerical limit are the finding of pathogenic and toxin-forming bacteria. The conclusive proof of the mere presence in meats of bacteria which are pathogenic to man is sufficient to condemn such meats. It is reasonable to assume that most bacterial invasions of meats are of the putrefactive kind and hence objectionable, and it is therefore fair and just to all concerned to fix a numerical limit at which such foods are still reasonably wholesome, as suggested. There are, however, those notable exceptions where meat contains toxins and ptomaines in quantities sufficient to produce serious and even fatal poisoning without bacteria being present, as when fresh meat has been in contact with decomposed

and toxin-bearing meats from which it has taken up the poisons by absorption. It is therefore desirable and often necessary to supplement the bacterial count by the toxicity test.

The numerical limit above suggested (1,000,000 per gram of the meat substance) pertains to bacteria found upon the exterior of the meat bulk or in the outside cells and tissues of the meat bulk or meat particles. Proper care must therefore be observed in taking samples and in preparing the sample for plating. In the case of bulk meats such as whole slaughtered animals, hams, bacon, etc., pieces as nearly cubical as possible (about 1 gram each) are removed with a sharp sterilized scalpel, the outer surface of the meat forming one face of the cube. This is to be weighed and pulped in a sterile mortar with an equal amount of sterile normal salt solution and this pulped material is then made up to the desired dilutions for plating, using normal salt solution. Gelatin media should be used for culturing and incubation should be done at 20° C. for a period of 3 days and the counts made. In the case of sausage meats and comminuted meats generally, take 1 gram quantities, pulp thoroughly and mix thoroughly with the required amount of normal saline and plate. In the case of soups and soup stocks having a meat or meat derivative base, take 1 cc. quantities, from the thoroughly mixed sample, dilute and plate.

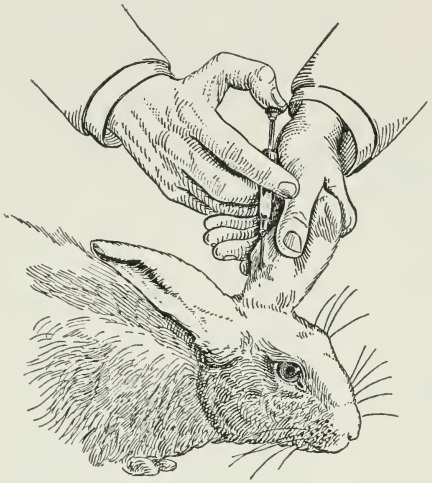


FIG. 58.—Illustrating the method of making an intravenous injection into a rabbit. The ear is manipulated to induce hyperæmia and the surface vein is compressed near the base of the ear, to facilitate the inserting of the syringe needle.—(*McFarland.*)

Weinzirl and Newton describe a method of determining the bacterial content of meat, in which the meat is ground in a mortar with sterile sand and normal salt solution to obtain an emulsion for inoculation into the culture media, and report the application of this method to the determination of the bacterial content of a number of samples of market Hamburger steak. The result showed that the standard of 1,000,000 bacteria per gram advocated as a maximum limit for the salable product is much too low, as nearly all the samples examined would be condemned on this basis, though showing no taint or other evidences of putrefaction. The authors propose a limit of 10,000,000 bacteria per gram.

For making toxicity tests of meats, broths, sausage meats, soup stocks and other meat products, the following general method is recommended. In case of solids such as meats (raw, smoked, cooked, canned or pickled), sausages, sausage meats, etc., 10 grams of a well-mixed average sample are well pulped in 10 cc. of boiled distilled water. Let stand for 20 min. with frequent stirring. Express and filter the extract through a clay bougie. The toxins being soluble will be found in the filtrate. Inject 2 cc. of the clear filtrate into the subdermal connective tissue or intraperitoneally into guinea-pigs or white mice, using three animals for each test. If one or more of the animals thus inoculated die within 48 hr., or if they show marked symptoms of intoxication without dying, the meat is unfit for consumption. In the case of soups, broths, soup stocks, chop suey and other meat products which contain liquid, the procedure is much simpler. Take suitable quantities of the thoroughly mixed sample and filter, first through filter paper and finally through the clay bougie, as for the meat extract already described, and inject 2 cc. quantities as already explained. The toxicity tests should in all cases be supplemented by the plate count.

Botulism or sausage poisoning is due to a toxin (botulin) formed by the *Bacillus botulinus* (Lat., *botulus*, a sausage), a

large anaerobic sporogenous saprophyte especially common in sausages and sausage meats, particularly in liver sausages, blood sausages, jelly sausages, in hams, in liver pate, canned meats, etc., etc. The bacillus, inclusive of the spores and the highly virulent toxins which it forms, are destroyed by boiling and thorough cooking. The digestive ferments do not destroy the toxin. The usual smoking of hams and sausages does not destroy the toxin or the bacillus. The bacillus is killed by strong brines, but this does not also destroy the toxin. The oval spores are quite readily killed by heat and chemicals. Heating to 80° C. for 1 hr. kills them. Ichthyotoxism (fish poisoning) and mytilotoxism (shellfish poisoning) are closely akin to botulism and are in all probability caused by the same bacillus or perhaps a varietal form of *B. botulinus*. The occurrence of the *Bacillus botulinus* is, however, not limited to pork and sausage meats. Well-authenticated cases are on record of the occurrence of this bacillus in canned vegetables and in domestically prepared string beans served without previous heating. There is no doubt that the heat employed in the canning process destroys the toxin formed, but the temperature may not always be high enough to kill all of the bacilli and their spores even though the spores are not very resistant to heat (80° C.). *Bacillus botulinus* does not multiply in the living organism. It grows readily in slightly alkaline media at a temperature of 18° to 25° C. At higher temperatures (35° to 37° C.) it grows only sparingly and without the formation of toxin. Cultures give out an odor of butyric acid.

In pickled, canned and otherwise prepared and preserved meats, and mixtures of meat and vegetables (chop suey, pork and beans, etc.), the processes of bacterial development are greatly modified. The use of deodorants, of preservatives and coloring agents mask or obscure many of the decomposition changes in meats. Very frequently the only cause for suspicion is an unusually heightened color or a lack of the normal meat flavor.

Sausage meats are found on the market so highly colored as to produce a red ink with the water in which they are boiled. The meat dealer tries to deceive the housewife by stating that the red color is derived from the rich red blood of the meat itself, whereas the red coloring matter of the blood is decomposed by the boiling and the boiled meat extract is only slightly colored. Very frequently pickled pigs' feet appear on the market which look quite normal, the only suspicious character being an unusual pallor of the surface with a smeary consistency and a lack in the flavor. On microscopical examination it will be found that the surface of the meat is covered or coated with yeast cells, mold hyphæ and mold spores and bacteria. The American method of making sausage and sausage meats from carelessly and promiscuously handled meat trimmings which accumulate during the day's work in the retail meat markets, is accountable for the high contamination with bacteria and other organisms (10,000,000 to 100,000,000 per gram). Such sausage meats are also very frequently colored to reduce the pallor due to the use of excessive amounts of fatty tissue trimmings, thus leading the customer to believe that there is a considerable amount of muscular (red meat) tissue present. The coloring also serves to hide the beginnings of decomposition changes in the meat. Preservatives are added to check and mask the decomposition changes which have begun to manifest themselves. It is unlawful to add coloring substances to sausage meats, but it is permissible to color sausage casings.

Numerous chemical tests for ascertaining the existence of putrefactive changes in meats have been recommended. The Ebers test appears to have met with considerable favor and is made as follows: Into a test-tube pour about 3 cc. of a mixture composed of 1 part. of pure hydrochloric acid, 1 part ether and 3 parts alcohol. This tube may be closed with a perforated rubber stopper carrying a glass rod which is pushed through the opening of the stopper so that the end almost touches the

liquid in the tube. Dip the free end of the tube into the meat pulp, meat extract or meat broth and, after shaking the tube in order to fill it with the acid vapors, insert the rod, closing the tube with the rubber stopper. If the juice or the meat particle is from decayed meat, a grayish smoky vapor appears at the end of the glass rod, which settles to the surface of the liquid. There must be no free ammonia in the room while making the test. The test is not applicable to pickled meats. This test should be made supplementary to the microscopical, bacteriological and toxicological examinations already explained. In place of the test-tube or reagent glass above recommended, the small perfume sample bottles with glass rod stoppers may be used in making the test.

FIG. 59.—*Bacillus tetani* as seen in a scraping from a wound. Some of the organisms show spore formation while others do not. The pale globules are blood corpuscles. (× 1000).—(*Kolle and Wasserman.*)

Sausage meat binders or fillers are very readily detected by means of the compound microscope. Corn starch and wheat starch fillers are most commonly employed, the object in adding them being to increase the water content of the sausage meat. Some brands of sausage contain corn meal and other cereal products. Egg albumen and tragacanth fillers are used occasionally, and it is said that it is possible to increase the water content of the meats by 30 per cent. with only 3 per cent. of the tragacanth filler. The increase in water content through the use of the starch fillers is about 5 to 10 per cent. In examining meats for starch fillers or added cereal it must not be forgotten that some of the spices used contain starch (pepper, allspice).

Graham, of the laboratory division of the Bureau of Animal Industry, has recommended a method for determining the percentage of starch added to sausages and sausage meats. A small pellet of a thoroughly mixed sample of the meat preparation is well pulped and teased out. Make the usual slide mount, using just enough of the prepared material to fill the space between slide and cover, using some pressure. Count the number of starch granules in the areas (squares) of the ocular scale and compare with the known number of similar starch granules in 1, 2, 3 and 4 per cent. mixtures of the same starch. Rarely does the amount of starch filler added exceed 3 or 4 per cent. Mr. Graham states that the method gives results accurate within 10 per cent., which is sufficiently accurate for all practical purposes.

It is suggested that the special spore and mold counter described elsewhere (*A* or *B*, Fig. 5) be used with the ocular counting scale (Whipple's) for making the starch determinations in sausage meats. The exact number of starch granules in mixtures containing 1 per cent. of starch should be carefully ascertained, following the general method recommended for finding the number of oil globules representing 1 per cent. of butter fat in milk. For determining the number of granules in 1 per cent. suspensions of the starch, it is suggested that weak solutions of gum arabic (1 per cent.) be used. The gum solution keeps the meat particles as well as the starch granules in suspension until the counting is completed. Having once determined the exact number of granules in 1 per cent. of the starch suspension, it is a simple matter to make comparative determinations of homologous starch in sausage meats, or in other substances, as may be required.

Add 1 gram of a well-mixed sample of the sausage or sausage meat to about 2 cc. of water in a suitable dish and mix thoroughly, in order to wash the starch from the meat particles. Next add enough of the gum arabic solution to make a total of 9 cc. of the liquid, thus making a dilution of 1–10. Mix thoroughly in order that the starch present in the meat may be uniformly

distributed and make the counts as for spores or yeast cells, and from the findings determine the percentage of starch which has been added. This quantitative method for determining added starch is applicable even if the starch has been dextrinized through the cooking of the sausages, provided the individual granules are still recognizable and provided also the identity of the starch is still ascertainable. Corn meal and corn starch are the more common sausage fillers used in the United States.

The above method for determining the percentages of starch in mixtures could also be employed, modified to suit special cases, in the examination of compounds of flour, of meals, for ascertaining the percentage of starch in baking powders, in almond meal, in adulterated mustard and in other products where starch or flour is used for purposes of adulteration, and to ascertain the proportions in flour or meal compounds, etc.

In frozen meats the red blood corpuscles are almost completely decolorized and disintegrated (hemolyzed), changes which are readily observed under the compound microscope. The microscope will also prove useful in the detection of added coloring substances. The micro-sublimation test will readily demonstrate the presence of benzoic and salicylic acids in meats and meat products.

The microphytic examinations of meat include the following groups of the plant kingdom:

1. **Penicillium Species.**—Especially common on hams, bacon and smoked meats generally. These molds are essentially aerobic saprophytes and are therefore found on the exterior of meats.

2. **Aspergillus Species.**—These molds are apt to occur on and in fish meats, in gelatin, in canned meats and in pickled meats.

3. **Mucor Species.**—These small molds are less common than the above. They may occur on pickled meats and on meats that are kept in damp places.

4. **Yeasts.**—Yeast cells may occur on pickled meats and,

more especially, in meats and meat products which contain starch and sugar.

5. Bacteria.—It is not necessary to enter into any extensive discussion of the different species and varieties of bacteria which may occur in and upon meats. The more important bacterial invasions of meats have already been mentioned. The following is a partial list of the more important species which the food bacteriologist may be called upon to look for in meats:

a. Bacillus botulinus.—Most common in sausages, as already

FIG. 60.—*B. tetani*, showing flagellæ.

stated elsewhere. Forms highly virulent toxins and produces rancid changes.

b. Bacillus tuberculosis.—Will be found in meats of tuberculous animals.

c. Bacillus tetani.—May occur in meat products, more especially in gelatin. It is essentially anaerobic but thrives better in association with aerobes, and it produces one of the most virulent toxins known which is, however, very unstable in its chemical composition and easily destroyed. A temperature of 60° to 65° C. destroys it and it is also very quickly destroyed on exposure to air and light. The danger from the tetanus bacillus pertains to possible inoculation with the bacillus rather than the

ingestion of the toxins, which might be formed outside of the body and absorbed by the meat.

d. Cadaver bacilli.—Under this head are included a variety of bacteria which cause putrefactive changes in dead animals and in meats, with toxin and ptomaine formation, and to which reference has already been made.

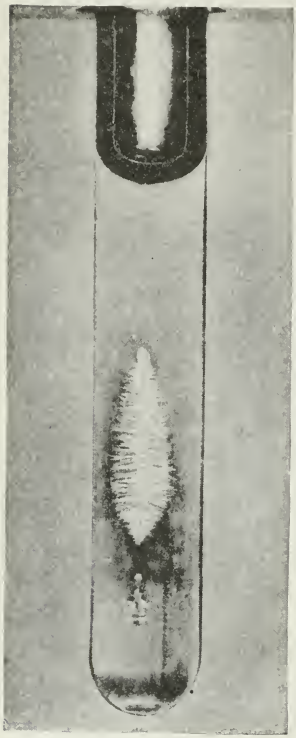


FIG. 61.—Tetanus bacillus stab culture in glucose-gelatin 6 days old.—(*McFarland, after Fraenkel and Peiffer.*)

e. Bacillus anthracis.—The anthrax bacillus may occur in all food-producing animals, and its isolation from beef and other meats may become an occasional necessity in the food laboratory.

f. Staphylococcus group.—These may occur in great abundance in living animals, causing septic decomposition changes in tissues and organs.

g. Streptococcus group.—Like the Staphylococci, these organisms produce pyemic or septic changes in living animals.

h. Numerous other bacteria may on occasion come to the attention of the food bacteriologist, as the bacillus of hog cholera, of swine plague, of swine erysipelas and others. In this connection we must not forget the possible presence in beef, and less frequently also in pork, sheep and horses, of the ray fungus (*Actinomyces bovis*) which is the primary cause of "lumpy jaw" in cattle and which disease is transmissible to man.

Examination of meats for the presence of encysted trichinæ (*Trichinella spiralis*) is incidental rather than a routine in the

food laboratory. Even if the meat is found to contain trichinæ it does not warrant condemnation procedures, because these organisms are harmless provided the meat is properly cooked before eating; however, it cannot be denied that no consumer could be persuaded to use meat thus infected. The examination of pork for the presence of encysted trichinæ was at one time a regular routine in the larger slaughtering houses of America because of the European (largely German) boycott against Ameri-

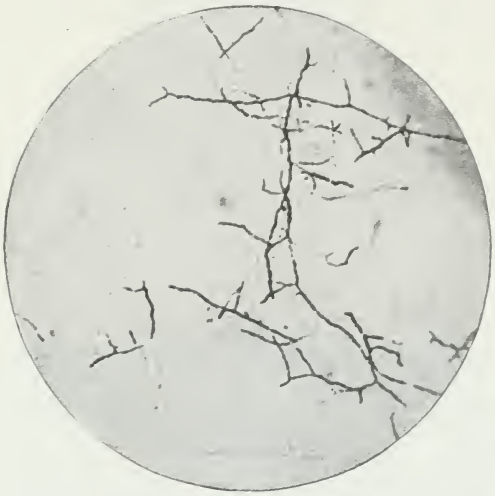


FIG. 62.—*Actinomyces bovis* from broth culture (× 1000).—(*Williams.*)

can pork. In recent years the routine examination for trichinæ has been very generally abandoned.

Trichinæ are not uniformly distributed in the muscular tissue of the animal. They are most abundant in the diaphragm, next in the base of the tongue, in the laryngeal, lumbar, masticatory, and abdominal muscles and nearest the tendinous insertions of the bones. They are never found in adipose tissue. They may occur in wild hogs, in dogs and in bears and of course also in man. To examine meat for trichinæ, cut bits

from the organs of chief distribution of the parasite. From these samples cut small flat pieces and compress between two

FIG. 63.—Colony of *Actinomyces bovis* from cow.—(*Williams.*)

glass slips and examine under the low power of the compound microscope. As a clearing agent a solution of acetic acid (1–30)

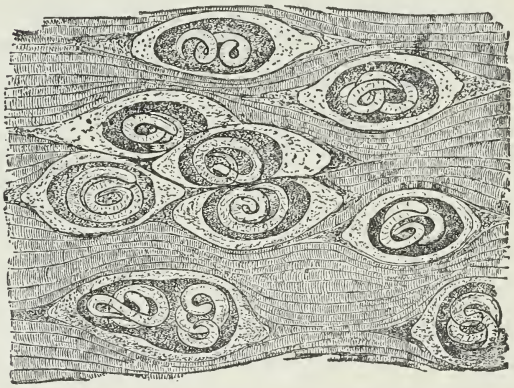


FIG. 64.—Encysted *Trichina spiralis* (*Trichinella spiralis*) in muscle tissue.—(*Stilt, after Ziegler.*)

may be used. To clear sections of salted hams or other meat, use diluted potassium or sodium hydrate. Examining minced

meats and sausages for trichinæ requires greater care and persistency.

Encysted trichinæ retain their vitality for a long period of time when kept at a low temperature, and persist even after the meat has undergone decomposition through bacterial infection. The wandering embryos are harmless and the muscle trichinæ continue their development only in another host, as man, dog or bear. In the intestinal tract of this second host they become sexually matured, growing to a length of 0.5 to 0.75 mm., and produce young in large numbers. Trichinella does not produce ova.

The inexperienced analyst might mistake vinegar eels (in pickled meats), Miescher's bodies (*Sarcocystis*), lime concretions, muscle degenerations and trichinæ-like worms (*Pseudo-trichinæ*), found in the muscles of the rat, mouse, rabbit, fowl, fish, mole and other animals, for trichinæ.

18. The Bacteriological Examination of Eggs and Egg Products

Among the foods which require the attention of the bacteriologist are eggs and egg products such as evaporated eggs, frozen eggs and dried egg albumen. Many fresh eggs are quite free from bacteria, or if bacteria are present they do not exceed negligible quantities, usually not over 500,000 per cc. Extensive investigations made by Stiles (Bureau of Chemistry) show that the contamination of eggs is in proportion to age and favorable temperature. Thus during warm weather the bacterial development is quite rapid, whereas cold retards such development. Placing contaminated eggs in cold storage checks bacterial development temporarily and even causes a reduction in the number of organisms present at the time the eggs were placed in storage, but within a short time the temporary numerical reduction in bacteria is not only regained but there is a steady

increase in proportion to the time of storage, until a maximum development is reached. The bacterial flora of the white and of the yolk of the egg differs quantitatively as well as qualitatively. It may happen that the yolk is badly infected while the white is in comparatively good condition. As a rule, however, if the yolk is highly contaminated the white is similarly affected. In fact the first decomposition changes generally take place in the periphery of the egg albumen, the infection taking place *via* the exterior of the shell.

Commercially, eggs are designated as fresh, stale, storage; firsts, seconds and thirds (when sorted as to size); watery and weak when the white is thin; heat eggs; leakers, checks and mashed when the shell is more or less broken; eggy, strong, musty, sour and stale as to odor; blood ring, sour rot, white rot, light rot, spot rots, moldy, black rots, etc., when more or less rotted and decomposed; green or grass eggs when the white is more or less green colored through the invasion of bacteria. These terms have no scientific importance and are of no significance to the food bacteriologist, beyond that of indicating the probable or likely condition and contamination and probable cause of the change or deterioration of the eggs so designated.

The old-time popular methods of testing eggs by candling, by shaking to determine "looseness," floating on brine, noting discoloration of the shell, and by the odor, have their value in practice but are far from reliable. An egg which gives off the odor of sulphuretted hydrogen is universally recognized as bad, rotten or spoiled. In Germany eggs are pronounced spoiled if the white is gelatinous in consistency (as in old eggs from which moisture has escaped) or yellowish in color (also due to age), or if the yolk is more or less adherent to the shell or is more or less mixed with the white. A fresh egg broken in the manner customary in the kitchen allows the entire contents, yolk and all, to fall out into a receptacle without rupturing the yolk. The white should be of uniform consistency, uniformly trans-

lucent and without marked yellowish or amber coloration. The yolk should be uniformly soft and entirely free from all lumpiness and should not be adherent to the shell.

