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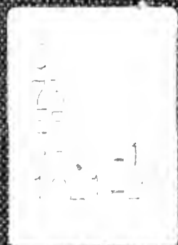

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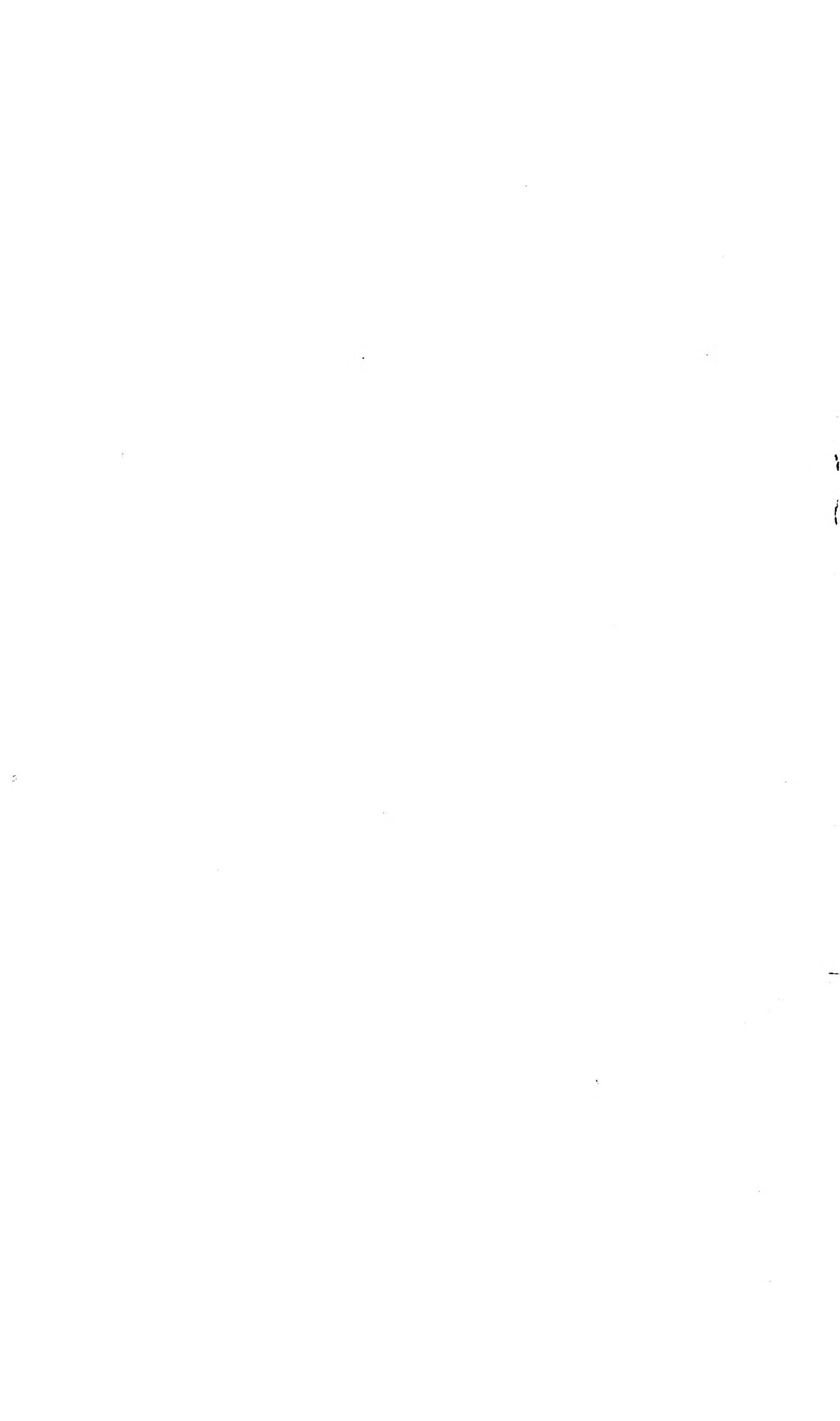

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AGRICULTURAL EXPERIMENT STATION

TECHNICAL BULLETIN No. 4 NOVEMBER, 1921

DEVELOPMENT AND PATHOGENESIS

OF THE

ONION SMUT FUNGUS

By P. J. ANDERSON

Onion smut is the most destructive of all onion diseases in New England. In the Connecticut Valley it is probably responsible for more loss to the growers than all the other diseases of this crop combined. This paper embodies the results of fundamental work on a project having for its chief aim the control of onion smut. Beginning with the germination of the spores, the development of the fungus is followed through the saprophytic stage, infection of the host, distributive stage within the host, and final sporogenesis.

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TECHNICAL BULLETIN No. 4.

DEPARTMENT OF BOTANY.

DEVELOPMENT AND PATHOGENESIS OF THE ONION SMUT FUNGUS.[1]

BY P. J. ANDERSON.

I. Introduction.

Onion smut is the most destructive of all onion diseases in New England. In the Connecticut Valley it is probably responsible for more loss to the growers than all the other diseases of this crop combined. Despite the fact that a method of control by the use of formaldehyde has been developed, many fields are now planted to other less profitable crops on account of the ravages of smut; every year sees fields plowed up because smut has so reduced the stand that it is not worth while to tend them; more important in the aggregate, perhaps, is the smaller toll which the disease exacts from each onion grower throughout considerable sections of the valley.

Investigation of the disease with the primary object of finding better methods of control was begun by the Department of Botany of the Massachusetts Agricultural Experiment Station in 1918, and has been continued to date. Since control measures are necessarily conditioned by the normal life history of the pathogene, and since a review of the published research of other investigators showed that the development of the fungus had been inadequately studied, this phase of the problem has been made the subject of no inconsiderable part of the writer's study. Results of the work which deals directly with control are to be presented in another publication. The present paper concerns certain phases of the life cycle of the causal organism (*Urocystis cepulae* Frost) in which it seemed to the writer that further investigation was desirable. Beginning with germination of the spores, the development of the fungus will be followed through its saprophytic stage, infection of the host, distributive stage within the host and final sporogenesis.

[1] This paper embodies the results of preliminary and fundamental work on a project having for its chief aim the control of onion smut. A report on the more practical phases of this project is to be published shortly.

II. Germination of the Spores.

The spore of *Urocystis cepulae* is compound, having one large central fertile cell to the surface of which are attached 15 to 40 smaller hemispherical sterile cells. There are said sometimes to be two fertile cells at the center, but in a three-year study of the fungus the writer does not remember ever having seen a spore with more than one. To conform to the nomenclature of certain other genera of smuts, the entire structure is usually called a spore ball, the peripheral cells being termed pseudospores. Since we have here only one cell capable of germination, it is perhaps better to term the whole structure a spore and then distinguish between fertile and sterile cells. The hemispherical cells are attached to the fertile cell by their flat surfaces, but do not cover it entirely. They stand apart as indicated in Fig. 1 (page 109). The sterile cells are tinted brown, while the central cell is a more solid opaque brown. Sterile cells average 5μ in diameter by 4.25μ in height. The fertile cell is usually spherical, but frequently oval or ovate, averaging about 12μ in diameter. The entire spore averages about 19μ in diameter.

For the germination of most fungous spores it is only necessary to place them when mature in a drop of water, and, after a few hours, or, at most, a few days, the whole process may be watched under the microscope. But for *Urocystis cepulae* the case is not so simple. Germination tests, conducted in the same way in which the writer had brought to germination the spores of many species of fungi, were entirely without result for the onion smut fungus. Apparently there are other essential conditions which had not been obtained in these trials. This preliminary failure led to a thorough search through the literature to find what conditions were essential for the germination of spores of other species of Ustilaginales. It seemed probable that the same conditions which brought about germination in other smuts might also be successfully applied to *Urocystis cepulae*. A condensed summary of the literature of this phase is given below, followed by a description of the experiments with the spores of *Urocystis cepulae*.

Review of the Literature on Essential Conditions for Smut Spore Germination.

The Water Requirement. — No spores will germinate without water in some form, sometimes, to be sure, merely as vapor in a saturated air. In the simplest cases, and, in fact, for the majority of the smut fungi, it is only necessary to immerse the spores, as soon as mature, in a drop of water on a slide, or in a hanging drop. Enough air to satisfy all requirements seems to be present dissolved in the water, or else the spores remain on the surface of the drop. Brefeld (3), in his experiments, germinated the spores in a film of water which adhered to the inside of the walls of flat glass chambers after the bulk of the liquid had drained out. This probably insured greater access to air than where the hanging drop or drop on slide has been used, and this fact should be kept in mind in interpreting his results.