Eggs are preferably used in the comparatively fresh state, that is, within a few days or at the longest 8 days after they are laid. It is, however, not always possible or practicable to use the eggs while still fresh, and egg preservation has become a very important industry. Eggs may be preserved in brine, in liquid glass and in various chemical preservatives. They may also be preserved in oil, in lard, or coated with tallow, wax or paraffin,

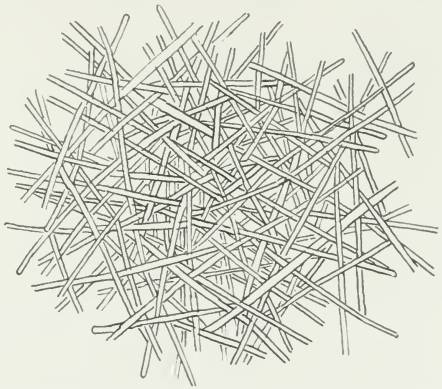


FIG. 65.—Egg membrane as seen under the high power of the compound microscope (× 450).

in order to keep out air bacteria and molds and also to check the evaporation of moisture from the interior. There is an opinion among some poultrymen that eggs will keep much longer if placed in a definite position (vertically with narrower end down). The now generally employed and preferred method for preserving eggs is to keep them in storage at a temperature as low as it is possible to make it. This is perhaps the simplest and cheapest method for keeping eggs in the natural state. However, as already stated, cold storage eggs gradually deteriorate, through bacterial invasion and through loss of moisture, in direct ratio to

time, until they finally become unsuitable for consumption. If fresh-laid eggs were thoroughly cleansed and sterilized externally, coated with sterilized wax, tallow, paraffin or placed in liquid glass and then put in cold storage, they would no doubt remain wholesome for a period of 5 months to 1 year. Under the usual conditions, cold storage eggs show marked deterioration in the course of 2 or 3 months as indicated by loss of moisture, yellowing of the albumen, softening of the yolk, loosening, increase in size of the air chamber and by the increase in the bacterial count. The increase in the size of the air chamber is due to the shrinkage of the egg mass, resulting from the loss of moisture. According to Greenlee,[1] the loss in weight of eggs is due to evaporation of moisture to the external atmosphere but the decrease in moisture of the white is not wholly due to external evaporation, as the yolk takes up a part of the moisture, thus increasing the moisture and weight of the yolk and also accounting for the increased liquidity and explaining the tendency on the part of the yolk to rupture and the white to gelatinize. Fresh eggs break well, whereas old eggs, including those kept in storage, break badly as a rule. The egg mass does not leave the shell readily, the yolk may be adherent to the shell, likewise the white, and the yolk membrane ruptures easily and the result is generally a mess.

Evaporated, dried and frozen eggs have come into extensive use in recent years. For these purposes, the cheapest and hence the poorest market eggs are generally employed. There is indeed an attempt made to cull the bad eggs at the factory, but this is, as a rule, not done in an efficient manner. The eggs are usually broken by women and the egg mass is thoroughly mixed and dried by spraying into a drying chamber or by spreading on a drying belt, or the drying may be done in very shallow pans. Of course the temperature must be kept below the coagulation

[1] Deterioration of Eggs as shown by Changes in the Moisture Content. Circular 83, Food Research Laboratory, Bureau of Chemistry, Aug. 20, 1911.

point of the albumen. Instead of drying, the egg mass may be preserved by freezing and keeping it frozen until wanted for use. The important factors are the use of fresh wholesome eggs and cleanliness. Pennington[1] summarizes the importance of cleanliness as follows: "The preparation of frozen and dried eggs parallels the milk problem. In dairying it is first necessary to obtain a cow giving good milk. Then her products must be so handled that it is maintained in good condition until it reaches the consumer, a question that has engaged the attention of sanitarians for many years and is still the subject of study. The hen seems to be more reliable as a producer of good eggs than is the cow of good milk. In either case the ignorance or carelessness of man results in the addition of multitudes of bacteria which will, and frequently do, spoil the product for food purposes. The fundamental in the handling of wholesome milk is *cleanliness*. The fundamental in the handling of good eggs is also cleanliness, a cleanliness based upon and adapted to the work to be accomplished."

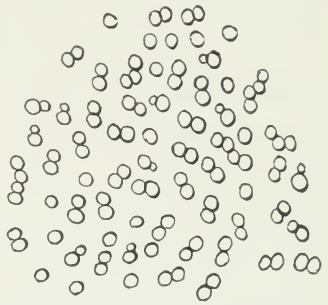


FIG. 66.—Diplococcus common in the white of storage eggs.

Recently (1913–1914), American poultrymen of the Pacific Coast have raised a hue and cry against the importation of storage eggs from China. Analysts in food laboratories have been called upon to examine these as to their suitableness for eating and cooking purposes. Barring differences incidental to accidents in shipping, the Chinese storage eggs compare favorably in quality with those cornered by the American egg trust or combine.

What is needed in food laboratories is a method for determining when an egg is or is not suitable for human consumption. Ac-

[1] Practical Suggestions for the Preparation of Frozen and Dried Eggs. Circular No. 98, Bureau of Chemistry, July 31, 1912.

cording to observations made, it would appear that the direct microscopical examination of the white of the egg will give this information. The yolk of the egg does not lend itself to direct examination because of the fat globules (cholesterin) and proteid granules present which interfere with the observation of the bacteria. The procedure as carried out in the laboratories of the California College of Pharmacy is to break the egg into a suitable sterilized dish, after having washed, dried and flamed the egg thoroughly. The egg mass is carefully tilted and poured from one portion of the shell into the other until most of the white is separated from the yolk. Mix the white thoroughly by means of a sterilized egg beater and examine under the compound microscope, making the counts with the hemacytometer. Fresh eggs contain bacteria in such small numbers as to make counting difficult. The principal organism found in the white of the egg is a coccus form, of fairly large size, having some of the characters of a diplococcus combined with those of yeasts. Multiplication appears to be by a modified budding process. After the cell has developed to maturity it sends out a second cell which at first appears as a scarcely perceptible speck or protuberance elevated above the surface. This protuberance grows larger and larger until it has the dimensions of the mother cell, whereupon the two cells separate. A chain of three cells is not uncommon and chains of fours and even fives may be found. At first these structures were believed to be proteid or perhaps lecithin particles, and in fact attempts to cultivate them in artificial media resulted in failure. There is, however, little doubt that they are micro-organisms which develop preferably in the white of the egg. They apparently do not increase in large numbers. The highest number recorded in cold storage eggs was about 180,000,000 per cc. They appear to increase in direct ratio to the age of the egg. They do not stain very readily. The most satisfactory stain appears to be carbol-fuchsin, though the organisms are not in the least acid fast. They do not stain

with methylene blue. This egg albumen organism requires further careful study.

The following general methods for making bacteriological examinations of eggs and of egg products are recommended.

To examine fresh eggs for bacteria proceed as follows: Scrub the egg well in clean sterilized water by means of a sterile hand brush. Soak in corrosive sublimate solution (1–1000) for 3 min., rinse in boiled water and wipe dry with a sterilized cotton cloth. Flame the end to be opened and set into a suitable holder (the ordinary breakfast table egg holder will answer the purpose after being sterilized), and with a sterile instrument crack open the flamed end and by means of a sterile forceps pick away small pieces of the shell without rupturing the egg membrane, making a hole large enough to introduce a sterile pipette. Rupture the egg membrane with a sterile forceps and take out 2 cc. of the white of the egg, place in a tared flask with broken glass and reweigh. Add 10 cc. of physiological salt solution and shake for 10 min., and then plate definite volumes.

To plate the yolk, break the egg in the usual manner merely being careful that the yolk membrane is not ruptured; remove the white of the egg by pouring back and forth in the two parts of the shell. Let the yolk rest in the larger part of the shell and puncture the vitelline membrane by means of a sterile forceps. Withdraw 2 cc. of the yolk and proceed as for the white of the egg.

To make bacterial counts of eggs which are quite badly spoiled (rotten eggs), simply break the thoroughly cleansed egg in the usual manner into a suitable sterilized dish and mix thoroughly (white and yolk) by means of a sterile egg beater. Let stand for 5 min., suitably covered to keep out air bacteria. Skim off the foam caused by the stirring and take up 1 cc. of the mixed egg mass and add to 9 cc. of boiled distilled water, and shake for 10 min. as for the examination of the white of fresh eggs. Make direct counts from suitable dilutions and also plate

definite quantities (dilutions as 1–10 and 1–100). Certain rotting bacteria attack eggs very readily. As all housewives know, eggs which are broken become unfit for use in a very short period of time, because of decomposition changes. The dried eggs of the market are very likely to show high bacterial counts and the manufacture of evaporated eggs, dried egg albumen and other egg products intended for use as food should be carried on under suitable methods, keeping in mind the speedy decomposition of the egg material.

Dried and evaporated eggs and dried egg albumen are examined for bacteria by the direct method and also by the plating method.

It is certainly evident that no complicated bacteriological testing is necessary to determine the unfitness of a rotten egg or an egg which is highly musty or discolored as shown by the candle test. The important problem in estimating the significance of rotten eggs is what percentage of rotten eggs may be present in an acceptable lot or shipment? It is evident that, under ordinary conditions, the bacterial count of eggs not sufficiently spoiled to be noticeable to the unaided senses (eggs taken from a lot in which there are numerous rotten eggs) will exceed many millions per cc. Condemnation of eggs for human consumption should not be based upon the percentage of rotten eggs present, but rather upon the finding of a given number of bacteria in a mixed sample of the whites of one dozen average eggs taken from the lot, exclusive of completely rotted eggs. Eighteen eggs are taken from the lot, cleaned as already suggested, the eggs broken one by one, pouring the white of each egg into a suitable container and rejecting all eggs in which the white cannot be separated from the yolk without mixing. If six or more out of the eighteen eggs are decidedly bad, the lot is to be condemned without further examination.

If twelve out of the eighteen eggs break in such a manner as to make it possible to separate the whites from the yolks,

then the whites are to be thoroughly mixed and the bacterial count made in the manner already explained. It is suggested that if the bacterial count (inclusive of the coccus form and motile forms or any other recognizable forms) exceeds 200,000,000 per cc. the eggs are not suitable for human consumption. In certain instances the limiting count should no doubt be lower. The analyst should take into consideration some of the other factors indicative of the quality of the eggs, as gelatinous condition of the whites, yellowing of the whites, tendency to adhere to the shell, etc. There is the possible occurrence of highly contaminated yolks with the white in passable condition. This is, however, a condition not likely to occur in the entire dozen selected for the count and may be ignored as a factor having any practical value in the rating of eggs as to quality.

In case the direct examination gives doubtful results, it is recommended that the plating method be resorted to for check purposes. For culturing it is advised that egg albumen be used for the bacteria in the white of the egg and yolk media for the yolk bacteria. In all media for egg bacteria egg peptone should be used instead of the ordinary meat peptone. The following media will be found useful:

WHOLE EGG MEDIUM

Contents of one egg	
Egg albumen peptone (Merck's)	1 gram
Distilled water	100 cc.

Mix ingredients thoroughly in a sterile container by means of a sterilized egg beater. Titrate to + 1.00. Filter through cotton. Tube and plate as may be desired. Coagulate carefully and sterilize as for culture media in general.

This medium is recommended for making plate counts of egg bacteria, of the yolk as well as those of the white of the egg. It will also be found an excellent medium for culturing the tubercle bacillus. For plating the egg albumen the following medium is recommended:

Egg Albumen Medium

Whites of two eggs	
Egg albumen peptone (Merck's)	1 gram
Distilled water	100 cc.

Prepare as for whole egg medium. If egg yolk bacteria are to be cultured, the following medium may be used:

Egg Yolk Medium

Yolk of two eggs	
Egg albumen peptone (Merck's)	1 gram
Distilled water	100 cc.

Several investigators have reported toxins in eggs. It is also known that some persons are peculiarly susceptible to eggs, being more or less injuriously affected on eating even perfectly fresh eggs. This phenomenon is by some ascribed to personal idiosyncrasy and others suggest that this is due to toxins present to which certain persons are perhaps peculiarly susceptible. The poisonous principles present in eggs should be more carefully investigated. The possibility of toxin formation in cold storage eggs also requires further careful study.

Eggs decompose very rapidly when the shell and membrane are broken and soon become unfit for use, due to bacterial development. The shell of the egg serves to prevent, or at least to check, for a time the development of the egg-rotting bacteria through exclusion of oxygen (of the air). It is, however, highly probable that the egg membrane keeps out bacteria even more effectually than does the shell. Shell-less eggs like those of the oviparous snakes are well protected against bacterial infection by the thick membrane, even though the eggs are deposited in the soil and in decaying rubbish. It has also been suggested that the egg membrane contains some bacteriolytic or perhaps bactericidal properties. It is declared, on fairly reliable authority, that fresh egg membrane applied to buccal inflammations and threatened abscesses will effect a prompt cure. The Chinese have used egg membranes as a medicine for many centuries.

In spite of shell and of egg membrane, the egg is gradually contaminated more and more, until finally complete decomposition has taken place. Tests made by European investigators show that fresh eggs inoculated with various molds resist penetration completely for about 1 month. After 8 weeks species of Cladosporum had entered. In 12 weeks *Phytopthora infestans* developed and still later *Rhizopus nigricans*. Other fungi which finally developed in the interior of the eggs were *Cladosporum herbarum*, *Aspergillus niger*, *Penicillium glaucum* and some yeasts. Fresh egg albumen is said to have marked bacteriolytic properties, but such properties are certainly quickly lost upon exposure to the air and light. Some eight or more species and varieties of bacteria are concerned in the decomposition of eggs, principally aerobes. Some of these liberate sulphuretted hydrogen (*Bacillus oogenes hydrosulphureus* group); others belong to the *B. coli* group and still others cause decomposition of the white without any pronounced color development. Liquefaction of the white as well as of the yolk is the most marked physical change in eggs undergoing bacterial decomposition. Mold infection is very generally indicated by an odor of mustiness. Pronounced mold infection is further indicated by spots shown in candling.

19. The Bacteriological Examination of Pharmaceutical Preparations

Thus far practically nothing has been done as to the bacteriological examination and standardization of medicinal substances. There is a popular belief that the ordinary pharmaceuticals, particularly the tinctures and the fluidextracts, are quite free from bacteria, the supposition being that these substances are in themselves highly antiseptic. This is only partially in accord with facts. Stronger alcoholic solutions of potent drug constituents no doubt inhibit the more rapid multiplication of most bacteria and higher fungi, but it is known that weak solu-

tions (1 per cent.) of pilocarpine, atropine, cocaine, morphine and of ergot, on standing for a time, show many millions of bacteria per cc., often also molds, mold spores and some yeasts. The variation in the resisting power of different bacteria to different medicinal substances is noteworthy. The pus staphylococci die at once in ether and in a saturated solution of quinine, but will remain active in a 10 per cent. solution of cocaine, while a 2 per cent. solution of morphine kills them in 24 hr. The same organisms will resist the action of pure glycerin for 6 to 8 days. Ten per cent. iodoform, glycerin, camphorated oil (1–10), solutions of apomorphine (0.2–20), quinine (1–10), antipyrin (1–2), and cocaine (1–10) are usually quite free from bacteria. The coal-tar derivatives are generally considered antiseptic in property. Aquæ are frequently found to contain bacteria in enormous numbers and the syrups are generally more or less contaminated with yeasts, bacteria and also with molds. It is known that weak solutions of substances intended for hypodermic and intravenous use, when left exposed to the air for a time, show numerous bacteria. Tinctures and fluidextracts are always more or less contaminated, showing organisms in direct proportion to age and the degree in unsanitary factory conditions. Certain medicinal substances, as those intended for hypodermic and intravenous use, are presumably free from living organisms. Undoubtedly the extensive bacteriological examination of medicamenta would reveal some of the causes which are responsible for irregularities in drug action, and would explain some of the hitherto perplexing phenomena of poisoning resulting from the administration of certain medicamenta in ordinary medicinal doses.

The bacteriological examination of medicamenta may be outlined as follows:

Direct microscopical examination.
1. Bacteria.
2. Molds.

3. Mold spores.
4. Yeasts.

Plating methods.
Colon bacillus test.
Tetanus bacillus test.
Tests for the staphylococcus and streptococcus groups.

In securing samples for examination, the precautions necessary to guard against outside contamination must be observed. Only rarely will it be necessary to pack the samples in ice. In the preliminary routine of the laboratory, many of the more extensive contaminations will be apparent to the unaided senses. Thus a change in color, in odor, in taste, and opacities in substances that should be clear, sediments in substances that should be free from deposits, etc., generally indicate decomposition changes due to bacteria and other organisms. More or less cloudy deposits with a clear supernatant liquid indicate possible spore sedimentation. Extensive contamination by bacteria, yeasts and molds may be estimated quantitatively by means of the hemacytometer and other suitable counting devices. All are agreed that the counts for medicinal substances intended for administration per mouth should not be very high. However, no numerical standards have as yet been adopted. It is suggested that such remedial agents are unfit for use when they contain bacteria in excess of 5,000,000 per cc. and yeasts and spores in excess of 500,000 per cc. Plating methods should be resorted to in order to ascertain the number of living bacteria present. The potassium tellurite may also be tried in order to ascertain microbic invasion.

All substances containing gelatin, intended for hypodermic use or for application to mucous membranes or to abraded skin surfaces, should be tested for the anaerobic tetanus bacillus (*Bacillus tetani*). Comparatively large quantities should be plated in large Petri dishes or in Erlenmeyer flasks (using agar media), and incubated at 37° C. in the absence of oxygen. Oxygen may be excluded by pouring a layer of sterile olive oil over the

medium or by displacing the air by means of the hydrogen apparatus. The colonies which appear should be examined microscopically to ascertain whether or not the characteristic spore-forming tetanus bacillus (drum stick bacillus, the Trommelschläger Bacillus of the Germans) is present. As a confirmatory test, a suspension of the suspected colony should be injected hypodermically into guinea-pigs or white rats and symptoms noted. Should other than the spore-forming bacteria be present in the anaerobic culture, these may be killed by pasteurizing for 1 hr. at 80° C., at which temperature all organisms excepting the spores of the tetanus bacilli are killed. Again incubate at 37° C. for several days and examine microscopically, and make inoculation tests as already suggested. The finding of a single tetanus bacillus (as represented by a single colony in the anaerobic culture) in the gelatin renders it unfit for use.

To test medicinal substances of all kinds in powdered form, the plating method must be relied on very largely, as the bacteria which might be present would be hidden or obscured by the granular particles present. However, extensive yeast and mold contamination could be detected readily and estimated quantitatively by the direct microscopical method. The qualitative test for this class of substances is very largely limited to the determination of the absence or presence of the colon group, the staphylococcus group and the streptococcus group. Face powders and dusting powders should be free from any considerable contamination with the pus-forming organisms. There should be uniformly standard methods governing the manufacture of all medicamenta, intended for internal or external use, which must be touched by the hands of the manufacturer. There must be absence of all skin diseases and of transmissible contagions of all kinds. There should be specific requirements as to personal cleanliness and the sanitation of the laboratory. Just as typhoid carriers, cholera carriers and diphtheria carriers employed as servants in the household and as laborers in the factory

may spread infections among those with whom they are brought in daily contact, so may the manufacturing pharmacist convey disease to his customers, through the articles which he offers for the cure of disease. This subject should receive more attention on the part of health officials.

Skin and scalp infections (acne, boils, abscesses, carbuncles) are traceable to the use of powders and ointments. The more common infections which may be carried by the usual hand prepared face powders and face and scalp lotions and ointments are pus streptococci and staphylococci, the colon bacillus and the tubercle bacillus. The most common of these infections are the staphylococcus group of pus germs and the germ of tuberculosis. Very few women who use face powders persistently for a long time escape without more or less severe facial infections. Particularly is this true of women who use the more or less irritating chemical skin renewers, that is, so-called cosmetics which act by removing the superficial epithelial layers of the skin. The use of these highly irritating agents is generally followed by the application of the germ-carrying dusting powders and ointments, the more or less raw skin favoring the infection.

The finger-nail deposits carry many different kinds of germs accumulated by the skin and scalp scratching process and through the manifold manipulations of all manner of articles during the daily work. These various contaminations may be transmitted to the hand manufactured toilet and face preparations offered for sale in the retail drug stores. Among the bacteria most commonly found with the finger-nail deposits are streptococci, staphylococci and the colon bacilli. Less commonly the itch mite and the larvæ of intestinal parasites and molds are found among the finger-nail deposits.

To examine finger-nail deposits, scrape the nail of the thumb and second and third fingers of the right hand (in the case of right-handed persons) and make the ordinary smear mounts, using such stains and reagents as may be required to bring out

the staining properties and the morphological characteristics of the different kinds of bacteria and of other organisms. Cultural methods may be desirable for purposes of identification.

The bacteriological testing of ampuls and all medicamenta intended for hypodermic, intravenous and intramuscular use is reduced to great simplicity. Since these substances must be absolutely sterile, the finding of living bacteria (by the plating method) would be proof that they are unfit for use. It must be borne in mind, however, that cloudiness in ampuls (containing substances which should be clear) does not necessarily indicate bacterial contamination, as this condition is frequently the result of chemical change, possibly occasioned by the alkalinity of the glass used in making the ampuls. However, all ampuls which show cloudiness when they should be entirely clear are to be rejected, even though no living organisms are present. Oils, salves and plasters may be examined directly (microscopically), noting in addition to bacteria and other living and dead organisms, possible decomposition changes in oils and fats, as indicated by the presence of the characteristic fat crystals. For plating, oils may be emulsified with measured quantities of the liquefied gelatin or agar media and measured quantities poured into Petri dishes; or definite quantities (0.1 cc., 0.01 cc., 0.001 cc., etc.) may be planted into the Petri dishes in the regulation manner and the liquefied gelatin or agar poured over it and spread. Incubate for from 3 to 4 days at a temperature of 20° C. and count the colonies formed. Plasters and salves may be plated by liquefying them at a temperature not to exceed 40° C., making the desired dilutions with sterilized olive oil.

20. The Microscopical and Bacteriological Examination of Syrups

Syrups are very important products extensively used in medical and pharmaceutical practice and at the soda fountain, and for practical purposes may be grouped as follows:

1. Medicinal syrups.
   a. Officinal, simple and medicated.
   b. Patent and proprietary medicated syrups.
   c. Medicinal preparations containing syrup or saccharine substances.
2. Soda fountain syrups.
3. Fruit juices containing sugar. Fruit juice concentrates.
4. Syrups, molasses, treacle.

Syrups contain cane sugar in variable amount. Many of them also contain variable amounts of invert sugars. The pharmaceutical syrups are numerous and may be prepared with sugar or simple syrup. The chemicals and therapeutic agents which are added undoubtedly have some influence on the keeping qualities of the preparations, but thus far no one has made any extensive report on the contaminations of medicinal preparations containing sugar or syrup. The soda fountain syrups are essentially sweetening and flavoring agents used in the preparation of the familiar soda fountain beverages, ice creams and sundaes. They may also contain physiologically active ingredients such as caffeine, cocaine, cocoa, ginger, etc.

Manufacturers have more or less difficulty in preparing syrups which will endure without spoiling. Simple syrup and the fruit juices in particular are likely to undergo yeast fermentation, and in many instances there is also mold development, more commonly the *Penicillium glaucum*. The contaminations of medicinal syrups and medicines containing syrup are very variable. Some of these preparations keep for a long time, while others appear to be quite susceptible to the invasion of yeasts. In many instances the initial yeast invasion is soon followed by the development of bacteria and also mold.

Among the organisms which are most likely to attack syrups and solutions containing sugar are the so-called potato group of bacilli. The most common and most destructive members of this group are *Bacillus vulgatus*, *B. liodermos*, *B. mesentericus fuscus*, *B. mesentericus ruber* and *B. levaniformans*, which latter species is really the group type. These bacteria are widely distributed in

nature, occurring in the soil and in surface waters, and because they very frequently comtaminate potato cultures, are known as the potato group. They are spore forming, which spores are

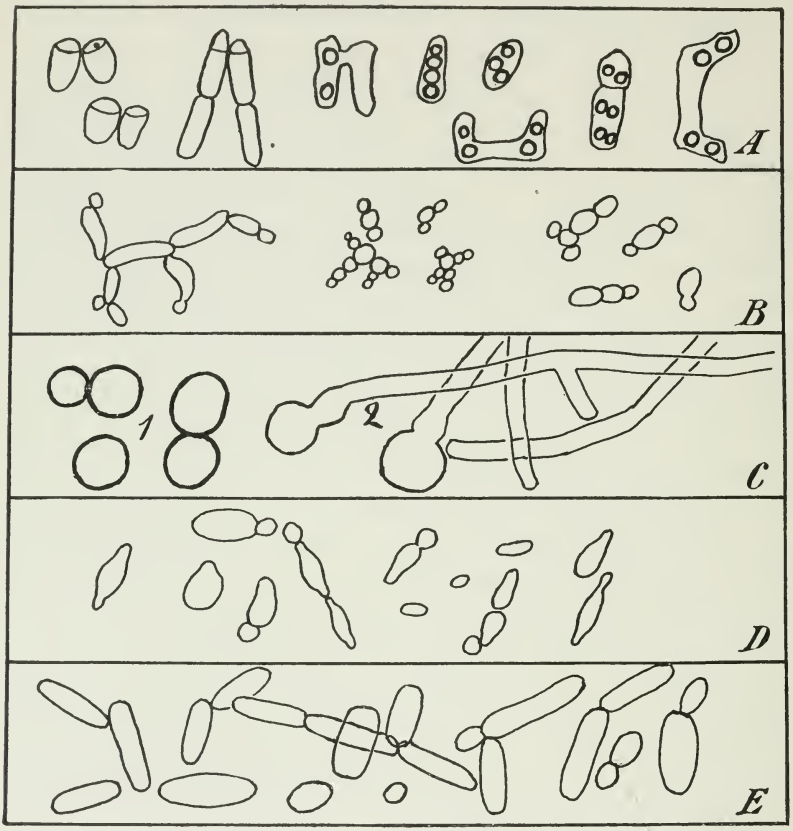


FIG. 67.—Wild and pseudo-yeasts. *A*, *S. pombe.* (*After Lindner*); *B*, *Torulæ.* (*After Pasteur*); *C*, *Mucor*, (1) spores; (2) germinating spores and mycelium; *D*, *S. apiculatus; E*, *Mycoderma vini.*—(*After Bioletti.*)

remarkably resistant to heat, being able to withstand boiling for 2 hr. Lafar maintains that they will resist the temperature of streaming steam for 6 to 7 hr., thus making them the most re-

sistant of all bacterial spores. They are further characterized by their ability to form gummy products on potato, on bread and in sugars.

Bacillus mesentericus niger and *B. granulatus mesentericus* are also members of the potato group and may be found in sugars and syrups. *Leuconostoc mesenteroides* and *Bacterium gelatinosum betæ*, which are frequently found in sugar beet juice, are evidently related to the potato group. *Bacillus gummosus*, also a gum former, is frequently found in digitalis infusions. This latter bacillus is comparatively large, feebly motile, forms spores and produces lactic and butyric acids. Another organism which is probably closely related to the potato group is the *Bacterium mesentericus panis viscosum*, the cause of stringy or slimy bread. All of the organisms named are Gram positive, are spore formers, liquefy gelatin and are motile, having flagellæ. The principal biologic characteristics of the group may be given as follows:

1. They form gums (levan) from sugars.
2. The spores are very resistant to heat.
3. They have a very low nutrient requirement.

Very minute quantities of sugar are sufficient to induce them to grow and multiply, resulting in the transformation of some of the sugar into a gum known as levan.

From the above general characteristics of the sugar bacteria it is almost self-evident how they may cause very serious harm to solutions of all kinds containing sugar. The highly resistant spores make thorough sterilization difficult and, since high temperatures cause inversion of sugars, the use of the autoclave and repeated and prolonged heating at the boiling temperature are frequently not permissible. In cases where the use of the autoclave is permissible, a single exposure at a temperature of 120° C. for a period of 30 min. is sufficient to kill the spores. More generally the fractional method of heat sterilization must be employed.

It is a noteworthy fact that after the gum formers have

once gained access to the syrup they are not easily exterminated. This emphasizes the importance of great care and cleanliness in the preparation of all syrups. It is also a fact that high concentrates of sugar are not so liable to be attacked as are the weaker solutions. The most favorable strength of sugar solution

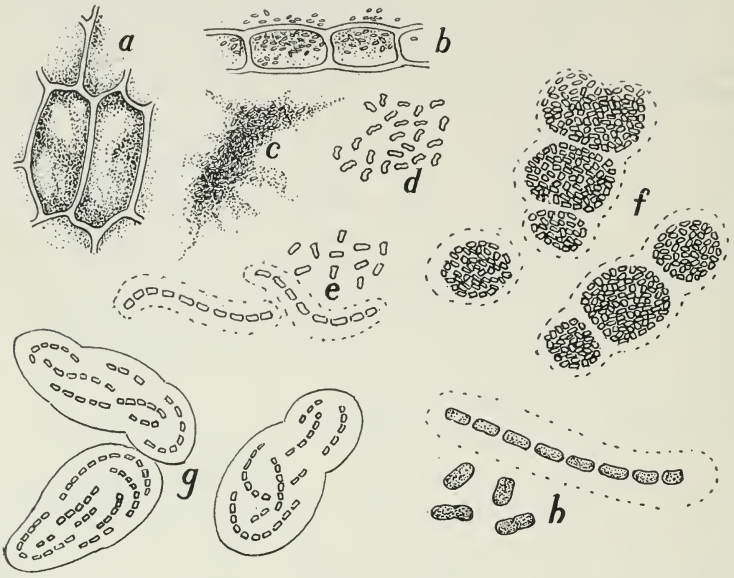


FIG. 68.—*Bacillus californiensis* isolated from the roots of the sugar beet. A typical gum former found in the soil, on the roots and in the surface tissues of the sugar beet and in the juice of the sugar beet. *a*, Beet root cells showing a mixture of cell plasm and *B. californiensis* (mycoplasm); *b*, epidermal cells of the root showing bacteria within the cell and also on the exterior; *c*, a bit of mycoplasm removed from the cell by pressure; *d*, *B. californiensis* removed from the cells by pressure; *e*, *B. californiensis* from a pure culture in beet root gelatin; *f*, zoogloea form of *B. cal.*; *g*, gelatinized form of *B. cal.*; *i*, a single chain highly magnified (× 450 to 1000).

is about 20 per cent. Development may, however, take place in 60 per cent. solutions. The organisms are facultatively anaerobic and their growth is greatly accentuated by free aeration. Completely filling the containers will materially check and even completely prevent the growth of the bacteria.

The bacterial diseases of syrups and substances containing sugar are by far the most important and the most difficult to combat. We must, however, not forget the far more common fermentation and decomposition changes induced by the yeasts and molds. Yeasts are very likely to attack the openly exposed fruit juices and fruit syrups at the soda fountain. Crushed fruits of the soda fountain may contain yeasts and also bacteria and molds. Fruit products invaded by yeasts are no longer suitable for use at the soda fountain. The attempt to render fermenting fruit juices usable by heating is not practicable as the natural flavor is lost, and the attempt to use such fruits or fruit juices would prove disastrous to the soda fountain business.

Little is known regarding the influence of the therapeutically active ingredients of the medicinal syrups on the development of the sugar-destroying bacteria but it would appear from the reports of some observers that they do not materially check them. It is quite evident that the carbonated sugar-bearing soda fountain drinks are just as liable to bacterial invasion as are the noncarbonated soft drinks. The sugar-destroying gum formers frequently do great damage to the soda bottling business, at times ruining the entire output. The trouble may be slow in making itself evident. For a long time all appears to be well at the factory but gradually complaints come in from the dealers (distributors) and the consumers. It is claimed that "gelatinous lumps" appear in the drink and that the taste is insipid and not sufficiently sweet. Very naturally these complaints are detrimental to the business. With proper attention to details at the factory the trouble could have been avoided. In the manufacture of soda fountain syrups and medicinal syrups, one of the most important essentials is thorough sterilization of the syrup to be used. The other ingredients which enter into their composition should also be thoroughly sterilized. All containers should be thoroughly sterilized and they should be completely

filled while the containers and the syrup are still hot and then hermetically sealed by means of sterilized stoppers.

In the case of medicinal syrups and sugar-bearing medicines which are contaminated by bacteria, it must be borne in mind that the active constituents present are also more or less completely decomposed. Such substances should be quite free from contamination and the presence of marked contamination should

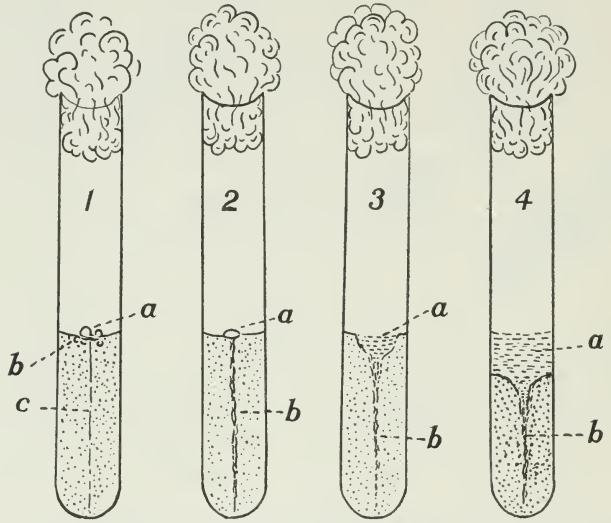


FIG. 69.—Stab culture appearance of *B. californiensis* in beef extract gelatin tubes. 1, Appearance of growth on third day after inoculation; 2, deep stab culture 24 hr. old, from tube (1); 3, same as (2) 36 hr. old. Liquefaction of gelatin is noticeable; 4, same as (2) 3 days old. In the course of 2 weeks the entire contents of tube became liquefied.

form the basis for the condemnation of such products. The adoption of a numerical bacterial, yeast and mold standard would appear highly desirable.

Among the products which are classed with the syrups are syrup or molasses and treacle from the sugar cane, and the sorghum molasses of the central and northern states, maple syrup and other syrups of commerce including the so-called corn syrup which

is largely starch glucose, the glucose syrups, honey and other syrupy substances used as a food and condiment. As a rule these do not come to the notice of the bacteriologist. A microscopical examination may be made occasionally. For example, the finding of pollen grains may be the means of distinguishing between true and imitation honey. Molds occasionally attack syrups and less frequently yeast cells may be found in some of the improperly stored syrups.

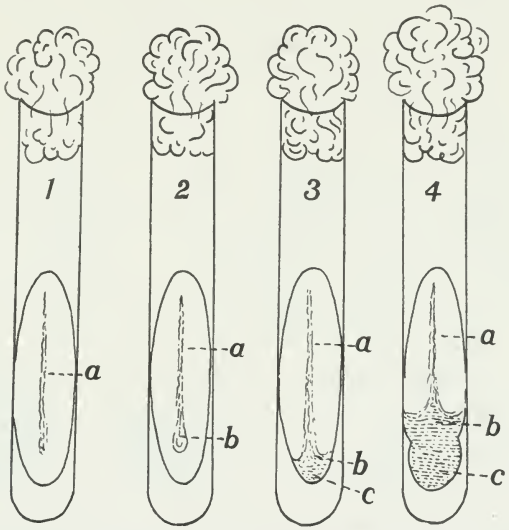


FIG. 70.—Streak culture appearance of *B. californiensis* on beef extract gelatin. 1, 24 hr. old; 2, 48 hr. old; 3, 72 hr. old; 4, 4 days old.

The bacteriological examination of sugars, candies and condiments is wholly incidental and need not be discussed. Analysis of this class of substances is left almost entirely to the chemist and the micro-analyst.

The bacteriological and microscopical examination of crushed fruits, fruit juices and fruit juice concentrates is much the same as for syrups. The crushed fruits at the soda fountain are very prone to yeasty fermentation during the hot summer months.

Grape juice is apt to become moldy. The nature of the contamination will depend upon the relative amounts of sugar and acids present.

21. The Microscopical and Bacteriological Examination of Fermented Foods and Drinks

The examination of alcoholic drinks and other fermented liquids and fermented food substances including certain fermented products used in the preparation of foods, on the part of the bacteriologist, is of minor importance and is largely supplementary to the analyses of the chemist and the organoleptic testings of the expert taster. The methods of procedure will be largely limited to the microscopical examination of concentrates, of natural and centrifugalized sediments, of sedimentary suspensions and of surface formations or deposits, with a view to the detection of added or other impurities and the recognition of abnormal fermentative changes, and invasions by objectionable bacteria, yeasts and molds. "Diseased" or "sick" wines, beers, porters, ales, vinegars, pickles, sauerkraut, etc., should be carefully examined as to the quantity and identity of the objectionable organisms present. In order that the report of the bacteriologist may supplement the report of the expert taster, it is absolutely essential that the bacteriologist have a thorough knowledge of the microscopical appearance of normally fermented products. This knowledge may be gained only through experience. The yeasts and other organisms concerned in normal wine fermentation are well known to the specialists who have made a long study of wine ferments.

There appears to be no recognized standard as to the number or kind of organisms which may be permissible in properly fermented and properly clarified wines and in other fermented drinks, nor does the present status of the subject warrant the adoption of numerical limits as to the organisms present. There

are, however, many instances in which the findings of the bacteriologist may be final and conclusive as to the quality and purity of the wine or other fermented alcoholic beverages or of fermented food products. If, for example, there is abundant mold forma-

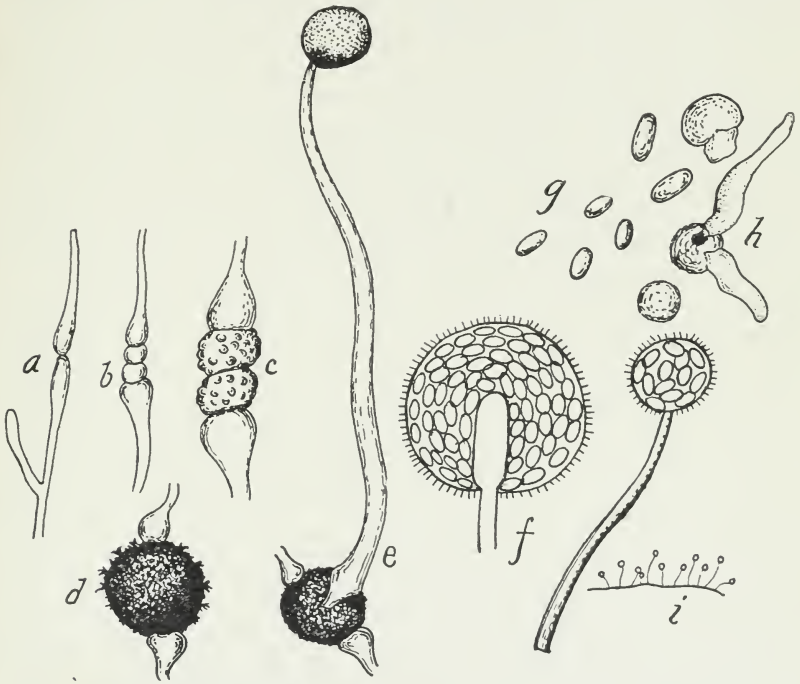


FIG. 71.—Development of *Mucor mucedo*. *a*, *b*, *c*, *d*, stages in the formation of the zygospore; *d*, mature zygospore; *e*, *f*, endospore formation; *g*, endospores; *h*, germinating spore, the beginning of the new zygospore forming cycle. The appearance of the stalks and the spore bearing capsules explains why this is called the "pin cushion fungus." Related molds occur on stale bread, on fruits, on damp gloves and leather generally. It is the cause of a fatal infectious disease in houseflies.

tion in a product which normally should be free from such organisms, then the product should be pronounced unfit for human use. Again it may be possible to recognize abnormal bacterial or perhaps abnormal yeast development as the causes of the deterio-

ration and undoubtedly the microscope alone will in most instances reveal the presence of numerous abnormal and objectionable organisms, even before the expert taster has been able to appreciate any abnormal alteration in flavor or in bouquet.

The following is a very brief outline of the principal fermentations concerned in the manufacture of alcoholic beverages.

A. Alcoholic Fermentation.—The alcohol forming ferments or zymases, or yeast ferments proper, are by far the most common and most widely distributed in nature and the most important from a commercial and economic standpoint. The zymases act upon sugars splitting these into alcohol and carbonic acid gas, thus acting upon the end products formed by the diastases and preparing them for the action of the acid forming ferments.

Zymases are formed by a great variety of plants and animals, more generally by the so-called yeast plants (the Saccharomyces and Torula groups). The alcohol-generating enzymes formed by these plants are capable of being isolated or separated from the living cells which form them and may continue the fermentative activities indefinitely. Alcoholic fermentation is by no means a simple process. The degree of alcohol production and by-product formation varies greatly, depending upon a great variety of factors and influences. To enter into a fuller discussion of the details of the fermentative processes and a description of the organisms involved, is not practicable or essential for the present purpose. The number of saccharine substances capable of undergoing alcoholic fermentation is legion, and it has thus far not been possible to ascertain the number and variety of yeast organisms and associated organisms which are involved in the multitudinous fermentations (natural and artificial) resulting in the formation of alcohol. In commercial practice (in the manufacture of wine, beer, brandy, etc.), a distinction is made between upper yeasts, lower yeasts, wild yeasts, etc. In some breweries lower yeasts are the chief fermenters used and in others the upper yeasts are preferred. For example, it is claimed

that the use of bottom yeast (Unterhefe) makes it easier to guard against the entrance of wild yeasts and other objectionable organisms. On the other hand it is claimed that the use of the upper yeast (Oberhefe) yields a better quality of beverage. These are factors of the greatest importance to the bacteriologists and zymologists employed by the breweries but concern the food bacteriologist but little.

The following are some of the more important yeast organisms concerned in alcoholic fermentation, giving the principal fermentative activities of each. Hansen's differentiation between the genera Saccharomyces and Torula is based upon sporulation. Saccharomyces forms spores (Ascospores; usually four spores in each ascus or spore sac, rarely eight) whereas Torula does not form spores. According to some authorities this is not a practical basis of differentiation.