The following species can be germinated in water as soon as mature: *Cintractia densa* McAlp. (11); *C. Sorghi vulgaris* (Tul.) Clint., 12 hours,[1] (11); *Entyloma canescens* (14); *Schizonella melanogramma* D. C. (4); *Sorosporium Reilianum* (Kühn) McAlp., tap water, 17 hours (11); *Tilletia zonata* Bref. (4); *Urocystis occulta* Wallr. (11); *U. primulicola* Magn., 10 hours (14); *U. Violae* Sow. 5 days (4); *Ustilago Avenae* (Pers.) Jens., 6 to 8 hours (8), (11) and others; *U. Bouteloua humulis* Bref. (4); *U. Carbo* Tul., 6 to 10 hours (5); *U. flosculorum*, 5 to 6 hours (7) (he finds that fresh spores germinate most quickly); *U. grandis* Fr., 24 hours (3); *U. longissima* Sow., 3 to 4 hours (3), (7); *U. major* 24 hours (14); *U. Panici glauci* Wallr., 8 days (3); *U. Readeri* Syd. (11); *U. segetum*, 6 to 8 hours, "fresh spores germinate better" (14); *Ust. violacea* Pers. (3) and many others. In the most favorable cases germination begins within two to three hours, while at the other extreme McAlpine (11) mentions species the spores of which did not begin to germinate until they had been in water for several weeks. Where such extreme lengths of time are required, the question arises as to whether this is not really the time required for the weathering process such as takes place when they are kept in damp soil, as in Brefeld's experiments.

Air. — Some spores require only a moist air for germination, and will not germinate at all or only abnormally when immersed in water. Thus Fischer von Waldheim (7) writes: —

> For the normal germination of the different species of Ustilago, a certain quantity of water or moisture is usually necessary. For this purpose, the spores need only be placed in a drop of water, or upon moistened earth, or even merely in an atmosphere kept moist; for instance, under a glass globe placed over a dish of water. But Tilletia and Urocystis germinate only in damp air (for instance, under the glass globe mentioned), and their germinating spores, coming in contact with water, only show abnormal appearances.

In Brefeld's germinating apparatus the spores were never entirely immersed in water, but in the thin film clinging to the chamber walls must have always had a sufficient quantity of air. This probably contributed to his remarkable success in germinating the spores of a very large number of species. McAlpine also found that he was able to secure germination in many cases only by floating the spores in a watch glass over water. Both Brefeld (3) and Fischer von Waldheim (7) mention the fact that the spores of *Tilletia caries* germinate in damp air. Plowright (14) had a similar experience with *Tubercinia trientalis.* McAlpine (11) was able to germinate the spores of *Tilletia Tritici* (Bjerk) Wint. best by keeping them on moist filter paper or blocks of plaster of Paris kept moist by capillary water from a dish in which the blocks were partially immersed. He (11) makes the following interesting observation on the necessity of air for germination of spores of *Ustilago Readeri* Syd.: —

[1] Figures after the species and not in parentheses indicate the time required for germination to begin after the spores were placed in water. Omission of them indicates that the investigator gave no data as to time required. Numbers in parentheses refer to bibliography on pp. 132 and 133.

Immersed in the liquid they do not germinate as readily as when floating on the surface. Thus, after eighteen hours on one occasion, the spores in the water had failed to germinate, while by simply altering the focus and examining the spores on the surface they were all found, with very few exceptions, to have germinated.

In the descriptions of germination given by the majority of writers there is no way of determining just how much influence the presence of air had.

It seems probable that, in general, the presence of air is essential to the germination of smut spores, but that different species vary in respect to the amount required; some need scarcely any, others must have very free access to air, and there are probably all gradations between these two extremes.

Nutrient Solutions. — Very early in the investigation of smut spore germination it became apparent that the spores of some species could not be germinated merely by placing them in water when mature. Consequently solutions of various substances supposed to have nutritive qualities have been tested for their ability to induce germination. Hallier (6), in 1868, was apparently the first to use such solutions. He used a great many substances such as albumin, starch, milk, sugar solution, etc. Others, since then, have used almost every kind of a salt, acid, or other substance for which one could imagine any germinative influence. One should consult Osner's (13) bulletin on "Leaf Smut of Timothy" to gain some idea of the number of substances that can be used for that purpose. McAlpine (11) seems to have had most success with a hay infusion, although he also used various other solutions. Sugar solutions and decoctions of the host plant have proved fairly successful.