Saccharomyces

cerevisiæ Hansen. A typical top yeast.
pastorianus, Hansen. A bottom yeast.
intermedius, Hansen. A rather feebly acting top yeast.
validus, Hansen. A top yeast.
ellipsoideus, Hansen. A typical bottom yeast.
turbidans, Hansen. A bottom yeast the cause of turbidity.
willianus, Saccardo. A flavor-producing yeast.
boyanus, Saccardo. Causes turpidity in beer and wine.
logos, van Laer. A bottom yeast developing a flavor.
thermanitonum, Johnson. A rapidly acting ferment.
ilicis, Grönlund. A bottom yeast; isolated from Ilex.
aquifolii, Grönlund. Also isolated from Ilex species.
pyriformis, Ward. Found in ginger beer.
vordermanni, W. and P. Isolated from Arrak.
sake, Yabe. Active in the fermentation of saké.
batatæ, Saito. In yam brandy.
cartilaginosus, Lindner. Isolated from Kephir.
multisporus, Hansen. A top yeast.
mali, Kayser. A cider ferment.
marxianus, Hansen. A wine ferment.
exiguus, Hansen. In beer wort.
jorgensenii, Lasche. Causes turbidity.
zopfii, Artari. Found in syrup.

bailii, Lindner. In beerwort.
hyalosporus, Lindner. In beerwort.
rouxi, Butroux. Found in fruit juices.
soya, Saito. In soya sauce.
unisporus, Hansen. In dutch cream.
flava lactis, Krueger. Found in cheesy butter.
hanseni, Zopf. In cotton seed meal.
minor, Engelman. Found in bread.
membranaefaciens, Hansen.
anomalans, Hansen. Causing a fruity flavor.
saturnus, Klöcker. Isolated from soil.
acidi lactici, Grotenfeldt. A milk-curdling yeast.
fragilis, Jörgensen. Found in Kephir.
barkeri, Saccardo. In ginger beer.
ludwigii, Hansen. From oak bark extract.
comesii, Covara. From millet seed.
octosporus, Bevjerinck. On dried currants.
mellacei, Jörgensen. A top yeast developing a pleasant odor.
guttulatus, Robin. Found in a rabbit.
capsularis, Schionning. From soil.

According to Hansen, Torulas also occur in great variety. The *Levure de sel* is a yeast capable of developing in a 10 to 15 per cent. sodium chloride solution. Those desiring to obtain detailed information regarding the complete fermentation processes involved in the brewing of beer and other fermented drinks, must consult the special technical treatises of which there are many available.

B. Acid-forming Ferments.—Dilute alcohol upon standing exposed to the air, gradually becomes sour, losing its alcohol more and more. This loss of alcohol and gain in acidity is due to the action of ferments which split the alcohol into acetic acid and water. The organisms which produce the acid-forming ferments or enzymes mostly belong to the group bacteria (bacilli). The more common and important species are *Mycoderma* (*Bacillus*) *aceti*, *B. Pasteurianum*, *B. kützingianum*, *B. oxydans* and *B. acetosum*. The yeast *Saccharomyces mycoderma* is also capable of forming acetic acid. The vinegar organisms are most active at a temperature of 25° C. to 30° C. They are very slowly active

at 10° C. and are killed at a temperature above 35° C. The so-called mother of vinegar consists of an agglutinated mass of *Mycoderma aceti* and is used as a starter in the manufacture of vinegar. Thus far it has not been possible to isolate the vinegar ferment or enzyme from the living cells which form it.

There are also acids of nonalcoholic origin formed by living ferments, such as oxalic acid, malic acid, citric acid and others,

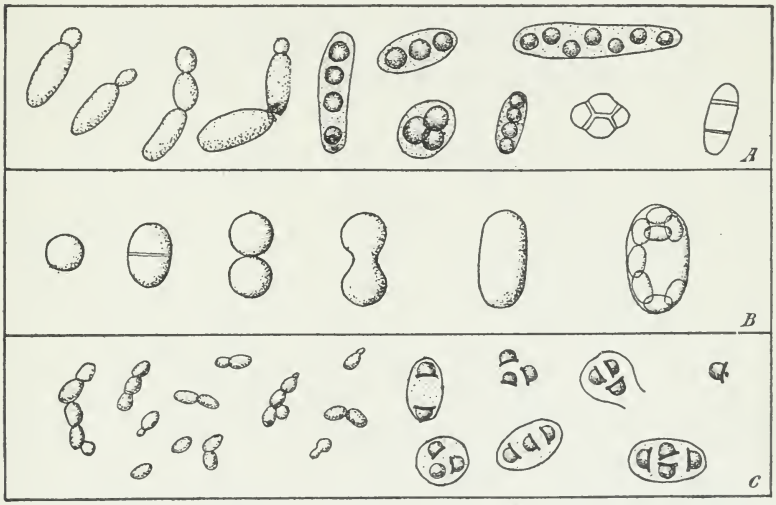


FIG. 72.—Types of yeast organisms and yeast sporulation. *A*, *Saccharomyces pasteurianus* showing spore formation in fours and eights (*after Bioletti*); *B*, *Schizosaccharomyces octosporus*, showing simple septation instead of budding, and spore formation (*after Schiönning*); *C*, *Saccharomyces anomalus*, vegetative cells and spore sacs.—(*Marshall, after Kayser.*)

which appear to be derived from the direct fermentation of sugars. Citric acid is formed from sugars through the activity of two fungi, *Citromyces pfefferianus* and *C. glaber*. *Saccharomyces hansenii* forms oxalic acid from mannit and galactose, without the intermediary alcohol formation.

The following are the more important products in which there is alcohol formation through the action of yeast organisms.

1. **Whiskey and Brandy.**—Whiskey and brandy are alcoholic beverages with an alcohol content ranging from about 44 per cent. to 55 per cent. (by volume). Whiskey (*Spiritus frumenti* of the U. S. P. and Schnapps of the Germans) is usually made from grain as rye, wheat, barley and corn. Brandy (Branntwein)

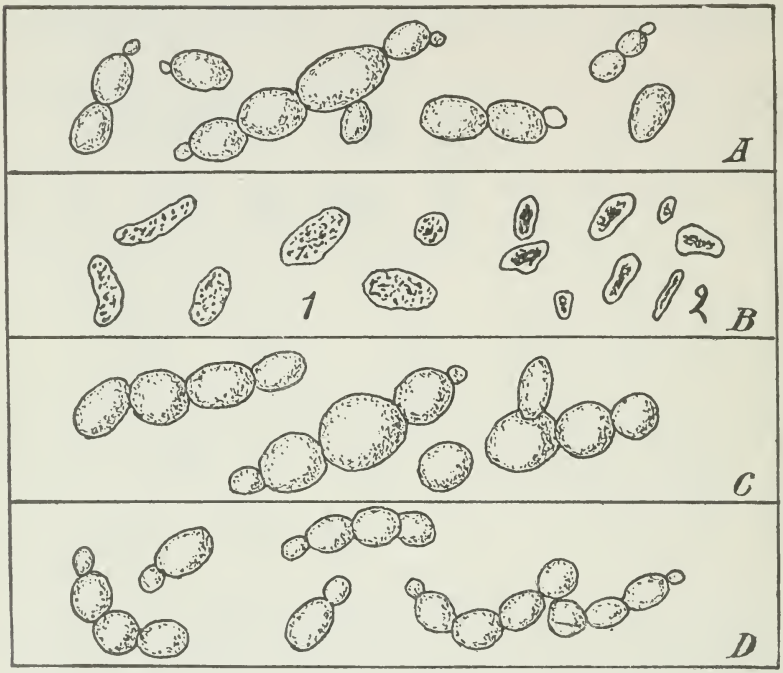


FIG. 73.—Wine and beer yeasts. *A*, *Saccharomyces ellipsoides* showing the young and vigorous cells; *B*, the same cells old (1) and dead (2); *C*, *S. cerevisiæ* as top yeast and *D*, *S. cerevisiæ* as bottom yeast.—(*Marshall.*)

is usually made from grapes. In the manufacture of both whiskey and brandy there is alcoholic fermentation followed by distillation, with or without the addition of coloring substances, as caramel. In both whiskey and brandy, certain collateral products of distillation known as congeners, such as flavor (bouquet), aldehydes, ethers, trace of fusel oil, trace of fruit or grain color, of

acids, etc., are present. These congeners are normally present and vary somewhat, dependent upon variations in the method of distillation, slight differences in the quality of the grain or fruit used, the process of fermentation, temperature, etc. With ageing whiskey as well as brandy undergo complex changes (chemical as well as fermentative) indicated by changes in color, odor and taste. These slow changes appear to be largely zymotic in nature but they are not well understood.

Whiskey and brandy may be made from all substances capable of undergoing alcoholic fermentation, such as rice, wheat, barley, rye, oats, potatoes, apples, pears, berries of all kinds, etc. Any apparatus so constructed and equipped as to vaporize and carry over and condense the alcohol existing in the fermented product, may be used in distillation. In the process of distillation certain congeners are always carried over with the alcohol and these constitute normal inclusions of the brandy or whiskey. If the congeners are poisonous or otherwise objectionable, then the distillate containing them is also poisonous or otherwise objectionable and may render the product unsuitable for human use. These poisonous congeners evidently exist in certain products of alcoholic distillation and should be more carefully investigated.

Alcohol *per se* (less all congeners) is a protoplasmic poison. Small quantities, when taken into the system are oxidized and in so far as it is oxidized, alcohol is a food, but because of its toxic character, alcohol can never be used as a food having practical value as such.

Rectified whiskey or brandy is redistilled or double distilled whiskey or brandy. As a result of this redistillation there is an increase in the alcoholic strength, with a decrease in the amount of fusel oil, a change or decrease in the congeners, a change in the color and bouquet or flavor, etc. As generally comprehended rectification implies purification and increase in alcoholic strength, without foreign additions of any kind. Adding coloring sub-

stances or flavoring agents to raw (unaged) whiskey or brandy so as to imitate the product which has been allowed to age naturally, constitutes adulteration under the Federal Pure Food and Drugs Act. Adding whiskey or brandy to alcohol (ethyl), commonly known as rectified spirits, does not make rectified whiskey or brandy.

Various medicamenta may be added to whiskey and brandy, such as caraway, aloes, juniper berries, absinthium, etc. Many alcoholic beverages are sold to the unsuspecting public under the guise of tonics and blood purifiers.

2. Beer.—Beer is a fermented drink generally made from barley. The carefully selected grain is washed in running water and then macerated in water to induce germination. This process liberates the ferment diastase which occurs in the grain and this enzyme acts upon the starch present converting it into saccharine compounds. The saccharine compounds are next acted on by the yeasts (*Saccharomyces cerevisiæ* and other species) which convert the sugars into alcohol. Hops are added to give the beer a bitter taste and also for the purpose of influencing the fermentation process favorably. After the alcoholic fermentation is completed, the product is filtered, clarified, pasteurized and occasionally preserved by adding salicylic acid or other preservative. The alcoholic content of beer varies from about 1.50 to 6 per cent. Some beers are fortified by adding alcohol. There are many kinds or brands of beer, differing in color, flavor, taste and consistency.

Brewers must observe great caution to guard against the invasion of objectionable organisms as bacteria, yeasts and mold, which might vitiate the normal or desirable process of fermentation. In spite of all precautions, things often go wrong. The wort may undergo sour or other objectionable fermentation and as a result the entire lot may have to be rejected. Wild yeasts may gain the upper hand and ruin the beer. Even after the product is finished and placed in the containers, abnormal fer-

mentations may be set up by various bacteria, yeasts and mold, causing more or less serious spoiling and even complete deterioration. The following are the more common beer diseases which may be brought to the attention of the food bacteriologist.

a. Ropiness.—This is quite common. The beer becomes thick and mucilaginous capable of being drawn out into threads. Two species of bacteria cause ropy beer; *Bacillus viscosus* I and *B.*

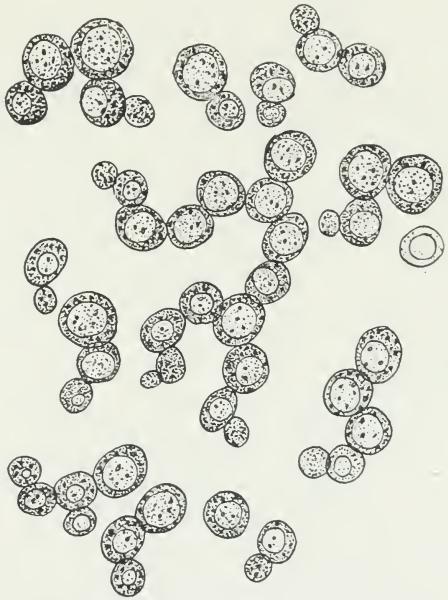


Fig. 74.—*Saccharomyces cerevisiæ.* The variety known as brewers' top yeast.—(*Oberhefe.*)

viscosus II. These bacteria are rod-shaped and measure 0.8 by 1.6–2.4 microns. Bacillus I gives rise to yellowish-white viscous patches on the surface of the beer whereas bacillus II does not develop such patches. *B. viscosus* III has been isolated from British ropy beer. The ropiness results from a change in the cell-wall of the bacterium and not from any chemical change in the beer itself. Ropiness may also be caused by a mold, *Dematium*

pullulans, which shows septate branching hyphal filaments and yeast-like sporulation, which might be mistaken for yeast cells.

b. Turning or Souring of Beer.—Soured, turned or spoiled beers have a disagreeable taste and odor and are no longer clear or brilliant and sedimentary deposits are usually found. Beers

FIG. 75.—*Saccharomyces cerevisiæ.* The variety known as brewers' bottom yeast (*Unterhefe*). *a,* Spore formation; *b,* elongated cells (rudimentary filaments or hyphæ).

containing only a small amount of hops or of hop extract and which are low in alcohol and inadequately filtered, pasteurized and improperly bottled, are likely to spoil. The most common cause of this kind of spoiling is due to the aerobic acetic acid bacteria which are particularly apt to do great damage in the top fermented beers where the conditions for their development

(aeration) is more favorable than in the bottom fermented beers. The three most common and best known beer acidifiers are *Bacterium aceti*, *B. pastorianus* and *B. kützingianum*.

Lactic acid and butyric acid bacteria may gain access to the fermenting vats and render the beer wholly unfit for use. Of these two kinds of bacteria, the butyric acid formers are by far the most objectionable because of the very disagreeable odors which they form.

FIG. 76.—*Saccharomyces ellipsoides*. The common wine ferment. Also common in jams, jellies and canned fruits.

c. Bitterness.—Bitterness of beer may be caused by several species of so-called wild yeasts, principally *Saccharomyces pastorianus* I, II and III, and of these, variety I is the most common and most injurious. It is stated that small amounts of varieties II and III are not objectionable as they transmit to the beer a stronger taste and smell.

d. Turbidity.—Turbidity of beers may be the result of a variety of factors which may be outlined as follows.

1. Gluten turbidity, due to the precipitation of protein substances.

2. Starch turbidity, due to the presence of unchanged starch.

3. Yeast turbidity, due to a high content of yeast cells. If wild yeasts are the cause of the turbidity then there may be noticeable a bad taste and bad odor.

4. Bacterial turbidity, due to the development of bacteria. In this case there may be noticeable bad odor, bad taste and ropiness.

5. Sarcina turbidity, caused by the members of the sarcina group. Unless certain species are present in large numbers the beer may not be appreciably affected in quality. It must be remembered that some of the sarcinas cause disturbances in gastric digestion.

3. Wines.—Wine is grape juice which has undergone alcoholic fermentation through the action of yeast organisms. To enter into the details of wine production is not necessary. Wines vary in the amount of alcohol (8 to 16 per cent.) which they contain, in color, in taste, in the amount of unchanged or added sugar, in the amount of acid, etc. *Saccharomyces ellipsoides* is the most common yeast concerned in the alcoholic fermentation of grape juice. Wine diseases are frequently met with and are not unlike those of beer. Ropiness, turning and lactic acidification are perhaps the most common, induced by bacilli and cocci. It may be stated that the greater natural acidity of wines in general tends to retard or check bacterial invasion. Bacterial invasion is also in a measure checked by the greater alcohol content of wines over that of beers. Souring of wine is the most common malady, induced by acetic acid bacteria which reduce much of the alcohol into acetic acid. A tough membranous scum forms on the surface of the wine, composed of a nearly pure culture of the acidifying bacteria. *Bacterium* (*Mycoderma*) *aceti*, *B. pastorianum* and *B. kützingianum* are the most common of the acid formers and the three species appear to be very closely related. Souring of

wine is in reality a normal process and which must be expected to develop under ordinary condition. It is, however, most desirable to retard or check this process as much as possible.

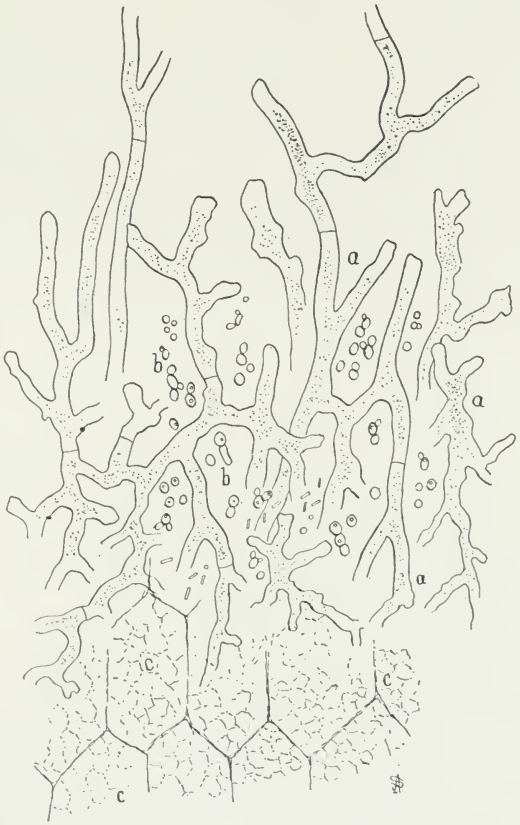


FIG. 77.—Saké. Steamed rice cells (*c*) attacked by the fungus (*Aspergillus oryzæ*). The fungus changes the starch into saccharine substances. Yeasts and bacteria are usually associated with the hyphal fungus, feeding upon the saccharine substances formed.

4. Saké or Japanese Rice Wine.—Saké is a fermented drink quite popular in Japan, China and in Corea. It is made from rice which has been steamed to soften the grain and starch so that

the fungus *aspergillus oryzæ* may convert the starch into saccharine compounds. The fungus is kept on hand in pure culture and

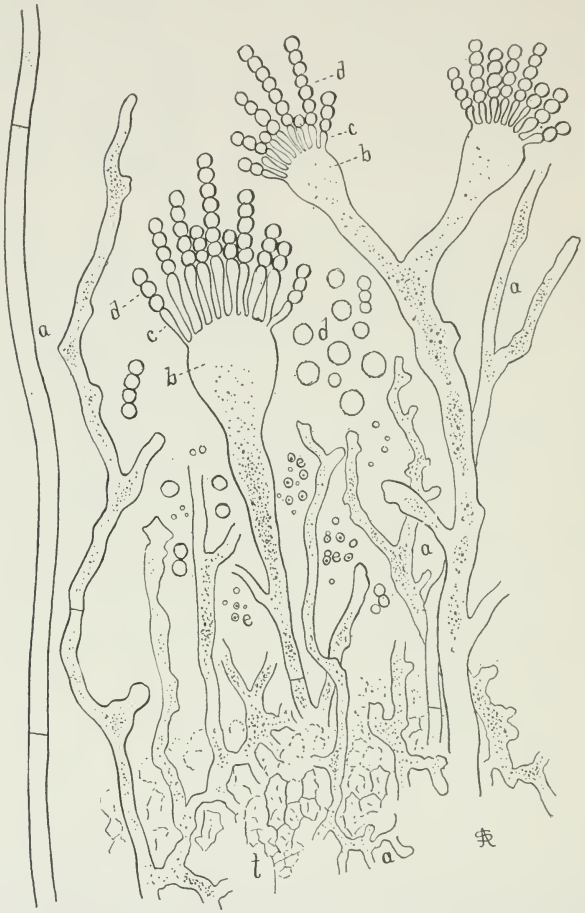


FIG. 78.—Sakè. *Aspergillus oryzæ*, showing vegetative hyphæ (*a*) and the spore-forming hyphæ (*b*, *c*, *d*).

mixed with the steamed rice and the sugar fermentation takes place in a warm room. The alcoholic fermentation, which follows, is much like that in beer making, likewise the final processes of

clarifying and pasteurizing. This drink contains from 14 to 18 per cent. alcohol and is essentially a wine. It may be taken cold or hot. The Japanese usually drink it hot. There are several brands of saké differing in quality. There is a sweet variety

FIG. 79.—Saké. *A*, Dead or dying yeast cells (*Saccharomyces saké*). Vacuoles are wanting, the cell walls are generally more thickened and the cells are somewhat shrunken in appearance; *B*, living yeast cells showing distinct vacuoles; *C*, *D*, actively budding yeast cells (*S. saké*) and hyphæ of aspergillus from the fermenting vats.

(Mirin) and a white variety (Shiro). Saké has a peculiar aroma or flavor which may be likened to that of bad champagne.

5. Arrak.—This Javanese alcoholic drink is made from rice which is acted upon by a fungus (*Ragi*) similar to *Aspergillus*

oryzæ, and subsequently the alcoholic fermentation is carried on by the saccharomyces. The method of preparing arrak is therefore similar to that of making saké. More generally, however, arrak is made from fermented molasses.