The nutritive solution which has been used most extensively and probably most successfully is the "nährlösung," a sterilized aqueous decoction of horse dung which was employed first by Brefeld (3, 4). In this "nährlösung" he was able to bring to germination the spores of many species which showed no sign of germination in water, *e.g.*, *Cintractia spinificis* (Ludw.) McAlp. (McAlpine (11) also confirmed Brefeld's results), *Doassansia Limosellae* Kunze, *Ustilago Andropogonis tuberculati* Bref., *Ust. Arundinellae* Bref., *Ust. Coicis* Bref., *Ust. Cynadontis* Hem., *Ust. Ischdemi* Fckl., *Ust. major* Schroet., *Ust. Panici leucophaei* Bref., and *Ust. Tulasnei* Kühn. Other species, *e.g.*, *Ust. Maydis*, which gave scanty or only occasional germination in water, germinated to almost 100 per cent in this "nährlösung." In almost every case the growth and size of the germ tube (promycelium) was increased; and frequently sporidia were produced in this nutritive solution where none at all were developed in water. On the whole, however, it should be kept in mind that in by far the majority of cases the function of the nutritive solution was to bring the germling to complete development after it had started, rather than to cause it to start in the first place. Only in the case of the comparatively few species mentioned above did he fail to get some germination in water also, and very commonly the percentage of germination was as high in water as in "nähr-

lösung." On the other hand, he found that *Tilletia Tritici* would not germinate at all in nutritive solution, but could be germinated easily in water.

His experiments with nutritive solutions led Brefeld to believe that smut spores in the soil are brought to germination and further development through the influence of manure which has been used to fertilize the soil. On this theory he explains the common observation of German farmers that cereal smuts are more destructive on freshly manured fields.

Host Stimulus. — One might expect that some stimulus from the host plant would be necessary for germination, and consequently that a decoction from the host, or the presence of bits of it in the germinative medium, would be necessary for starting germination. Although such host decoctions have been successfully used, we find in the literature no instance in which they furnished the only conditions under which the spores would germinate. There seems, then, to be no evidence to indicate that a smut spore must be in close proximity to, or in actual contact with, its host before it will germinate.

Period of Rest. — But, even with the aid of nutritive solutions, and all other conditions which have been tried, there is a considerable number of species, the spores of which cannot be brought to germination immediately after maturity. For these species, a period of "rest" is necessary during which they must be exposed to certain natural conditions which operate in some way to bring them into the proper condition for germination. For our knowledge of this phase of the problem we are indebted, above all, to Brefeld, and we cannot present it better than by quoting from his summary of it ((4), page 128):[1] —

> Only a part of these forms germinate at once even in nutrient solution, more rarely in water; many will not germinate at all, but must be made capable of germination by special methods. . . . The spores of many species are so adapted in their time of germination that they do not proceed at once, but only after passing through a shorter or longer resting period. In cases of this kind one has only to wait until after the expiration of the resting period in order to bring them to germination. But one would often wait long and in vain, if he only kept the spores dry in the house. Under these circumstances, the external influences are not brought to bear, which operate in nature during the period of rest, and which must operate in order to bring about those changes on which the initiation of germination depends. For the most part, when simply kept dry the spores die without germinating, except in a few cases, as, for example, the corn smut, . . . but even here germination is always incomplete. It is necessary to obtain the conditions which in nature operate on the spores and influence them to germinate, if one wishes to succeed in observing germination. The simplest method would be to expose the spores in nature or leave them in their natural habitat and observe from time to time whether germination has begun. But in most cases it is entirely impossible in this way to get and keep the material pure.

He then describes in detail his method of keeping the material in sterilized damp sand in pots in a cool cellar. Then he continues: —

---

[1] Translated by P. J. Anderson.

> By this method it has been possible to bring to germination most spores which otherwise would not germinate. The length of time required to bring about germination varies greatly. The spores of some species usually germinate after a few months, others after a half or an entire year, others require several years before germination, some even five years. . . . In this methodical way, which is, to be sure, nothing but an imitation of what takes place in nature, ultimately all spores can be induced to germinate. Therefore it can be scientifically proved that the earlier or later germination is only an adaptation, a resting period, which under the natural conditions must be passed through, if the inner and apparently chemical changes are to operate, through which the germination of the spores is slowly prepared and finally made possible.

In this way Brefeld was able to germinate the spores of the following species none of which would germinate when first mature (length of time in moist earth given after each): *Anthracoidea* (*Ustilago*) *Caryces* Bref., over winter; *Anthracoidea subinclusa* Bref., 1 year; *Doassansia Alismatis* Nees, 1 year; *D. Limosellae* Kunze, 1 year; *D. punctiformis* Niesse, more than a year; *D. Sagdlariae* Fckl., over winter; *Melanotaenium cingeus* Bref., 4 years; *Neovossia Barclayana* Bref., 2 years; *Sphacelotheca Hydropiperis* Schum., 6 months; *Tilletia controversa* Kühn, 2 years; *Tilletia decipiens* Pers., 3 years; *Tolyposporium bullatum* Schroet., 9 months; *Tol. Junci* Schroet., 6 months; *Tol. Penicillariae* Bref., 1 year; *Urocystis Anemones* Pers., 6 months; *Ur. Filipendulae* Tul., 1 year; *Ustilago Adoxae* Bref., 1 year; *U. anomola* Kunze, over winter; *U. Bistortarum* D. C., 1 year; *U. Coicis* Bref., 2 years; *U. domestica* Bref., 6 months; *U. Holostei* D., 3 years; *U. utriculosa* Nees. Other writers also have found that for various species, a weathering under natural conditions was necessary in order to secure germination.

Substitution of Nutritive Solution for Weathering Period. — In the case of some species Brefeld believes that the same changes which are ordinarily induced by storage in damp soil for a long period may be induced at once by the use of his "nährlösung." For example, he finds that the corn smut spores when first mature will not germinate in water, but if kept until the following spring they germinate in water. If, however, the freshly matured spores are put in nutritive solution, they germinate overnight almost without exception. He concludes, therefore, that the changes induced by one are the same as those induced by the other, or, in other words, that each may be substituted for the other.

Freezing. — Whether or not freezing has any influence on germination seems never to have been determined. Brefeld makes no mention of freezing, and one infers from his publications that his buried spores were never frozen. Since the spores of practically all species of smuts have been successfully germinated without freezing, it may be safely said that freezing is not a necessary condition of the process.

Essential Conditions for Germination of Urocystis cepulae Spores.

Search through all available literature on the subject revealed only one reference to previous attempts at germination of the spores. Thaxter (18) was unable to germinate fresh spores either in water or in moist air. When,

however, the smutted onions were stored until January, then mixed with wet earth and frozen for a week or more, the spores germinated when kept moist in a warm room. They also germinated in an onion decoction. He also made pure cultures in onion decoction from fresh spores and from sporiferous hyphæ, but does not mention germination in this respect. Such, in full, is the extent of our present knowledge of the necessary conditions. The purpose of the writer's experiments was twofold: (1) to duplicate Thaxter's work and (2) to extend the inquiry in order to determine more exactly many points which Thaxter either did not touch or treated insufficiently. The experiments are summarized below.

Fresh Spores in Water. — Spores from a fresh but mature lesion were scattered in a drop of water on a slide kept in a Petri dish with water in the bottom of the dish to prevent evaporation of the drop on the slide. This common and familiar method was used in all the experiments where water or a water solution was tested. Both distilled water and tap water were tried. The spores were examined daily for over two weeks, but no indication of germination was observed. The experiment was repeated many times, and the temperature and light relations were varied in different sets, but always without result. Spores taken from lesions which had been kept dry for a year in the laboratory gave no better results.

Fresh Spores in Soil Water. — A soil extract was made by filling a beaker with good onion soil (taken from a field where smut was abundant), adding water until the soil was saturated and the water was 1 cm. deep on top of it, stirring thoroughly several times and filtering off after several days. Results were the same as with tap and distilled water.

Influence of the Germinating Onion Seed. — These tests were in every way like those described above with water, except that a few germinating onion seeds were placed in each drop in addition to the spores. With one exception, in these tests the spores failed to germinate. On one slide a very few spores germinated in close proximity to the young cotyledon.

Fresh Spores in Soil Decoction. — A mixture of soil and water was cooked for one hour on two successive days in the autoclave at 14 pounds' pressure, filtered, tubed and sterilized. It was hoped that in this way more of the soil substances would be brought into solution, and that they might bring about germination. But, just as in the case of the soil extract, so with this more concentrated soil decoction, there was no germination.

Fresh Spores in Dung Decoction. — This decoction was prepared just as Brefeld prepared his "nährlösung" which he used so successfully on the spores of a large number of species. Fresh spores failed to germinate in it. In these experiments the solution was concentrated. It is possible that if it had been more diluted the results might have been different.

Fresh Spores in Onion Decoction. — This decoction was prepared by boiling a sliced onion in a pint of water for one hour. It was then filtered, tubed and sterilized one-half hour at 15 pounds' pressure. This appears to furnish an excellent medium for the growth of bacteria and fungi, and in working with it every possible precaution must be used to prevent contamination. These organisms grow so fast that they soon obliterate the

more slowly germinating smut spores. It was found necessary not only to sterilize, by boiling, the slides, Petri dishes and all instruments used, but also to wash the seedlings from which the spores were taken, first, in mercuric chloride, 1 to 1,000, and then in sterile water, before the lesions were opened. In drops of this decoction some of the spores began to germinate within three days at laboratory temperature. The percentage of germination, however, was always very low. In dozens of slide tests made in this way, not over 25 per cent germination has ever been observed; and in most cases it is lower, averaging 5 to 10 per cent. It is apparent from these tests that there is some substance in the onion which is capable of inducing germination of fresh spores. In the light of other tests described below, however, one would not be justified in concluding that this substance is peculiar to the onion alone.