6. Yoghurt.—This is Bulgarian sour, thick or klabbered sheep's or cow's milk. The milk is boiled and evaporated to about half its volume, then cooled to about 45° C. and the ferment known as *maya* or *podkoassa* is added. The maya is simply the dry residue from a previous fermentation. The fermented product has a sour aromatic taste. The most important organism in this

FIG. 80.—Showing a Kephir granule or mass natural size and three types of bacteria found in Kephir.—(*Marshall.*)

fermentation is the *Bacillus bulgaricus*. Other bacilli, cocci and yeasts are also present. The Yoghurt tablets of the market are presumably pure cultures of the *Bacillus bulgaricus*.

7. Kephir.—Kephir is an effervescent alcoholic sour drink made from the milk of the cow, sheep or goat. This is also a Bulgarian preparation. The kephir granules or seeds are simply more or less dry residues of a previous fermentation and may be obtained in the market. These granules are composed of the organisms which give rise to the fermentation products, principally *Dispora* (*Bacillus*) *caucasica* and several species of streptococci. These several organisms are supposed to form a mutualistic as-

sociation and cause alcoholic and lactic acid fermentation in the milk.

8. Koumiss.—This drink is similar to kephir, made from mare's milk, by the inhabitants of southern Russia and of Siberia. The active organisms in the ferment are a yeast, a lactic acid bacillus and a second species of bacterium which is characteristic of the koumiss and which appears to be active only in association with the other organisms, thus also indicating a mutualistic association. The fermented milk contains lactic acid and alcohol.

9. Soja Sauce.—This Chinese sauce or relish is made from the fermented soja bean (*Glycine hispidus*). The beans are boiled

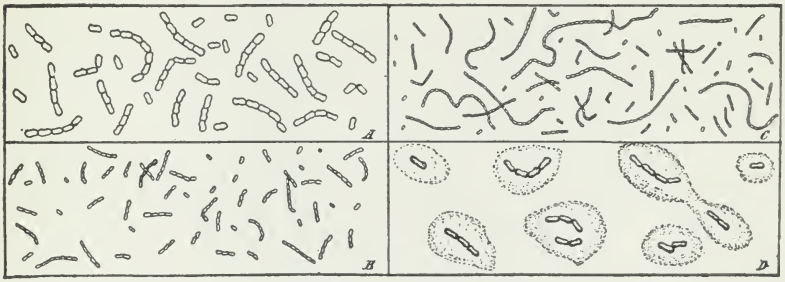


FIG. 81.—Bacteria of slimy wine. *A*, *B*, *C*, pure cultures of various forms; *D*, mucilaginous sheath of slime bacteria. (*After Kayser and Manceau.*)

and mixed with parched flour and then exposed to the ferment *Aspergillus oryzæ*. Salt and water are added and the mixture is allowed to ferment slowly, sometimes for years. The final product assumes a rich brown color and a characteristic aroma. It is then put in bags and almost a clear juice is expressed which is then further clarified and pasteurized. In the second or long process of fermentation several organisms are active along with the *Aspergillus*, as *Saccharomyces soja*, *Bacillus soja* and *Sarcina hamayuchia*.

10. Mazun.—This, like the kephir and koumiss, is a fermented milk, usually of the cow and of the goat, which is much used in

Armenia. The active organisms in the ferment are a bacillus which appears to be identical with *Bacillus subtilis* and also several different kinds of lactic acid bacteria.

11. Leban.—This sour aromatic drink is very closely similar to mazun and is made from boiled buffalo's, cow's and goat's milk. It is of Egyptian origin. It is said to contain less alcohol than does kephir. Leban fermentation is due to a streptobacillus which coagulates milk and forms lactic acid. A diplococcus is also present which ferments glucose, saccharose and maltose. A strep-

FIG. 82.—*Sarcina ventriculi.*—(*McFarland, after Migula.*)

tococcus hydrolyzes lactose and another organism is capable of fermenting glucose and maltose but not lactose.

12. Ginger Beer.—This is a fermented sugar solution to which ginger has been added. The essential fermenting organisms are a saccharomyces (*S. pyriformis*) and *Bacillus vermiforme.* *Mycoderma aceti* is also present. The two essential organisms are evidently in close mutualistic relationship. The drink produced is acid and effervescing. The so-called ginger beer plant is simply a mass or matrix of the active organisms and is used for the purpose

of starting the fermentation. The ferment is evidently closely related to the following.

13. Bebee Wine.—Bébées or California Bees, also known as Japanese Beer Seeds, is a ferment composed largely of dried yeast cells which when added to solutions of sugar or molasses causes a quick alcoholic fermentation, resulting in a pleasant alcoholic drink (bebee wine). The bebees or bebee granules resemble dried peas somewhat, though they may be quite variable in size. Some 15 years ago this ferment was quite common in the United States, having been reported from California, Minnesota,

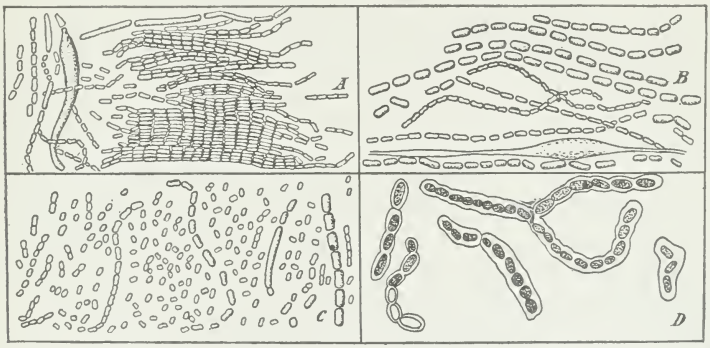


FIG. 83.—Vinegar organisms. *A*, *Bacterium* (*Mycoderma*) *aceti; B*, *Bacterium pasteurianus; C*, *Bacterium kützingianum; D*, *B. pasteurianum*, showing the mucilaginous sheath. This mucilaginous material causes the cells to stick together in large masses, forming the so-called "mother of vinegar."—(*Marshall.*)

Kentucky and other states. It is evidently of Japanese origin. Kebler and Lloyd made brief reports on this ferment several years ago, and it is reported that the ferment has disappeared from the American market.

Additional products of fermentation are vinegar, sauerkraut, pickled cucumbers, apple cider, yeast cakes, sour dough and a host of other substances used as food or employed in the preparation of foods. These may occasionally come to the notice of the food bacteriologist. Vinegars, yeast cakes, sauerkraut and pickles, in particular, may be attacked by objectionable organisms.

Diseases may enter the pickling vats and ruin the entire contents within a short time. "Hard cider" is apple wine or cider in which the alcohol has been largely changed into acetic acid by the *Mycoderma aceti*, forming cider vinegar. Cider vinegar in turn may be invaded by bacteria which decompose the acetic acid (*Bacillus xylenum*).

22. Standardization of Disinfectants

The success in modern surgery, preventive medicine and sanitation is based upon the use of disinfectants. This statement indicates the importance of disinfectants as articles of commerce, suggests the necessity of adequate supervision of the manufacture and commercial handling of these substances and points out the necessity of guarding against adulteration and misrepresentation. A vast array of so-called antiseptics have been placed on the market, the manufacturer claiming therefor properties which they do not possess. These fraudulent and exaggerated claims have impelled investigations of the marketed disinfectants with a view to determining their true merit.

Methods for standardizing disinfectants on the basis of their power to kill or destroy bacteria have been proposed by various investigators, some of which have proven quite satisfactory. The Rideal-Walker method and the Lancet method of England and the Anderson-McClintic method of the U. S. Public Health Service appear to find most favor, the latter method being a modification of the two former. In the Rideal-Walker and Anderson-McClintic methods the test organism used is the typhoid bacillus, exposing definite quantities of pure cultures of this organism to varying quantities of the disinfectant to be tested in order to ascertain the killing strength as compared with the standard which is pure phenol. The method is rather complicated and demands great care and precision in technique in order that the results may be reliable and uniform in the dif-

ferent laboratories. A simplified method will no doubt be substituted for the Anderson-McClintic method. A method has been proposed based on the percentage of bacteria killed within a unit of time by a unit quantity of the disinfectant when added to a unit quantity of a typhoid bacillus culture known to contain a definite number of organisms. The Ohno-Hamilton method is simpler than the Anderson-McClintic method and is included for purposes of comparison.

An efficient disinfectant for general purposes should comply with certain requirements which may be stated as follows:

1. Should be highly potent as destroyers of bacteria.
2. Should be readily soluble in water and should readily permeate or penetrate solutions of organic substances.
3. Should be comparatively nontoxic to man, when applied externally or when taken internally.
4. Should have a minimum albumen coagulating power, and conversely should be capable of penetrating organic substances readily.
5. Should be comparatively cheap and should be readily usable by those of average ability and intelligence.

The ideal disinfectant, that is, one which is highly potent, readily soluble in all organic solutions and capable of penetrating such substances readily, and at the same time nontoxic and cheap, does not exist. There is no disinfectant which is highly efficient as a destroyer of bacteria and at the same time nontoxic, notwithstanding all claims to the contrary by manufacturers. It is true, however, that disinfectants vary greatly regarding the essentials above stated. Our present means for testing the efficiency of disinfectants may be summarized as follows:

1. John F. Anderson and Thomas B. McClintic[1] of the United States Public Health Service have worked out a method for determining the comparative germ-destroying power of disinfectants,

[1] John F. Anderson and Thomas B. McClintic. A Method for the Bacteriological Standardization of Disinfectants. The Journal of Infectious Diseases, Vol. VIII, No. 1, Jan. 3, 1911.

which method is generally designated as the "Hygienic Laboratory Method for Determining the Phenol Coefficient of Disinfectants."

2. Worth Hale[1] of the United States Public Health Service has worked out a method for determining the comparative toxicity of coal tar disinfectants.

3. A method for determining the albumen coagulating power of disinfectants has been worked out in the bacteriological laboratories of the California College of Pharmacy.[2]

The rate and amount of solubility of disinfectants in water is generally known or can be ascertained very readily. The solubility of disinfectants in organic substances (as sputa, excreta, pathological secretions, sewage, etc.), is of the greatest importance and is conversely indicated by the albumen coagulating power. Certain disinfectants not only do not coagulate albuminous substances but have the power of penetrating such substances. As is generally known most disinfecting solutions do not penetrate or permeate coagulated albumen. Certain tests which have been made at the California College of Pharmacy would indicate that some of the coal tar disinfectants, such as lysol, cresols with alkali, and others do actually penetrate coagulated albumen, though very slowly. A better knowledge of colloids and of colloidal solutions would throw much light on the behavior of disinfectants when added to organic substances and would no doubt greatly modify the practical use of disinfectants. It is generally known that the solubility or penetrability of disinfecting solutions in the presence of organic substances is increased by the addition of certain substances, thus very materially increasing the efficiency of such disinfectants.

It is most desirable to adopt a practical method for rating disinfectants according to their efficiency value. In other words,

[1] Worth Hale. A Method for Determining the Toxicity of Coal Tar Disinfectants. Bull. No. 88, United States Public Health Service, April, 1913.

[2] Albert Schneider. An Albumen Coagulation Coefficient for Disinfectants. The Pacific Pharmacist, Vol. V, No. 11, March, 1912.

it is desirable to know what are the most efficient and cheapest disinfectants for general use. It is suggested that such a method of rating be in terms of comparison with phenol.

U. S. Public Health Service Phenol Coefficient

The following is a detailed description of the method for determining the comparative (phenol) germ (bacteria) destroying power of disinfectants as given by John F. Anderson and Thomas B. McClintic of the Hygienic Laboratory. In order that the results by different workers may be uniform the details must be followed out exactly.

Media.—Standard extract broth is used, both for the culture to be tested and for the subcultures made after exposure to the disinfectant. The broth is made from Liebig's extract of beef and is in exact accordance with the standard methods adopted by the American Public Health Association for water analysis. Ten cc. of the broth are put into each test-tube. This amount of broth has been found sufficient to avoid any antiseptic action of the disinfectant carried over. It is important that the reaction of the media is just + 1.5.

Organism.—For the test organism, a 24 hr. broth culture in extract broth of the *B. typhosus* is used. Before beginning a test the culture should be carried over every 24 hr. on at least 3 successive days. For carrying over the culture one loopful of a 4 mm. platinum loop is used.

Before being added to the disinfectant the culture is well shaken, filtered through sterile filter paper, and placed in the water bath in order that it may reach a temperature of 20° C. before being added to the disinfectant.

Temperature.—A standard temperature of 20° C. has been adopted for all experiments. This temperature is obtained by the use of a specially devised water bath. The culture and dilutions of the disinfectant are brought to this temperature before beginning the test.

Proportion of Culture to Disinfectant.—One-tenth cc. of the culture is used, added to 5 cc. of the disinfectant dilution. The amount of culture is measured with a pipette graduated in tenths of a cubic centimeter.

Inoculation Loops.—For making the transfer of the culture after exposure to the disinfectant a platinum loop 4 mm. in diameter of 23 U. S. standard gauge wire is used. We have found it of advantage to have at least four, and preferably six, loops. In order to save time in flaming the following method was devised:

A block about 3 in. wide, 10 in. high, and 12 in. long, containing four or six grooves, spaced 2 in. apart, is used. Into each of the grooves the platinum loop is laid so that the ends of the loops extend about 5 in. beyond the side of the block. The first step in the operation is to sterilize each loop by flaming with a fantail Bunsen burner before beginning the experiment.

When ready to begin the operation the loop farthest from the operator is taken in the right hand and the inoculation made. It is then replaced in the groove with the right hand and the Bunsen burner (fan tail) placed under it with the left hand. The next loop is then used, replaced in its groove, and the Bunsen burner placed under it with the left hand, the first loop having been heated to redness while the second loop was in use. This procedure is then continued until all the inoculations have been made. The time required in making the inoculations and in replacing the loop is short, it being found that 15 sec. is ample.

Incubations.—The subcultures are incubated 45 hr. at 37° C., and the results then read off and tabulated.

Dilution.—Capacity pipettes for the original dilutions are invariably used. For the phenol controls a standard dilution of pure phenol (Merck) is made and standardized by the U. S. P. Method (Koppeschaar) to contain exactly 5 per cent. of pure phenol by weight. From this stock solution the higher dilutions are made fresh each day for that day's test.

For the dilutions of the disinfectant a 5 per cent. solution is

made by adding 5 cc. of the disinfectant to 95 cc. of sterile distilled water. A standardized 5 cc. capacity pipette is used for this and after filling the pipette all excess of the disinfectant on the outside of the pipette is wiped off with sterile gauze. The contents of the pipette are then delivered into a cylinder containing 95 cc. of sterile distilled water and the pipette washed out as clean as possible by aspiration and blowing out the contents of the pipette into the cylinder. The contents of the cylinder are then thoroughly shaken and the dilutions up to 1 : 500 made from it, using delivery pipettes for measuring. For those disinfectants which do not readily form a 5 per cent. solution we make a 1 per cent. stock solution and from this make the dilutions greater than 1 : 100 in accordance with the second table of dilutions. If greater dilutions than 1 : 500 are to be made, a 1 per cent. solution is made from the 5 per cent. solution, and the higher dilutions made from this.

We had adopted the following scale for making dilutions:

For dilutions up to 1:70, increase or decrease by a difference of 5 (*i.e.*, 5 parts of water).

From 1:70	to 1:160	by a difference of 10
From 1:160	to 1:200	by a difference of 20
From 1:200	to 1:400	by a difference of 25
From 1:400	to 1:900	by a difference of 50
From 1:900	to 1:1800	by a difference of 100
From 1:1800	to 1:3200	by a difference of 200

and so on if higher dilutions are necessary.

It is important that the cylinders used for making the dilutions be correctly graduated, as we have found disregard of this factor an important source of error. It is preferable to use standardized cylinders and pipettes, and we recommend that they be used whenever possible. They of course should be perfectly clean. For making the dilutions in accordance with the above scheme we have found the following table of much service:

TABLE I. STOCK 5 PER CENT. SOLUTION, (FOR DILUTIONS)
(5 cc. disinfectant + 95 cc. distilled water)
Solution A

cc. of A	cc. Dist. Water		cc. of A	cc. Dist. Water		cc. of A	cc. Dist. Water
1:20	20 + 0	or	10 +	0	or	4 +	0
1:25	20 + 5	or	10 +	2.5	or	4 +	1
1:30	20 + 10	or	10 +	5	or	4 +	2
1:35	20 + 15	or	10 +	7.5	or	4 +	3
1:40	20 + 20	or	10 +	10	or	4 +	4
1:45	20 + 25	or	10 +	12.5	or	4 +	5
1:50	20 + 30	or	10 +	15	or	4 +	6
1:55	20 + 35	or	10 +	17.5	or	4 +	7
1:60	20 + 40	or	10 +	20	or	4 +	8
1:65	20 + 45	or	10 +	22.5	or	4 +	9
1:70	20 + 50	or	10 +	25	or	4 +	10
1:70	20 + 50	or	10 +	25	or	4 +	10
1:80	20 + 60	or	10 +	30	or	4 +	12
1:90	20 + 70	or	10 +	35	or	4 +	14
1:100	20 + 80	or	10 +	40	or	4 +	16
1:110	20 + 90	or	10 +	45	or	4 +	18
1:120	20 + 100	or	10 +	50	or	4 +	20
1:130	20 + 110	or	10 +	55	or	4 +	22
1:140	20 + 120	or	10 +	60	or	4 +	24
1:150	20 + 130	or	10 +	65	or	4 +	26
1:160	20 + 140	or	10 +	70	or	4 +	28
1:160	20 + 140	or	10 +	70	or	4 +	28
1:180	20 + 160	or	10 +	80	or	4 +	32
1:200	20 + 180	or	10 +	90	or	4 +	36
1:200	20 + 180	or	4 +	36	or	2 +	18
1:225	20 + 205	or	4 +	41	or	2 +	20.5
1:250	20 + 230	or	4 +	46	or	2 +	23
1:275	20 + 255	or	4 +	51	or	2 +	25.5
1:300	20 + 280	or	4 +	56	or	2 +	28
1:325	20 + 305	or	4 +	61	or	2 +	30.5
1:350	20 + 330	or	4 +	66	or	2 +	33
1:375	20 + 355	or	4 +	71	or	2 +	35.5
1:400	20 + 380	or	4 +	76	or	2 +	38
1:450	20 + 430	or	4 +	86	or	2 +	43
1:500	20 + 480	or	4 +	96	or	2 +	48

TABLE II. STOCK 1 PER CENT. SOLUTION (FOR DILUTIONS)
(1 cc. disinfectant; 99 cc. distilled water)
Solution A

cc. of A	cc. Dist. Water	cc. of A	cc. Dist. Water	cc. of A	cc. Dist. Water
1:100 =	100 + 0 or	10 +	0		
1:110 =	100 + 10 or	10 +	1		
1:120 =	100 + 20 or	10 +	2		
1:130 =	100 + 30 or	10 +	3		
1:140 =	100 + 40 or	10 +	4		
1:150 =	100 + 50 or	10 +	5		
1:160 =	100 + 60 or	10 +	6		
1:160 =	100 + 60 or	10 +	6		
1:180 =	100 + 80 or	10 +	8		
1:200 =	100 + 100 or	10 +	10		
1:200 =	100 + 100 or	10 +	10 or	4 +	4
1:225 =	100 + 125 or	10 +	12.5 or	4 +	5
1:250 =	100 + 150 or	10 +	15 or	4 +	6
1:275 =	100 + 175 or	10 +	17.5 or	4 +	7
1:300 =	100 + 200 or	10 +	20 or	4 +	8
1:325 =	100 + 225 or	10 +	22.5 or	4 +	9
1:350 =	100 + 250 or	10 +	25 or	4 +	10
1:375 =	100 + 275 or	10 +	27.5 or	4 +	11
1:400 =	100 + 300 or	10 +	30 or	4 +	12
1:400 =	10 + 30 or	4 +	12 or	2 +	6
1:450 =	10 + 35 or	4 +	14 or	2 +	7
1:500 =	10 + 40 or	4 +	16 or	2 +	8
1:550 =	10 + 45 or	4 +	18 or	2 +	9
1:600 =	10 + 50 or	4 +	20 or	2 +	10
1:650 =	10 + 55 or	4 +	22 or	2 +	11
1:700 =	10 + 60 or	4 +	24 or	2 +	12
1:750 =	10 + 65 or	4 +	26 or	2 +	13
1:800 =	10 + 70 or	4 +	28 or	2 +	14
1:850 =	10 + 75 or	4 +	30 or	2 +	15
1:900 =	10 + 80 or	4 +	32 or	2 +	16
1:900 =	5 + 40 or	4 +	32 or	2 +	16
1:1000 =	5 + 45 or	4 +	36 or	2 +	18
1:1100 =	5 + 50 or	4 +	40 or	2 +	20

TABLE II. STOCK 1 PER CENT. SOLUTION (FOR DILUTIONS)
(1 cc. disinfectant; 99 cc. distilled water)
Solution A

cc. of A	cc. Dist. Water	cc. of A	cc. Dist. Water	cc. of A	cc. Dist. Water
1:1200 =	5 + 55 or	4 +	44 or	2 +	22
1:1300 =	5 + 60 or	4 +	48 or	2 +	24
1:1400 =	5 + 65 or	4 +	52 or	2 +	26
1:1500 =	5 + 70 or	4 +	56 or	2 +	28
1:1600 =	5 + 75 or	4 +	60 or	2 +	30
1:1700 =	5 + 80 or	4 +	64 or	2 +	32
1:1800 =	5 + 85 or	4 +	68 or	2 +	34
1:1800 =	5 + 85 or	4 +	68 or	2 +	34
1:2000 =	5 + 95 or	4 +	76 or	2 +	38
1:2200 =	5 + 105 or	4 +	84 or	2 +	42
1:2400 =	5 + 115 or	4 +	92 or	2 +	46
1:2600 =	5 + 125 or	4 +	100 or	2 +	50
1:2800 =	5 + 135 or	4 +	108 or	2 +	54
1:3000 =	5 + 145 or	4 +	116 or	2 +	58
1:3200 =	5 + 155 or	4 +	124 or	2 +	62

Seeding Tubes.—The seeding tubes are glass test-tubes 1 in. in diam. and about 3 in. long, with round bottoms. In order to

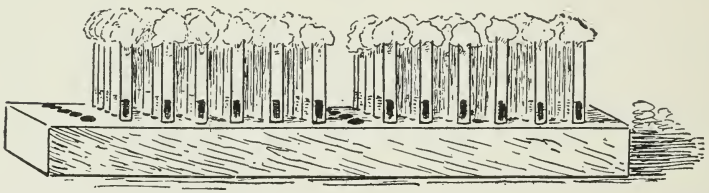


FIG. 84.—Block for holding the subculture tubes.—(*Anderson & McClintic, Hygiene Laboratory Bulletin No. 82, U. S. Public Health Service*)

measure the disinfectant into them they are placed in a suitable wooden stand to receive them. We found it convenient to use a wooden block containing six rows of fifteen holes each for the disinfectant to be tested and a separate stand for the phenol controls. The tubes are placed in the stand and each marked with the strength of dilution it is to contain. The rows of tubes run-

ning crosswise represent the same strength dilution, while the rows running lengthwise represent the different strengths to be used in the experiment.