Fresh Spores in Sugar Solutions. — Sterile solutions of ½, 1, 2, 3, 5, 7 and 10 per cent cane sugar were used just as the onion decoction mentioned above. There was some germination in all of them, but very little in the ½ per cent and the 10 per cent. The highest percentage of germination was in the 2 per cent solution, where 50 per cent of the spores *which were on the surface of the drop* germinated. When spores are mixed with a water solution of any kind, some of them remain on the surface while others sink to the bottom. Only a very small percentage of those which were immersed germinated. Since the spores on the surface are better located for obtaining air, it is apparent that air is an important factor in germination. It is also apparent that sugar is at least one of the substances which may induce germination. Since onions contain a high percentage of cane sugar, it seems probable that this is also the effective element in the onion decoction which induces germination.

Fresh Spores on Onion Decoction Agar. — Onion decoction agar was prepared by adding 2 per cent of agar to the onion decoction. Sterile plates were poured and permitted to become hard. Spores were mixed with onion decoction or water and floated over the surface of the hard agar. After permitting the spores to settle to the bottom the liquid was poured away and the spores were left distributed over the agar. This insured a sufficient quantity of air, and at the same time access to nutrient substances in the agar. The percentage of germination varied with different experiments, but always it was as high as 10 per cent; sometimes 50 per cent. This was found to be the most reliable of all the methods and was largely used. Here also it was noticed for the first time that the spores did not all germinate on the same day, but that there was a progressive germination, new ones starting each day for as long as three weeks, after which the plates had dried too much, or possibly the supply of food had become exhausted.

Fresh Spores on Czapek's Agar, Sugar Potato Agar, etc. — The Czapek's agar contains 3 per cent of cane sugar. Several other agars containing sugar were tried and always with a small percentage of germination, but none higher than on onion decoction agar.

Fresh Spores in Soil Decoction Agar. — After the rôle played by air was determined, it seemed that the writer's previous failure to induce germination in soil decoction might have been due to exclusion of air. Therefore a medium was prepared by adding 2 per cent of agar to the soil decoction. Tests were made as with the onion agar, using soil decoction, however, for floating the spores over the surface. After five days, germination of 1 to 2 per cent was observed. With each day, however, more of them germinated, and this continued for several weeks until the plates became too dry or were exhausted. We may conclude from these experiments that (1) the soil contains all the essential stimulating elements for germination, and (2) not all the spores germinate at once, but there is a progressive preparation.

Fresh Spores on Dung Decoction Agar. — This medium was prepared by adding 2 per cent of agar to the dung decoction mentioned above. Since the soil used in making the soil decoction had been heavily manured during the previous season, it was thought that some element in the manure might furnish the stimulus and a higher percentage of germination would be secured. The percentage of germination in this medium, however, was scarcely as high as for the soil decoction. Here is proof, however, that stable manure contains some substance which is capable of inducing germination.

Effect of Freezing the Spores. — It has been previously mentioned that Thaxter froze smutted mature onions in the soil and then found the spores capable of germination. This experiment was duplicated as nearly as possible by the writer, but he was entirely unable to get the spores free from bacteria and other fungi, and abandoned the method rather than work with contaminated cultures.

An attempt was next made to freeze the spores under sterile conditions. Smutted seedlings were sterilized with mercuric chloride 1 to 1,000, washed in sterile water, and sealed in sterile test tubes with a drop of water in the bottom of each tube. After being exposed for nine days during December, during which there were some light freezes, they were tested in onion decoction. There was a germination of about 2 per cent. In similar tests during January, in which they were frozen solid for ten days or more, buried under the snow in zero weather, the spores were apparently killed. No germination at all was observed, although tried on or in the various media described above. In view of the fact that the mycelium in culture is not killed by freezing, these results are difficult to explain. In a later series of tests smutted seedlings, sterilized on the surface, were buried in sterile soil in test tubes and then frozen out of doors for eight weeks. On onion decoction agar plates, varying percentages of germination were then secured, but it was never as high as for spores which had been kept in damp soil during the same length of time, but not frozen. The conclusion seems warranted that freezing does not kill spores in the soil, but it does not render them more capable of germination, and is not necessary.

Effect of a Period of Rest in Damp Earth. — Seedlings with unopened lesions were sterilized and buried in sterile soil in test tubes. The tubes

were then sealed and kept in the laboratory. After two weeks, germination was found to be somewhat higher than in the case of spores from fresh lesions. Tests at the end of four weeks gave 50 per cent germination. At the end of three months the average percentage was not higher, though in individual slides it mounted to about 65 per cent. A higher percentage of germination has not been seen in any test. In removing these seedlings from the damp earth it was constantly noticed that the soil remained clinging to the lesions and could be washed off with difficulty, while it was very easily removed from other parts of the plant. Microscopic examination showed that the soil particles were attached by numerous fungous hyphæ. When these hyphæ were transferred to sterile agar tubes they gave pure cultures of Urocystis. It was not possible to determine whether these hyphæ arose from germination of spores in the sori, from vegetative hyphæ in the seedling or from both. If the spores germinate while still inside the lesion, this may explain why not all the spores taken from the weathered sori germinate; they may have already germinated. This experiment demonstrates clearly one way, at least, in which the smut mycelium gets back into the soil from the diseased plants.

Natural Conditions of Germination. — It is a common impression among laymen that the spore remains dormant in the ground, lying in wait until an onion starts to grow near it, upon which it germinates and infects the onion. Such, however, is apparently not the case. The seedling does not seem to furnish any stimulus which causes the spore to start. Whatever substances are necessary for starting the process are in the soil itself. As soon as the spores are released into the soil — if not before — a few of them germinate; the others become capable of germination gradually, and it seems likely that all of them finally come to germination, but that the period of preparation differs in length for different spores, so that the germination extends over many months and possibly years. This period of preparation may be shortened artificially by the use of certain stimulating substances, such as cane sugar.

The Process of Germination.

Germination begins in three to six days after the spores are placed in the water solutions or on agar plates as previously described. The time varies somewhat with the medium used, and also apparently with other factors which have not been explained. Three days are usually sufficient in onion decoction or onion agar, while on soil decoction agar six days were found necessary.

The first indication of germination is the appearance of a hyaline hemispherical vesicle (Fig. 1, B) on one side of the spore. This is apparently an extrusion from the central fertile cell, but whether it comes out by a rupture of the spore wall or by a regular pore could not be determined. The covering of sterile cells renders exact observation of this point difficult. This vesicle when first observed is of about the same size as one of the sterile cells, and can at first be distinguished from the latter only by the

fact that it is hyaline while the sterile cells are brown. During the succeeding stages, however, it increases rapidly in size until it may be almost as large as the spore itself (Fig. 1, K). This hemispherical or subglobose

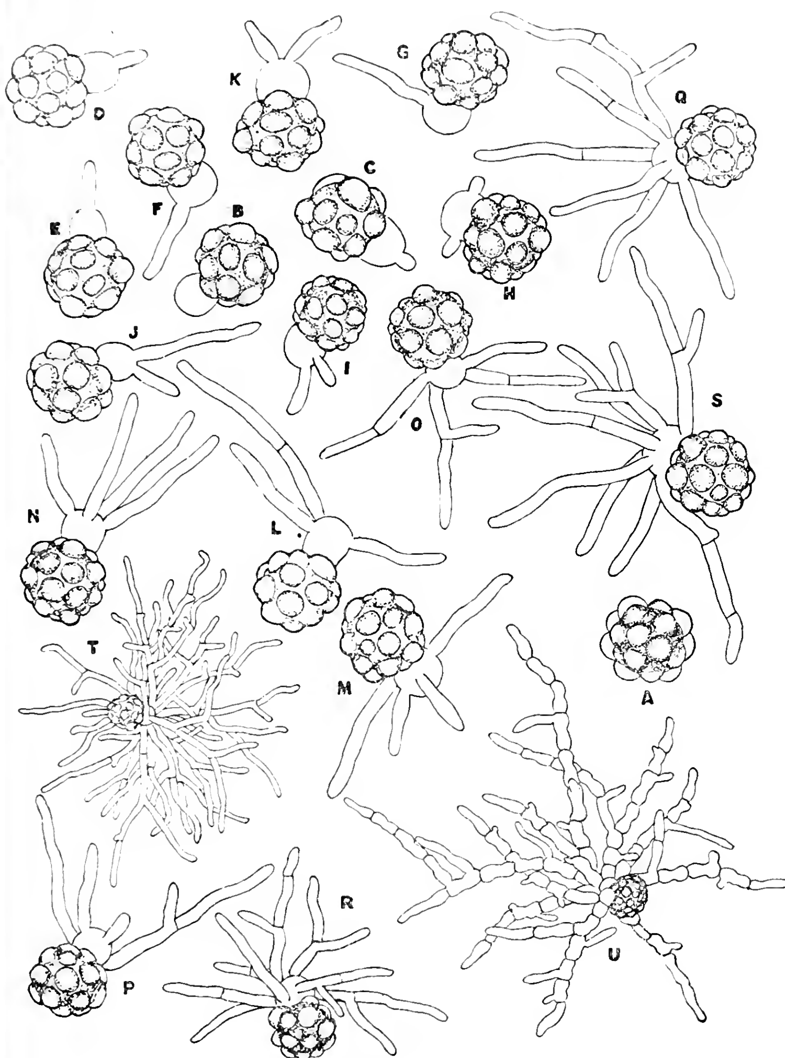


FIG. 1. — Germination of spores.