Starting with the lowest dilution (*i.e.*, the strongest), the cylinder is shaken, then 5 cc. are measured into the tubes of the row to receive that strength, using a 5 cc. delivery pipette. In order to economize glassware, the same pipette is used for measuring out the next dilution, first blowing out as much of the remaining liquid as possible, then drawing a pipette full of the next dilution to be used and discarding that, then filling the pipette a second time, which is then emptied into the seeding tube.

The measuring out being completed, the tubes are placed in the water bath and allowed to stand a few minutes in order that the disinfectant solution may reach the standard temperature. We have not found it necessary to use cotton plugs in the seeding tubes. They are sterilized in paper-lined wired baskets, with the closed end of the tubes up.

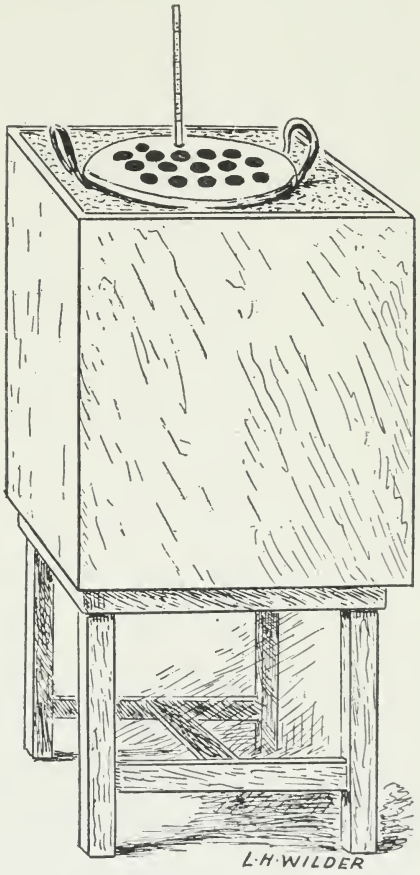


FIG. 85.—Water bath showing position of holes for the thermometer and the seeding tubes.—(*Anderson & McClintic, Hygiene Laboratory Bulletin No 82, U. S. Public Health Service.*)

Subculture Tube Racks.—Wooden racks, with five rows of

fourteen holes each, are used for holding the subculture tubes, and as plants are made from each mixture of culture and disinfectant every 2½ min. up to 15 min., six tubes are required for each dilution. Thus in each rack we have ten rows of six tubes each with two empty cross rows of holes left, which are utilized by placing over in the next row each tube as it is planted. This makes it easy to keep run of the tubes that are planted. It is

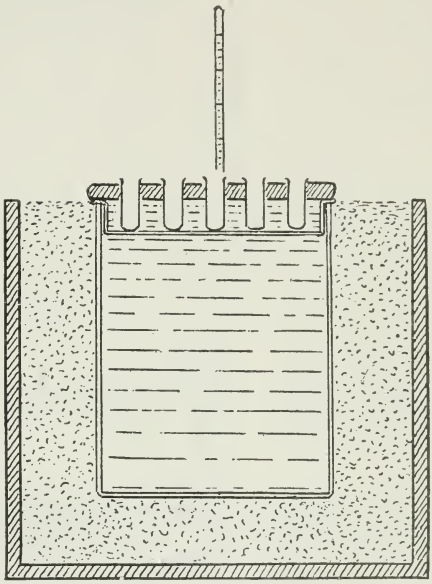


FIG. 86.—Cross section of water bath showing seeding tubes in position.—(*Anderson & McClintic, Hygiene Laboratory Bulletin No. 82, U. S. Public Health Service.*)

well also always to plant from the seeding tube in a certain hole in the water bath into a certain row of tubes in the rack. This, after a little practice, will help to avoid errors in planting.

Method of Conducting the Test.—If there are in one experiment more than ten dilutions of the disinfectant, including the phenol controls, the stronger solutions of the disinfectant and

phenol are tested first, as it will not be necessary to plant them after 7½ min. The weaker solutions are then immediately done and are planted every 2½ min. for 15 min.

For keeping the time a stop watch can be used, but an ordinary watch will serve the same purpose by simply starting on the 2½ or 5 min. periods.

When everything is in readiness the culture is added to the disinfectant solutions with a sterile pipette in tenths of a cubic centimeter.

To add the culture, the seeding tube containing the disinfectant is removed from the water bath with the left hand and slanted at an angle of about 45°, and with the right hand the end of the pipette containing the culture is introduced and lightly touched against the side of the tube where the liquid has run away on account of slanting. At the proper time the culture is allowed to run into the disinfectant solution, the pipette removed, the tube straightened up, gently shaken three times, and replaced in the water bath. The other tubes are done the same way in succession, and it will be found that 15 sec. is ample time for each tube. By adding the culture to the disinfectant with a pipette touched against the side of the seeding tube, accurate measurements can be made and each tube receive exactly the same amount of "seeding," which is not the case when the culture is added by the "drop."

If ten tubes are to be inoculated, only a few seconds will remain after inoculating the last tube before a plant from the first tube will have to be made.

The mixing tubes are not removed or disturbed in making the planting except to insert the loop or spoon into them, touch the bottom, withdraw, and then make the plant in broth. Every effort is made to insert and withdraw the loops and spoons in a uniform manner. The loops and spoons are bent to an angle of about 45° where they are joined on to the shank, and therefore are always filled with the mixture when withdrawn from the seeding

tubes. After making the plants, the loops or spoons are flamed as already described.

After an experiment is finished the date and any necessary details can be marked on one of the broth tubes and the rack placed in the incubator at 37° C. for 48 hr. At the end of this time the results are recorded on a chart especially devised for the purpose. (See Table III.)

Determining the Coefficient.—After a large number of experiments, we have concluded that the method employed by the Lancet

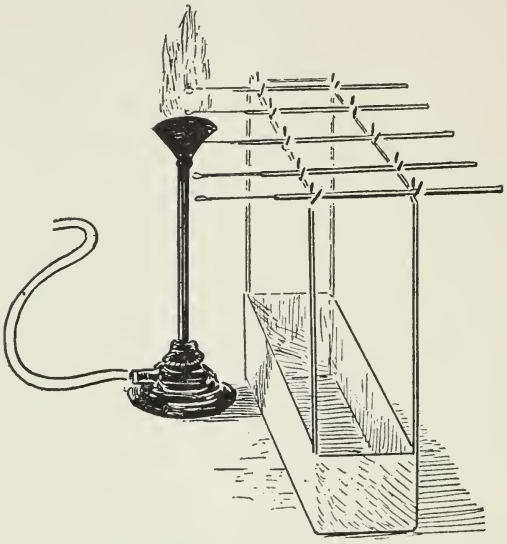


FIG. 87.—Device for holding and flaming inoculating loops.—(*Anderson & McClintic, Hygiene Laboratory Bulletin No. 82, U. S. Public Health Service.*)

Commission, with certain modifications, is the best one for determining the coefficient, *i.e.*, the mean between the strength and time coefficients.

In performing the test, plants are made every 2½ min. up to and including 15 min. To determine the coefficient, the figure representing the degree of dilution of the weakest strength of the disinfectant that kills within 2½ min. is divided by the figure

representing the degree of dilution of the weakest strength of the phenol control that kills within the same time. The same is done for the weakest strength that kills in 15 min. The mean of the two is the coefficient. The method of determining the coefficient will be seen in Table III.

TABLE III

Name, "A." Date: May 18, 1913.
Temperature of medication, 20° C.
Culture used, B. typhosus; 24 hr.; extract broth filtered.
Proportion of culture and disinfectant, 0.1 cc. + 5 cc.
Organic matter, none; kind, none; amount, none.
Subculture media, standard extract broth. Reaction, + 1.5; quantity in each tube, 10 cc.

Sample	Dilution	Time Culture Exposed to Action of Disinfectant for Minutes						Phenol Coefficient
		2½	5	7½	10	12½	15	
Phenol.....	1:80	−	−	−				
	1:90	+	−	−	−			
	1:100	+	+	+	−	−	−	
	1:110	+	+	+	+	×	−	
Disinfectant "A"	1:350	−	−	−				
	1:375	−	−	−				
	1:400	+	−	−	−			
								80)375(4.69
	1:425	+	+	−	−	−	−	110)650(5.91
	1:450	+	+	−	−	−	−	2)10.60
	1:500	+	+	−	−	−	−	5.30
	1:550	+	+	+	−	−	−	
	1:600	+	+	+	+	−	−	
	1:650	+	+	+	+	+	−	
	1:700	+	+	+	+	+	+	5.30 = phenol
	1:750	+	+	+	+	+	+	coefficient

To Determine the Comparative Cost per Unit of Efficiency.—When bids are solicited for supplying disinfectants they should be required to be made so as to show the comparative cost per 100 units of efficiency of the disinfectant as compared with 100 units of pure phenol. It is manifestly cheaper to purchase a

disinfectant that sells for 60 cents a gallon than one that sells for 30 cents a gallon, if the former has four times the efficiency of the latter.

The true cost of a disinfectant can be determined only by taking into consideration the phenol coefficient and the cost per gallon of the disinfectant.

The following table (IV) is a good illustration of the value of a determination of the comparative cost per 100 units of disinfectant in terms of 100 units of pure phenol:

TABLE IV

Disinfectant	Phenol Coefficient	Price per Gallon	Relative Cost per 100 Units of Efficiency as Compared with Pure Phenol
Car	2.12	$0.30	5.2
Chl	4.44	1.00	8.4
Phi	1.40	0.37	9.9
Cre	1.13	0.44	14.5
Nap	0.44	0.41	34.8
Zod	0.25	0.40	59.6
Pure phenol	1.00	2.67	100.0

It will be seen that the substance Chl has a higher coefficient than any of the others in the table, but its high cost per gallon results in its being placed second in cost per 100 units.

The cost per 100 units of efficiency as compared with pure phenol is obtained by first dividing the cost per gallon of the disinfectant by the cost per gallon of pure phenol; this gives the price ratio between the disinfectant and pure phenol; the cost ratio is then divided by the phenol coefficient, which gives us the cost per unit of efficiency as compared with pure phenol = 1. The cost per unit is then multiplied by 100 to give the cost per 100 units.

THE OHNO-HAMILTON PHENOL COEFFICIENT

Tatsuzo Ohno and H. C. Hamilton of the Parke, Davis Research Laboratory have proposed a method for the bacteriological

standardization of disinfectants, which is a decided simplification of the Anderson-McClintic (U. S. Public Health Service) method, and it is hereby given in somewhat abbreviated form (American Journal of Public Health, May, 1912).

I. The organism used is a vigorous culture of *B. typhosus* grown for 24 hr. in standard bouillon culture medium at 38° C. It is taken from the incubator at least ½ hr. before using, to allow gradual adjustment to changed conditions of temperature before exposure to the germicide. The culture and germicidal agent should always be at the same temperature before interaction takes place.

The 24 hr. bouillon culture is removed from the incubator and kept at room temperature without agitation for about half an hour. Then, without shaking the culture, as is usually done, it is decanted into a specially constructed cotton filter, thus leaving scum and large clumps on the filter and filtering the individual bacteria in a practically isolated state. It is then filtered into a sterile test-tube, which is subsequently shaken in order to obtain a homogeneous filtrate and make it ready for use.

The cotton filter for the filtration of bacterial cells is an ordinary test-tube drawn out at one end like a centrifugal tube, the small end cut open and the edges smoothed with a flame. Into the large end a small pledget of good quality ordinary cotton is introduced as far as the constricted portion of the tube, pushed gently with the forceps, taking care not to form any fissure in the cotton or to leave any spaces between the cotton and tube. The open end of the tube is then plugged with cotton and the whole wrapped in cotton and parchment paper and sterilized by dry heat as usual. Before using the cotton filter after sterilization, the cotton in the tube should be gently pushed back to the proper place by means of a sterile pipette, so that it is in exactly the same position as before sterilization. During sterilization the cotton is pushed up by the tension caused by the heat and its own

elasticity, producing an undesirable space between the cotton and the constricted part of the tube.

II. Culture Medium.

500 grams chopped beef.
20 grams peptone (Witte).
5 grams sodium chloride.
1000 cc. water.

The beef is digested at 50° C. for ½ hr., then boiled, strained, the other ingredients added, then boiled again, filtered and adjusted to + 1 reaction.

III. The dilutions of sample and standard are made either by weight or volume, depending on the character of the disinfectant to be tested. In the case of liquids such as the coal-tar disinfectants, both sample and standard should be diluted by volume.

The Sample.—Dilutions of an emulsive coal-tar product should be made by adding water gradually to the measured quantity of disinfectant. The reason for this is that in some cases the character of the emulsion is greatly altered by the method of making the dilution.

An emulsion is less likely to break if it is made as follows: To make a 1 per cent. solution, moisten the measuring flask or cylinder with about 2 cc. water. With a capacity 1 cc. pipette measure the disinfectant and mix it with the water, using this mixture for a partial cleaning of the pipette. Then add more water, stirring just enough to mix but not to make the mixture foam. Wash out the pipette by drawing up and expelling the dilution, then make up to the mark.

This method requires more care in measuring the final dilution if the meniscus is obscured by the emulsion. If the last addition of water is made by carefully running it down the side of the container, the surface liquid will not greatly obscure the reading.

This 1 per cent. solution is further diluted to the desired ex-

tent by mixing with distilled water in proper proportion, in each case adding the measured disinfectant to the measured quantity of water to make the desired dilution.

The Standard.—Merck's pure phenol is diluted by volume by weighing out any desired quantity, dividing by the specific gravity, 1.08, and dissolving in distilled water, diluting to twenty times the volume of the carbolic acid used, to make a 5 per cent. solution by volume in volume. Further dilutions can be made from this as desired, since the solution is practically permanent.

The dilutions of carbolic acid ordinarily used with the results to be expected are as follows:

	Minutes				
Dilutions	1	2	3	4	5
1–110	+	−	−	−	−
1–120	+	+	+	−	−
1–130	+	+	+	+	−

The sample is usually diluted in such a way that not more than a half unit in the coefficient lies between two dilutions. For example

1–480 1–540 1–600 1–660 1–720

the intention being to compare with the carbolic acid dilution 1–120. A disinfectant value based on growth at 1 min. only would be far from exact. Much more accurate results can be obtained where the dilutions compared are those which kill the organism in from 3 to 5 min.

IV. The proper mixture of culture and disinfectant is carried out as follows: five drops of bacterial filtrate are introduced into 5 cc. of germicidal solution, contained in test-tubes 5½ in. long by ⅝ in. in diam. This is added drop by drop in rapid succession, by means of a sterile pipette, 8½ in. long and 0.219 in. in diam., the narrow end measuring about 0.075 in. in diam., held vertically at the mouth of the test-tube containing the germicidal solution, so that the pipette with the germs will not

come into direct contact with any part of the test-tube containing the germicidal solution. If any of the organisms adhere to the sides or to threads of cotton in the mouth of the tube they might be inoculated into the bouillon without being exposed to the germicide. As soon as the last one of the five drops is added to the germicidal solution, they are mixed thoroughly for 15 sec. or longer by holding the test-tube in the left hand and shaking it with the fingers of the right hand; a formation of air bubbles shaped like a long funnel extending from the bottom of the tube toward the surface of the liquid shows that the mixing is efficiently done, thus bringing every bacterial cell into direct contact with the germicidal solution.

V. The subcultures are taken each minute for 5 min. by means of a 23 platinum wire loop of 4 mm. inside diam.

Tests of two dilutions can be carried on at the same time by one person, as a half-minute is more than is ordinarily necessary for taking out a subculture and planting into the tube of culture medium. Tests of five dilutions of a sample and three of the standard can therefore be made in about 25 min.

To obtain a loopful of the mixture, the test-tube should be tilted so as to prevent getting the foam formed on the surface of the mixture during shaking instead of getting a liquid portion of the mixture. The wire loop should be plunged almost to the bottom of the tube before withdrawing.

These loopfuls are inoculated into test-tubes containing about 6 cc. of the standard culture medium above described. They are placed in the incubator at 38° C. for 48 hr. or for such a time as it is found that no further development of bacteria takes place.

VI. Conclusions as to the value of a disinfectant from a test conducted as described are drawn by comparing the dilutions of the sample and the standard, which are equally efficient. While in general one dilution of each is used for comparison, it is often necessary to take the mean of a number, since it is not uncommon

for two dilutions to give practically identical results. Note, for example, such results as these:

Carbolic acid	1–120	+	+	−	−	−
	1–130	+	+	+	−	−
Sample	1–720	+	+	−	−	−
	1–780	+	+	−	−	−
	1–840	+	+	+	+	−

In this case if no other dilutions of the sample are tested the value is determined by comparing both 720 and 780 with carbolic acid dilute 120 and the result is 6.25, the average of the two values.

It will be noted that in the foregoing description no mention is made of the temperature at which the test is made. While temperature affects very vitally the process of disinfection, the changes in temperature of an ordinary working room rarely exceed 10° C., while the average change would not exceed 5° C., the year round; and since the standard is affected to practically the same degree by these changes it seems an unnecessary complication to carry out the test at a rigidly defined temperature.

It will also be noted that the time of contact between organism and disinfectant is only one-third as long as is recommended in most tests. While it may form in some cases a better picture of the value of a disinfectant to find its efficiencies at two periods such as 2½ min. and 15 min., practically no material change in its value results from such a course. The taking of a subculture each minute rather than at 2½ min. intervals makes for greater accuracy, but this does not materially affect the results.

The Worth Hale Toxicity Coefficient

Worth Hale of the U. S. Public Health Service Hygienic Laboratory has worked out a method for determining the comparative toxicity of disinfectants of which the following is a briefly summarized outline.

Test Animals.—The animals upon which the substance in question is to be tested shall be white mice of not less than 15 nor more than 30 grams weight.

Dilutions and Dosage.—The dose is to be calculated per gram of body weight and should when diluted equal between 0.03 and 0.04 cc. per gram weight; that is, 0.6 to 0.8 cc. for a 20 gram mouse. The diluent is to be distilled water and primary dilutions are to be made of such strength that the dose is easily measured with a 1 cc. pipette graduated into hundredths. This is most easily accomplished by the use of the substance in greater concentration than is required to kill in the above volume doses.

Administration of the Test Solutions.—After the required dose of the diluted disinfectant has been estimated it is measured into a suitable dish and is then diluted further to the required volume by adding sterile distilled water in sufficient quantity. A series of mice are then injected subcutaneously with varying amounts of the substance until the least fatal dose (L. F. D.) is determined, the mice being kept under observation.

Time Limit of the Observation.—After the animals have been inoculated they are kept under observation for a period of 24 hr. unless death results in a shorter period of time.

Phenol Comparative Test.—Mice of the same lot are similarly injected with pure phenol properly diluted to make the measurements of the dose easy and then further diluted in a small dish to equal a volume dose of 0.03 to 0.04 cc. per gram of body weight and the fatal dose determined as above. This least fatal dose (L. F. D.) of phenol is unity and the least fatal dose of the substance in question is estimated in per cent. of this.

Determining the Comparative Toxicity.—The phenol toxicity of the disinfectant tested is to the toxicity of phenol as x is to 100. The example given below would be represented in the following proportions: $4.5 : 18 :: x : 100 = 25$ per cent., that is disinfectant "A" is one-fourth as toxic as is pure phenol.

TABLE V

Name of Disinfectant	Mouse, Weight	Dose per Gm., Body Weight	Result	Time, Hr., Min.
Disinfectant "A".....	21.13	0.0012	Survived	
	20.64	0.0016	Survived	
	18.32	0.0018	Died	10:30
	19.05	0.0020	Died	2:15
Pure Phenol.....	18.46	0.0035	Survived	
	20.10	0.0040	Survived	
	19.23	0.0045	Died	1:15
	18.90	0.0050	Died	0:25

Valuable information regarding the comparative toxicity of many substances used as disinfectants may be obtained from a study of the comparative medicinal doses. For example, the medicinal doses of phenol, betol, resorcinol and corrosive sublimate are 1 grain, 3 grains, 4 grains and $\frac{1}{30}$ grain respectively. These doses are practically in proportion to the toxicity of the substances named and stating the dosage in the terms of the phenol toxicity coefficient as proposed by Hale, we would get the following results:

Phenol	100.00
Betol	33.30
Resorcinol	25 00
Corrosive sub	3000.00

As a rule, however, the exact composition of many of the proprietary disinfectants is either not made known to the users or is not disclosed by the manufacturers and in such cases the only thing to be done in order to ascertain whether or not the claims of the manufacturers are correct, is to make tests as above outlined. However, in cases where the composition of the disinfectant is definitely known, whether a simple or compound substance, its comparative toxicity can be determined by ascertaining the toxicity of the several ingredients and rating in comparison with the standard, namely, pure phenol.

The Albumen Coagulating Coefficient of Disinfectants

As is well known to surgeons and pathologists, the action of disinfectants and their value in tissue disinfection and in the disinfection of organic matter such as sputum, excreta, etc., varies according to their albumen coagulating power. Some disinfectants, as alcohol, mercuric chloride, silver nitrate, copper sulphate and others, coagulate albumen very actively and this property checks or prevents further penetration and action. Furthermore, inert combinations between the coagulating disinfectants (metallic ions) and the albuminous substances are formed which render a portion of the disinfectant unavoidable for further action. This behavior explains why some disinfectants are more active when diluted, as for example alcohol and carbolic acid. Even high dilutions of copper sulphate (1 : 50,000 to 1 : 4,000,000) will gradually kill bacteria in water or in other nonorganic liquids, due to a coagulation of the bacterial plasm, whereas solutions of 5 per cent. to 10 per cent. of the same substance are considered rather unsatisfactory disinfectants. The stronger solutions coagulate the albuminous matter in which the bacteria may be imbedded, no doubt quickly killing the organisms in the exposed outer layers of the albuminous particles or masses while the layer of coagulum encloses many of the bacteria effectually protecting them against further action of the disinfectant. These enclosed bacteria may become liberated after a time due to a breaking up of the coagulated covering or coating and, if pathogenic, may cause a single infection or an epidemic.

The following are some of the disinfecting agents which precipitate or coagulate albumen actively:

Alcohol.
Ether.
Salts of heavy metals.
Camphor.
Volatile oils.
Chloral hydrate.
Phenol.
Picric acid.
Mineral acids.
Some organic acids.
Tannic acid.

The following are disinfecting agents which do not precipitate or coagulate albumen:

Acetic acid.	Salts of light metals.
Phosphoric acid.	Lysol.
Alkalies and soaps.	Cresols.

While the albumen coagulating power of the different disinfectants varies greatly, it does not follow that a disinfectant which coagulates albumen actively in strong solution will do so when in weaker solution. For example, pure carbolic acid is a strong coagulant but in solutions of 5 per cent. and less it is indeed a very weak coagulant. It is therefore not exactly in accord with fact to designate carbolic acid as a disinfectant having a high coagulating power and hence comparatively unsuitable as a tissue (abscesses, infected wounds, etc.), and organic matter (ejecta, excreta, etc.), disinfectant, because in strengths of 2.5 per cent. and 5 per cent. it has only a slight coagulating power, but is still very active as a germ (nonsporebearing) destroyer.

It is not intended to imply that a disinfectant becomes useless as soon as it begins to coagulate albumen actively, but the indications are that the noncoagulating disinfectants are more satisfactory than those which are active coagulants. The coagulating coefficients give that solution strength of the disinfectants tested, which indicates or marks a retardation in disinfecting efficiency due to the coagulation of albuminous matter. This albumen coagulating coefficient is wholly independent of the germ destroying coefficient as well as that of the toxicity coefficient.

The following is an outline of the proposed method for determining the comparative albumen coagulating power of disinfectants, at the same time also indicating the solution percentage limit of optimum efficiency and usefulness as disinfectants.

Albumen Test Solution

The standard test solution shall be a 1 per cent. aqueous (distilled water) solution of pure dried egg albumen.

The following methods for making the albumen solution are submitted.

1. Gravimetric Method A.—Place 2 grams of pure powdered egg albumen in 100 cc. of boiled distilled water, shake and set aside for 6 to 12 hr., shaking frequently. Filter through a tared filter paper which has been dried (at 100° C.) to constant weight. Filtering is slow, requiring perhaps 1 hr. time. When the last drop has filtered through, dry the filter paper, with the unfiltered albumen residue upon it, to constant weight and weigh. Deduct from this weight the weight of the dried filter paper to obtain the weight of the albumen residue. From 1 gram of egg albumen dried to constant weight determine the percentage of moisture. From the data thus obtained it is easy to determine the amount of boiled distilled water which must be added to the filtrate (100 cc.) to make 1 per cent. dried albumen solution.

We will suppose that the dried filter paper to be used in filtering the albumen solution weighs 1.570 grams and this same paper with the undissolved albumen residue (also dried at 100° C. to constant weight) weighs 1.965 grams, then the weight of the undissolved dried albumen residue equals 0.395 gram. We will suppose that 1 gram of albumen loses 0.126 gram on drying, or 12.6 per cent. moisture. 0.395 gram raised to its normal air moisture (0.395 gram + 12.6 per cent of 0.395 gram = 0.444 gram) and subtracted from 2.00 grams leaves 1.556 grams, the amount of albumen that passed through the filter paper. 12.6 per cent. of 1.556 grams = 0.196 gram, and 1.556 grams less 0.196 gram = 1.360 grams which represents the amount of albumen, dried to constant weight, that passed into solution. Therefore to make a 1 per cent. solution it is necessary to add enough boiled distilled water to the filtrate to make 1: 100. In this case add water up to the 136.00 cc. mark. We now have a 1 per cent. solution sufficiently accurate for all practical purposes.

This albumen test solution is now ready for use but it must be

kept in mind that it is readily attacked by microbes. However, if carefully prepared with pure albumen, boiled distilled water, in sterile vessels, and put on ice or in a cool place, it will keep for perhaps 4 days.

Any quantity of albumen solution may be made, it merely being advised not to prepare more than may be required for the tests contemplated.

2. Gravimetric Method B.—In a dried and tared platinum dish place 5 cc. of the albumen filtrate (2 grams in 100 cc. of boiled distilled water), evaporate over water bath and dry to constant weight, and from this determine the percentage of albumen in the solution and the amount of water that must be added to the albumen filtrate to make 1 per cent.

The Phenol Standards

The standard of comparison is the opacity produced in 5 cc. of the 1 per cent. egg albumen solution when 5 cc. of 5 per cent. phenol solution are added (in a standard test-tube of about 15 cc. capacity). This phenol tube is placed against a black background. In making a test, varying dilutions of the disinfectant are added to the egg albumen solutions in a series of test-tubes until the opacity produced is the same as that in the phenol tube. In each test 5 cc. of the dilution are added to 5 cc. of egg albumen in a standard test-tube and the two tubes compared, placed against a black background.

Dilutions of Disinfectants to be Tested

The phenol control solution (5 per cent.) is made as for the Anderson-McClintic method of standardizing disinfectants, using only pure phenol crystals.

Of the disinfectants to be tested, 10 per cent. and 1 per cent. primary stock solutions are made; 10 per cent. solutions of liquid

disinfectants as alcohol, formalin and acids, and 1 per cent. solutions of the salts of heavy metals and of soluble substances generally. From these primary stock solutions the following secondary dilutions or substock solutions are made, always in those amounts which will serve the purpose, that is, in amounts for perhaps ten subdilutions for each and every disinfectant to be tested.

1 : 10 (of liquids only.)
1 : 100
1 : 1000
1 : 10,000
1 : 100,000

Method of Testing

1. Phenol Standard.—Pour 5 cc. of the egg albumen solution in a standard test-tube, using a standard 5 cc. pipette having a free outflow. Add to this 5 cc. of the phenol stock solution (5 per cent.). Set tube in the standard test rack (with black background made of cardboard covered with black tissue paper). The degree of opacity developed is to serve as the standard of comparison.

2. Preliminary Testing.—The albumen coagulating power of the disinfectant being unknown, much time and labor can be saved by testing with the four or five substock solutions, adding 5 cc. to 5 cc. of the egg albumen test solution, in order to find that dilution of the disinfectant which fails to show any opacity. We will suppose that the 1 : 1000 substock solution shows very marked opacity or precipitation, then the 1 : 10,000 solution might be tried, which may also show quite marked opacity, then the 1 : 100,000 may be tried. If this gives negative results then we know that the phenol standard lies between 1 : 10,000 and 1 : 100,000 with the probabilities that it is nearer 1 : 10,000.

3. Concluding Testing.—Going back to the 1 : 10,000 dilution, make ten subdilutions, increasing the dilutions by a difference of

1000 by simply adding the required parts of distilled water, using small quantities, thus:

10 parts of 1 : 10,000 + 1 part water = 1 : 11,000
10 parts of 1 : 10,000 + 2 parts water = 1 : 12,000
10 parts of 1 : 10,000 + 3 parts water = 1 : 13,000

Etc.

Any other quantity proportions may be used, however, as 10, 15, 20, etc., parts of the substock solution with the required parts of distilled water. If the 1 : 1000 substock solution is to be used then the dilutions should be increased by 100, as follows:

10 + 1 = 1 : 1100
10 + 2 = 1 : 1200
10 + 3 = 1 : 1300
10 + 4 = 1 : 1400

or any other equal proportion of stock solution and distilled water may be used, as

5 + 0.5, 100 + 10, or 1000 + 100, etc.

If the highest stock dilution (1 : 100,000) is to be used, then the increase should be 10,000, thus:

10 + 1 = 1 : 110,000
10 + 2 = 1 : 120,000
10 + 3 = 1 : 130,000

Determining the Phenol Coefficient

Having determined that dilution which gives the same coagulation opacity as the 5 per cent. carbolic acid, it is a very simple matter to determine the phenol albumen coagulating coefficient by simply dividing the strength of the dilution of the disinfectant tested by the phenol dilution (1 : 20).

The following are the coagulating coefficients of a few disinfectants:

Name of Disinfectant	Reaction Limit	Phenol Coefficient
Phenol	1 : 20	1.00
Copper sulphate	1 : 15,000	750.00
Mercuric chloride	1 : 10,000	500.00
Silver nitrate	1 : 9,500	475.00
Alcohol (95 per cent.)	1 : 3	0.15

The following table gives the efficiency value of some disinfectants. It will be seen that this value is of necessity variable, depending upon the variation in the market price of the disinfectants. It will also be seen that in the proposed rating the remarkably high coagulation coefficient of some of the more important chemical disinfectants lowers the efficiency value greatly.

Efficiency Values of a Few Disinfectants

Name of Disinfectant	Phenol Coeff.	Tox. Coeff.	Coag. Coeff.	Comp. Cost	Eff. Value	Special Properties
Phenol	1.00	1.00	1.00	0.15 1.00	1.00	Odor
Boracic acid	0.23	0.05	0.00	0.15 1.00	0.20	Odorless
Chloro-naphtholeum	6.06	0.16	0.00	0.15 1.00	5.22	Odor
Copper sulphate	3.30	1.00?	750	0.20 1.20	0.004	Odorless. Slight color
Lysol	2.12	0.45	0.00	0.65 4.33	0.44	Odorless
Mercuric chloride	43.00	50	650	1.27 8.46	0.06	Odorless. Corrodes metal
Neko	20.00	0.20	0.00	0.50 3.33	5.66	Odor
Potassium permanganate	0.85	0.50	0.00	0.25 1.66	0.04	Odorless. Deodorant. Stains
Silver nitrate	38.00	3.00	475	5.64 37.60	0.075	Odorless. Stains
Trikresol	2.62	0.90	0.00	0.40 2.66	0.73	Odor

The efficiency value of any disinfectant is found by dividing the phenol coefficient by the sum of the other coefficients, as follows:

$$\frac{\text{Phenol coefficient}}{\text{Tox. coefficient} + \text{coag. coefficient} + \text{comp. cost}} = \text{Efficiency value}$$

In the table the first figure in the comparative cost column is the market price *per pound* of the disinfectant and the second figure is the comparative cost (compared with phenol at 15 cents per pound).

The Toxicity and Germ Destroying Power of Some of the More Important Disinfectants

The values given are obtained from various sources and in some instances require further verification. The table will serve as a guide to a valuation of the disinfectants for purposes of general disinfection.

Germ Destroying Power and Toxicity of Disinfectants

Name	Germ Destroying Power (Phenol as 1)	Toxicity (Phenol as 100)
Alcohol	0.03	5.00
Alum	0.64	10.00
Ammonia	2.40	15.00
Ammonium chloride	0.03	10.50
Ammonium sulphate	0.015	5.00
Antozone	0.00	.....
Arsenious acid	0.50	5000.00
Arsenite of soda	0.33	3000.00
Bacterol	1.58	45.00
Benetol	1.23	33.00
Bichloride of platinum	10.00	.....
Boracic acid	0.23	5.00
Bromine	5.00	.....
Cabot's sulpho-naphthol	3.87	11.00
Calcium chloride	0.08	3.50

GERM DESTROYING POWER AND TOXICITY OF DISINFECTANTS—*Continued*

Name	Germ Destroying Power (Phenol as 1)	Toxicity (Phenol as 100)
Camphor	....	33.30
Carbolene	1.36	11.00
Carbolic acid	1.00	100.00
Carbolozone	1.48	6.40
Car-sul	2.00	16.00
Caustic acid	0.17	120.00
Chinosol	0.95	25.00
Chloride of gold	12.50	.....
Chlorine	12.50	.....
Chloro-naphtholeum	6.06	16.00
Chromic acid	15.00	100.00
Copper sulphate	3.30	100.00?
Corrosive sublimate	43.00	3000.00
Cre-bol-you	....	9.00
Cremolene	1.26	.....
Creo-carboline disinfectant	4.03	30.00
Creola	0.52	12.80
Creoleum (Dusenberry)	1.00	9.00
Creolin (Pearson)	3.25	18.00
Creolol (Rudish's)	1.24	13.00
Creosol (saponified)	1.03	6.40
Creo-Sul	.....	15.00
Creosoleum	2.90	11.00
Cresylone	.....	56.00
Crude carbolic acid	2.75	90.00
Cupric chloride	4.20	80.00
Cyllin	11.00	.....
Dioxygen	0.02	.....
Electrozone	0.90	.....
Ether	2.50	25.00
Ferrous sulphate	0.27	0.50
Formacone liquid	0.04	40.00
Formaldehyde	0.30	75.00

GERM DESTROYING POWER AND TOXICITY OF DISINFECTANTS—*Continued*

Name	Germ Destroying Power (Phenol as 1)	Toxicity (Phenol as 100)
Germol	2.12	16.00
Glycerin (sp. gr. 1.25)	0.015	0.50
Hycol	12.30	32.00
Hydrate of chloral	0.32	10.00
Hydrocyanic acid	7.50	10,000.00
Hydrogen peroxide	6.30	5.00
Hygeno A	3.56	17.00
Iodine	12.50	400.00
Iron sulphate	0.27	40.00
Izal	8.00	
Killitol	0.02	
Kreosota	1.26	5.60
Kreotas	1.10	5.60
Kreso	3.92	22.50
Kresolig	2.18	56.00
Kretol	0.92	14.00
Lead chloride	1.50	200.00
Lead nitrate	0.83	300.00
Lincoln disinfectant	1.48	17.00
Liquid creoleum	.....	9.00
Liq. cres. comp., U.S.P.	3.00	56.00
Lisapol	0.01	50.00
Listerine	.....	0.20
Lysol	2.12	45.00
Mercuric chloride	43.00	5000.00
Mercuric iodide	120.00	1000.00
Milkol	.....	11.00
Mineral acids	1–1.50	120.00
Naphthalene	2.50	7.50
Naphthol phenoline	6.40	6.40
Neko	20.00	20.00
Nitrate of cobalt	1.50	
Noncarbolic disinfectant	.....	7.50

GERM DESTROYING POWER AND TOXICITY OF DISINFECTANTS—*Continued*

Name	Germ Destroying Power (Phenol as 1)	Toxicity (Phenol as 100)
Osmic acid	20.00	1000.00
Phenaco	15.00	32.00
Phenol (pure)	1.00	100.00
Phenol disinfectant	0.61	.....
Phenol, disinfecting and cleansing	.....	7.50
Phenol liquid, U.S.P. (1890)	1.77	80.00
Phenol sodique	0.01	4.50
Phenosote	3.43	19.00
Phenotas disinfectant	1.37	9.00
Pi-ne-ex	.....	10.00
Pino-lyptol	0.27	3.20
Platt's chlorides	0.01	.....
Potassium bichromate	3.00	500.00
Potassium cyanide	3.00	1500.00
Potassium iodide	0.02	5.00
Potassium permanganate	0.85	25.00
Public health disinfectant	0.48	.....
Pyxol	.....	28.00
R. R. Roger's disinfectant	3.03	56.00
Salicylate of soda	3.20	6.50
Salicylic acid	0.30	5.00
Sanax	0.22	22.00
Sanitas	0.30	6.00
Saponified cresol	1.03	300.00
Silver nitrate	38.00	5.00
Sodium borate	0.04	00.15
Sodium chloride	0.02	00.05
Sulpho-naphthol	3.87	.....
Tarola	3.12	16.00
Trikresol	2.62	90.00
20th Cent. disinfectant	0.13	10.00
Veriform germicide	0.43	15.00
Victor sanitary fluid	.....	13.00

GERM DESTROYING POWER AND TOXICITY OF DISINFECTANTS.—*Continued*

Name	Germ Destroying Power (Phenol as 1)	Toxicity (Phenol as 100)
Wescol disinfectant	.....	22.00
Worrel's disinfectant	0.01	
Zenoleum	2.25	19.00
Zinc chloride	1.56	100.00
Zodane	0.04	
Zodone (4)	1.62	8.60
Zonol	2.37	10.00

THE NARCOTIC AND ANTISEPTIC PROPERTIES OF THE ESSENTIAL OILS

It is generally believed that the addition of spices to foods serves to preserve them, that is, prevent decomposition changes. In a general way this is in accord with facts. The antiseptic properties of essential oils are quite marked and the antiseptic properties of spices are largely due to the essential oils which they contain. Important investigations in regard to the narcotic and antiseptic properties of the more important essential oils have been made by Martindale, Coupin and Geinitz. The following is a summary of results by Geinitz as given in the Semi-annual Report of Schimmel and Co., for Oct., 1912.

The principal outcome of Geinitz' investigations is the establishment of the fact that the narcotic and disinfecting properties of the essential oils do not correspond with those of the active constituents of those oils; the sequence of the series differs widely. For example, Russian anise oil and its active constituent, anethol, have no antiseptic action whatsoever, but both have a pronounced narcotic action upon cold-blooded animals. It would appear that the group which exerts an antiseptic action and that which acts narcotically are not found in the same molecule of the odoriferous bodies; nay, in many of these substances one of the groups is wanting altogether. It is also necessary to abandon the theory that narcosis is determined simply by the great solubility of lipoid in the cells of the nervous system, and that the antiseptic action of essential oils depends upon solubility of the bacteria in the lipoids. The explanation of the facts which have been observed is probably that the organism of the bacteria with its peculiar metabolic process occupies in Nature a

position wholly for itself. For the results of narcotic experiments which have been obtained with essential oils in the case of cold-blooded animals and in that of the higher plants are altogether different from those which have been obtained with bacteria.

The results of narcotic experiments with fishes and tadpoles, of respiration experiments with toads and of injection experiments with frogs are reproduced in the form of tables arranged according to the degree of activity of the essential oils.

For the purpose of testing narcotic action on fishes, roaches (*Leuciscus rutilus*) were used. The limit of concentration taken was the dilution which produced perceptible narcosis in the fish within a period of 24 hr., that is to say, a condition when the animal, without displaying much spontaneous motion, floated in the water in an atactic condition and altogether failed to respond to squeezing with hooked pincers. Only those experiments were regarded as affording proof in which the fish recovered when replaced in fresh water.

For the purpose of estimating the antiseptic action, Geinitz added in each case to 10 cc. of fresh milk, placed in a test-tube of 16 to 18 cc. capacity, first as much *Sulphur depuratum* as would lie on the point of a knife, and afterward the antiseptic. After vigoro us shaking a piece of filtering paper soaked with solution of lead acetate was hung up in the upper part of the test-tube in such a way as not to come in contact with the milk, and the test-tube was closed with a wad of cotton-wool. The tubes were then kept 24 hr. in a water plug bath at about 38° C. If, after that lapse of time, the lead paper was found to be blackened, it was evident that the tube in question did not contain a sufficient proportion of the antiseptic. As a series of test-tubes was always being treated with an increasing quantity of antiseptic, it was easy to determine exactly when the limit of concentration was reached at which the activity of the bacteria was impeded.[1]

Experiments in Narcosis, Made on Fishes

Substance	Dilution	Substance	Dilution
Mustard oil (allyl*iso*-sulphocyanate)	1 : 1,320,000	Fennel oil	1 : 34,535
Cinnamon oil	180,000	Terpineol (liquid)	32,000
Citral	153,846	Coumarin	28,571
Carvacrol	134,010	Turpentine oil, fraction containing β-pinene	28,000
Thyme oil	133,333	*d*-α-Pinene	26,806
Carvone	125,918	Borneo camphor	26,087
Sandalwood oil	116,327	Eucalyptol (Cineol)	22,000
Eugenol	111,836	*l*-α-Pinene	20,105
Anethol	104,587		

[1] Abstracted from the *Sitzungsberichte und abhandlungen der naturforschenden Gesellschaft zu Rostock*, New Series, Vol. IV, 1912. Rostock, 1913. The paper was awarded a prize.

The evolution of sulphuretted hydrogen from milk diluted with sulphur is due to bacterial action.

EXPERIMENTS IN NARCOSIS, MADE ON FISHES—*Continued*

Substance	Dilution	Substance	Dilution
		Benzaldehyde	18,096
Greek turpentine oil	86,405	Oenanthol	16,788
French turpentine oil	79,493	Menthenone	16,000
Spanish turpentine oil	77,304	Umbellulone	15,000
American turpentine oil	77,304	Eulimene (artificial	
Calamus oil	71,862	limonene)	13,000
German peppermint oil	70,000	Rosemary oil	13,333
Russian anise oil	67,000	Juniperberry oil	11,320
Clove oil	67,000	Cymene	10,965
Mitcham peppermint oil	66,666	Terpineol (cryst.)	10,554
Caraway oil	62,500	Anisic aldehyde	10,164
Safrol	60,560	Lemon oil	9,157
		Chloroform	8,070
Heliotropin	45,454	Fenchyl*iso*valerate	6,345
Heptylalcohol	45,000	Bornyl*iso*valerate	6,300
Rose oil	41,715	Limonene	1,040
Lavender oil	38,764		
Mace oil	38,527	Chloral hydrate	286
Octylalcohol	38,090	Alcohol	190
Geraniol	37,500	Ether	166

23. Determining the Purity and Quality of Sera, Bacterins and Related Products

Sooner or later the regulatory work under the pure drugs laws of the land will cover the newer remedies which have come into prominence within recent years, such as therapeutically active sera, the so-called bacterial vaccines or bacterins, tuberculins, smallpox vaccine, rabies vaccine, glandular extracts, etc. It is, however, self-evident that such supervision on the part of the bacteriologists in drugs laboratories will not be necessary as far as the products manufactured under supervision of the U. S. Public Health Service are concerned. State and city authorities (inspectors) may perhaps find a supply of these products in the

more remote drug stores which have exceeded the age limit or which have become deteriorated in some manner, but even this must be, in the very nature of things, rather a remote possibility. It is therefore not likely that the drug bacteriologist will be called upon to examine any of the standard products put up in the Government inspected laboratories. There are, however, numerous preparations placed on the market which are said to have properties similar to the standard sera, etc., but which are of a fraudulent character. It then becomes necessary to resort to certain tests which will determine whether or not the article under consideration possesses the properties claimed for it. Such tests are both chemical and bacteriological. The chemical tests are largely qualitative and include certain color reactions, precipitation reactions, etc. However, much remains yet to be done in the way of devising methods which will prove practically useful, Some of the very recent laboratory guides to the examination of medicinal substances contain suggestions which will prove useful, and these may be applied in special cases. For example, a number of chemical tests have been suggested for determining the presence of ductless gland products and of various animal secretions. The absolute merit of these tests is seriously questioned by some authorities; however, their confirmatory significance is generally admitted.

Biological products the activity of which depends upon the presence of living germs are comparatively few and are not likely to be brought to the attention of the drug bacteriologist. The biological products are intended for hypodermic, intravenous, intramuscular or some similar mode of use and must therefore conform to certain specific requirements. They must be entirely free from all undesirable foreign bacteria, dead or alive, and must not contain undesirable foreign biological or toxicological products.

The complete examination of biological products comprises standardization and certain so-called safety tests, and is carried out in all of the laboratories operating under Government super-

vision. These tests, as carried out in the laboratories of Parke, Davis and Co., may be outlined as follows:

I. Standardization.
   1. Potency.—Determining the number of units per cc.
   2. Activity.—Ascertaining the power to produce the desired results. This is simply a check on the potency test.
   3. Serum Tests.—In some of the biological products certain tests are made to determine the difference between anti-sera and the normal serum of the same species.

II. Safety Tests.
   1. Freedom from bacterial contamination in those products which are supposedly free from living germs.
   2. Determining the purity of the cultures in those products which are composed of pure cultures of a given kind of germ.
   3. In case of products which are supposed to contain dead bacteria only, tests are made to determine the absence of all organisms capable of multiplying.

In order to test biological products as to the absence of viable or living organisms, about 2 cc. of the sample is cultured under aerobic and anaerobic conditions. To determine the purity of a product containing living bacteria, cultures are made in suitable media and these are carefully studied as to specific cultural characteristics and appearance under the compound microscope.

For the purpose of standardizing the products, a well-equipped laboratory is necessary, including the necessary experimental animals. The full routine followed out in the laboratory of the factory need not be carried out in the regulatory drug laboratory. In most cases the work will consist of making animal inoculation tests to determine the potency of the marketed article in order to ascertain whether or not it possesses the properties claimed for it. In some instances it may be necessary to determine the presence of toxic ingredients. Perhaps the most likely tests will be those which come under the head of potency and safety tests. For the present purpose the above outline will no doubt suffice. As to what methods may become desirable and necessary, only time and further experience will indicate.

The bacterial contamination of smallpox vaccine has received considerable attention on the part of American bacteriologists. Such vaccines are rarely wholly free from extraneous bacteria, no matter how carefully prepared. It is rather remarkable that the method of manufacturing the vaccine is not modified in accordance with modern progress in sanitation. Since the smallpox virus is filterable it would seem possible to pass the dissolved material through a porcelain or clay filter leaving behind the bacteria and other undesirable foreign matter. The filtrate could be tested for the possible presence of such bacteria as might have passed through the filter and these destroyed by suitable agents (such as will not interfere with the activity of the vaccine), and the filtrate perhaps concentrated to the desired degree or perhaps used in the liquid form. However, it is likely that the present method of manufacture and use of the smallpox vaccine will continue for some time. The marketed smallpox vaccine should contain but few viable bacteria, not to exceed 200 per dry point or per glycerinated tube. According to extensive tests made by Rosenau in 1902–1903, dry points and glycerinated tubes contained as high as 44,000 bacteria per point or tube, but tests made since that time (Nelson and others) show much lower figures, ranging from ten or fifteen to 300 bacteria per point or tube. Smallpox virus should also be examined (occasionally at least) for the presence of colon bacilli, streptococci, tubercle bacilli and the tetanus bacillus.

24. Special Biological and Toxicological Tests

Arsenic in Foods and Medicines—Biological Test.—Arsenic is widely distributed in nature and is extensively used in the arts and industries. Medicinally it is a very popular tonic and is also much used as an insecticide in the form of sprays and washes. Animal hides are frequently preserved by arsenic which accounts for the presence of this poison in gelatin made from such hides.

Fruits and vegetables which have been sprayed with arsenical compounds for the purpose of destroying insect pests, may contain enough of this substance to produce symptoms of poisoning. Arsenic is occasionally added to alcoholic beverages to give them a tonic effect. It has been demonstrated that very minute amounts of arsenic are normally present in various organs of the human body, as the thyroid gland, thymus gland and liver, although some investigators question the correctness of this claim. However these somewhat problematical traces of arsenic in organs of the human body and also in the organs of other animals need not concern the food and drug analyst as far as routine work is concerned.

As a rule, the tests for arsenic outlined in the majority of text-books are chemical and hence this work is usually relegated to the chemical laboratory. Within recent years attempts have been made to employ biological tests for determining the presence of arsenic in food substances, based upon the discovery that certain molds when growing in substances containing arsenic will give rise to garlic-like odors.

Gosio demonstrated that certain molds which when grown in and upon media containing very minute quantities of arsenic gave rise to gaseous compounds characterized by a garlic-like odor. Seven different kinds of molds have this power, more especially *Penicillium brevicaule*, which Gosio isolated from air and which he frequently found on decomposing paper. Crumbs of bread (wheaten) form the culture medium for this mold and the incubation is done at 28° to 32° C., a vigorous growth being produced within 48 hr. In the presence of not more than 0.00001 gram of arsenic in such culture there will be noticeable a distinct and very characteristic garlicky odor which may persist for months, if the culture is not killed. These arsenic molds do not produce garlic odors or gases with sulphur, phosphorus, antimony, boron, and bismuth compounds but they do have the power of converting selenium and tellurium compounds into

volatile substances having the garlic-like odor. The following procedure is recommended.

If the material to be examined is liquid, let the dry bread crumbs (either white or graham) absorb it to saturation, and then scatter a small quantity of fine crumbs over the surface. If the material to be tested is solid, grind or cut it into small pieces and mix with an equal amount of the bread crumbs and then moisten with a little sterile distilled water. Place the prepared material in sterile flasks of suitable size and plug with sterile cotton. Sterilize the flask and contents by the usual fractional method at 100° C., or for 30 min. in the autoclave. Absolute sterilization must be secured. There is no danger of volatilizing the arsenic at these temperatures. As soon as flask and contents are cold, inoculate with the mold, as follows. The mold cultures may be grown on bread or on pieces of potato. Remove a small quantity of the mold in the spore-forming stage and mix with peptone salt solution or sterilized water. Add enough of this mold suspension to just moisten the bread in the flask. Do not add more of the spore-bearing material than the mass (bread and arsenical substance) in the flask will absorb as too much moisture will retard growth. Cover the inoculated flask with a rubber cap and incubate at a temperature of 37° C., although the ordinary room temperature will answer the purpose. As soon as the growth is clearly visible to the naked eye, which may be in 24 hr., the characteristic garlic odor will be noticed upon opening the flask. If no odor is appreciable, again seal and incubate for another 24 hr. period or even longer. In case the substances to be tested are strongly acid, they may first be neutralized by means of calcium carbonate. It must also be kept in mind that *Penicillium brevicaule*, as well as other molds, will convert tellurium and selenium compounds into volatile substances having a garlic-like odor. The arsenic and tellurium odors are very closely similar but that from selenium is somewhat different in quality, more like that of mercaptan. The test is extremely delicate, 0.00001 gram of arsenic

can be recognized with certainty. A solution of 0.00001 gram of potassium tellurite in 10 cc. of mold infested gelatin medium in a cotton plugged test-tube gave out a strong odor of garlic for several weeks.

Biginelli ascertained that the gases formed by *Penicillium brevicaule* in arsenical cultures were completely absorbed by solutions of mercuric chloride with the formation of a double compound of mercuric chloride and diethyl arsine which is quite easily decomposed accompanied by the reappearance of the garlic odor.

The test is unlimited in its application and will respond in the presence of all manner of organic substances and bacterial contaminations. It is far more delicate than any of the chemical tests and can be carried out in much shorter time.

Toxicity Tests with Defibrinated Blood.—The older physiologists and toxicologists made the interesting observation that toxic substances of various kinds produced certain changes in the blood. Some poisons disintegrated the red corpuscles, some caused the corpuscles to clump or to agglutinate and still others reduced or even completely inhibited the coagulating power of the blood. These phenomena have suggested the possibility of estimating or measuring the toxicity of certain groups or classes of substances by noting the effects which they produce when brought in contact with red blood corpuscles. The more important groups of toxic substances which give rise to marked reactions with red blood corpuscles are the toxalbumins or toxins, the saponins and many of the toxic chemical compounds. The following tests may prove of value in the food and drugs laboratories.

Toxalbumins or Toxins.—Toxalbumins and toxins are poisonous substances formed in plants and animals as the result of microbic invasion and also as the result of metabolism in the plant or animal itself. Of special interest are the vegetable toxalbumins which possess the remarkable property of clumping, agglutinating and finally precipitating red blood corpuscles and have therefore been designated "vegetable agglutinins." A mere trace of these sub-

stances, when added to defibrinated blood in a test-tube, causes clumping into a mass resembling sealing wax. The most important vegetable agglutinins are abrin, ricin, robin and crotin. Of these, ricin, abrin and crotin also cause the coagulation of milk.

To make the agglutination tests, defibrinated blood is used. Whip the fresh blood (of ox, horse, guinea-pig or rabbit) by means of twigs, bunch of thin wires, wire mesh egg beater, or run the blood into Erlenmeyer flasks with iron filings and shake vigorously for several minutes. The fibrin is deposited on the twigs, wires, or on the iron filings, thus separating it from the corpuscles and the serum. Removing the serum from the blood and displacing it by physiological salt solution renders the reaction more pronounced, thus pointing to the existence of antiagglutinins in the serum. Ricin will agglutinate the blood of the guinea-pig in dilutions of 1 : 600,000. Abrin, crotin and robin react in a similar manner.

Saponins.—These substances are widely distributed in the plant kingdom and have chemical properties linking them with the glucosides. They have been designated nitrogen-free glucosides. The dry powder causes violent sneezing when inhaled and the aqueous solutions foam when shaken. Most of them are neutral in reaction and are capable of holding many finely divided substances in suspension. They dialyze with difficulty and incompletely. They dissolve in hot as well as in cold water but are insoluble in absolute alcohol and in ether.

Saponins have been found in many different species of plants. The more important and better known are digitonin (in *Digitalis purpurea*), saponin (*Saponaria officinalis*), githagin (*Agrostemma githago*), senegin (*Polygala senega*), saponin (*Chlorogalum pomeridianum*), struthiin (*Gypsophila struthium*), sapotoxin (*Quillaja saponaria* and *Sapindus saponaria*), and sarsaparilla-saponin (Sarsaparilla species). Saponins are highly toxic when introduced into the blood directly and some of them are well-known poisoning agents. American Indians have long made use of the roots of Chlorogalum for the purpose of stupefying fish. Most saponins

are however absorbed quite slowly which makes it possible for persons in good health to take comparatively large quantities of weak solutions without producing serious harm. They are protoplasmic poisons and it is due to this property that they hemolyze blood causing it to become laky. It has been demonstrated experimentally that saponins act more energetically upon blood corpuscles separated from the serum because the serum contains cholesterin which retards hemolysis. It is suggested that the hemolytic action of saponins is due to the removal of the lining membrane of the corpuscles which consists of lecithin, forming lecithin-saponin. Saponins also combine with cholesterin (forming cholesterin-saponin) and the affinities of any saponin being satisfied by the cholesterin, it no longer acts upon the lecithin. This explains why cholesterin retards or checks the hemolytic action of the saponins. The saponins also dissolve white blood corpuscles but to a much weaker degree.

In making the blood tests for the presence of saponins, isotonic (to blood serum) or physiological salt solution (0.9 per cent.) is added to the defibrinated blood, 100 parts to one of the defibrinated blood. Dilute the suspected saponin bearing substance with physiological salt solution and add it to the diluted blood suspension. If saponin is present the mixture at once becomes laky due to hemolysis. Githagin will develop the hemolytic action in dilutions of 1 : 50,000.

It must be borne in mind that a variety of substances will produce hemolysis, such as ether, chloroform, alkalies, gallic acid and solanine. The lytic test above outlined may be employed as a check or corroboration of the chemical and perhaps additional biological tests.

Chemical Hemolysis.—Vandevelde has suggested a method for determining the toxicity of chemical compounds by hemolysis. Defibrinated ox blood is used in addition to the following solutions. A solution of 0.9 per cent. of salt in 50 per cent. alcohol (by volume, specific gravity of 0.9548 at 15° C.); physiological salt solution

(0.9 per cent.) and a suspension of 5 per cent. defibrinated ox blood in 0.9 per cent. salt solution.

To make the experiments test-tubes are used and the compound microscope is not required. In a series of standard test-tubes place 2.5 cc. of the suspended blood (in the sodium chloride solution) and the same amount of the solution to be tested (in varying amounts of physiological salt solutions, therefore different strength solutions) in order to ascertain the exact point when hemolysis takes place. A solution which does not produce hemolysis after a definite period of time (3 hr.), but which does result in hemolysis on the smallest further addition of the substance under examination is spoken of as a "critical solution." The time limit in these tests is 3 hr. If after the expiration of this period of time, the trace addition of the solution does not produce the hemolytic effect, the test is negative and the next stronger or higher concentrate must be tried. The term critical coefficient refers to the number giving the concentration of the substance necessary to hemolyze or kill the red corpuscles.

The following is the result obtained by Vandevelde regarding the critical solution of ethyl alcohol.

Cc. of Suspended Blood	Cc. of NaCl Sol. in Alcohol	Cc. NaCl Solution	Alcoholic Sol. in Volume Per Cent.	Reaction in 3 hr.
2.5	2.20	0.30	22.0	Hemolysis
2.5	2.15	0.35	21.5	Hemolysis
2.5	2.10	0.40	21.0	Hemolysis
2.5	2.05	0.45	20.5	Hemolysis
2.5	2.00	0.50	20.0	Hemolysis
2.5	1.95	0.55	19.5	Negative
2.5	1.90	0.60	19.0	Negative

From this table it will appear that the critical solution of ethyl alcohol contains 19.5 cc. of absolute alcohol in 100 cc., or 15.489 grams of alcohol in 100 cc. According to Vandevelde, the addition of methyl alcohol diminished the toxicity of ethyl alcohol, whereas the higher alcohols were found to be more toxic than the

latter. Giving the toxicity of 100 parts of ethyl alcohol as 100, then 47 parts by weight of isopropylic alcohol, 29 parts of isobutylic alcohol and 12.5 parts of amylic alcohol were found to be isotoxic with that quantity of ethyl alcohol.

The term toxin, more accurately speaking, applies to poisonous substances elaborated by bacteria and which require an incubation period before forming antibodies or antitoxins. The toxins formed by the bacterial group appear to be intimately associated with the life processes of the living cell, but their chemical composition remains thus far unknown. We know that they are very readily destroyed by heating (60° to 80° C.) and that they are chemically very unstable, and that they are among the most highly poisonous agents known to science. They are far more toxic than the potent vegetable alkaloids and animal toxalbumins, as is shown in the following tabulation (Jordan):

Atropine, fatal dose to man	130 mg.
Strychnine, fatal dose to man	30–40 mg.
Cobra venom, fatal dose to man	4.375 mg.
Tetanus toxin, fatal dose to man	0.23 mg.

Various animals produce toxalbumins or toxins, as snakes (crotalin, viperine), scorpions, tarantulas, the Gila monster and other lizards. Rattle-snake venom evidently possesses a variety of properties. It will agglutinate blood, neutralize the fibrinogen, hemolyze red corpuscles, and is highly neurotoxic. Within recent years antibodies have been produced against these several toxic substances.

Muscarine, the toxic agent of *Amanita muscaria* (fly agaric), is an alkaloid which acts very quickly, whereas the toxic agents of *Amanita phalloides* and *A. verna* are toxin-like in that there is an incubation period of from 10 to 14 hr. before the toxic symptoms begin to manifest themselves. They are strongly hemolytic. It is supposed that the pollen grains of certain flowers contain toxin-like substances to which certain persons are peculiarly susceptible. All toxalbumins or toxins, whether derived from bacteria,

fungi or higher plants, possess the very characteristic property of forming antibodies or antitoxins. Thus there is antivenin used in the treatment of snake-bite, also antibodies used in the treatment of hay fever, etc., which products are more fully described in works on medical bacteriology.

Frog Tests for the Presence of Alkaloids.—Lively small frogs respond quite readily to the action of even high dilutions or very minute quantities of vegetable alkaloids. It is suggested that the food bacteriologists perform the following tests, which are frequently desired as a check or corroboration of the findings of the chemist and toxicologist.

The material used for the frog injections is the evaporated ether extract dissolved in a small amount of sterilized distilled water. The injections are made hypodermically in the lymph sac on the back of the frog. It is advised that the tests be made in duplicate and repeated as often as may be required to attain absolutely conclusive results. Each test should be checked by injecting approximately minimal fatal doses of the pure alkaloid itself, obtained from some reliable house. This will make it possible to note the toxic symptoms produced by the pure alkaloid, and compare with the symptoms produced by the suspected alkaloid in the substance under consideration. These check tests are generally omitted by the analyst who has had extensive laboratory experience and who is therefore in a position to recognize the nature of the poison (alkaloid) from the symptoms manifested by the inoculated frog. In many instances the frog alkaloidal tests may serve as checks upon the blood tests already described.

In extracting the suspected substances it must be kept in mind that alkaloids are very sparingly soluble in water, but when acidulated (hydrochloric acid about 1 per cent.) water is used the acid forms the salt (chloride) which is readily soluble in water. Alkaloids are soluble in ether and in mixtures of ether and chloroform, therefore these reagents should be used rather than the acidulated water, especially since they are also antiseptic and the extractives

which they yield are freer from extraneous impurities, and also because the residue which is injected into the frog (dissolved in the water) represents the alkaloid and not its chloride. Should, however, the evaporated acidulous extract be used for making the frog test, then the check test should be made with the corresponding pure alkaloidal salt.

If the inoculated frog shows no marked symptoms of poisoning in the course of 3 or 4 hr., it is very likely that the residue under suspicion does not contain any very poisonous substance. The primary object of the tests is to ascertain whether or not dangerously toxic substances (alkaloids) are present in foods, and not for the purpose of determining the identity of the alkaloid nor to demonstrate the presence of comparatively nontoxic alkaloids.

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