vesicle corresponds to the promycelium or hemibasidium of other smuts and rusts, and will be so designated. A stout tube grows out from the surface of the promycelium (Fig. 1, C–G) and is quickly followed by others in succession until a whorl of diverging branches is produced (Fig. 1, H–N). The number of branches in the whorl is not constant, neither

do they arise simultaneously but usually in succession. None has been observed, however, which showed more than eight branches on a promycelium (Fig. 1, S). These primary branches are 2 to 3μ in diameter, usually somewhat undulating, with broadly rounded tips. They soon become septate, and almost invariably a lateral secondary branch grows out from the top of the cell just below each septum (Fig. 1, O–S). The angle of divergence between the primary and secondary branch is very broad, often approaching a right angle. This manner of branching is characteristic of onion smut mycelium wherever it is found, and is a good diagnostic character. By continued branching, a dense mass of mycelium is developed about the spore, and it becomes increasingly difficult to follow the course of single hyphæ. Fig. 1, T, represents the latest stage in which the separate branches could be followed. On agar plates the older cells lose their dense protoplasmic content, and only the more distant tip cells appear to be alive. In onion decoction, as the hyphæ become older they become more constricted at the septa and the cells rounded in the middle until they appear almost separated from each other, the hypha having somewhat the appearance of a string of beads (Fig. 1, U). Frequently in the older mats of mycelium from the germinated spore it has been observed that some of the hyphal tips are recurved in the form of croziers. They have, however, never been seen to develop further, and it is impossible to say whether this development has any relation to development of spores, which are never produced except inside the tissue of the host. In hundreds of germination tests which have been made during three years in a large number of media, no conidia have ever been observed on the promycelium or its branches or anywhere else throughout the development of the organism. Sometimes the short lateral branches appear like conidia, but continued observation soon convinces one that they are merely vegetative branches which will elongate apically like other branches unless the supply of nutriment is exhausted.

Comparison with the Germination Process in Other Species of Urocystis.

Let us now compare this process with the process of germination which other investigators have described for other species of Urocystis.

Urocystis occulta Wallr., causing the flag smut of rye, was apparently the first species of this genus which was studied with respect to germination of spores, that process having been first observed and described by Kühn in 1858. It was later studied by Wolff (19), Brefeld (4), McAlpine (11) and others. According to Brefeld a promycelial tube of varying length is first produced, and at its apex it branches verticillately into a whorl of four to six branches. These branches increase in length by apical growth, and they, as well as the promycelial tube, become progressively septate, while the protoplasmic content of the older cells constantly disappears and the only living cells are those at, and just back of, the growing tips. The verticillate branches never produce conidia, but form mycelium by continued growth. McAlpine considers the verticillate branches them-

selves as conidia, but does not state that he ever found them detached. This resembles the process in *U. cepulae* in (1) the production of the whorl of branches, (2) the complete absence of sporidia and (3) the progressive emptying of the cells. The main point of difference is in the elongated, ultimately septate, promycelium in *U. occulta* which replaces the globose vesicle of *U. cepulae.*

In *U. Tritici* Koern. the process is almost identical with that of *U. occulta* according to McAlpine (11), but the promycelium is at times unicellular, a condition which suggests that of *U. cepulae.*

The germination process in *U. Anemones* (Pers.) Wint. has been studied by Fischer von Waldheim (7), Plowright (14) and Brefeld (4). As described and figured by Brefeld it is almost identical with the process which the writer observed in *U. cepulae* except that the promycelium is not so large. The whorl of 2 to 4 branches arises very close to the surface of the spore on a very much reduced promycelium, and they remain permanently sterile.

In *U. Filipendulae* Tul. (Brefeld (4)) the whole process is identical with that of *U. Anemones.*

Germination of the spores of *U. Violae* Sow. has been studied by Prillieux, Dangeard, Brefeld (4) and others, being a favorite subject for study because of the ease with which germination can be brought about in water. Each fertile cell of the spore ball produces an elongated promycelium which becomes septate just as in *U. occulta.* A whorl of three to eight diverging branches is produced at the apex. Each verticillate branch grows out at the distal end into a slender sterigma on which is borne a long cylindrical conidium. In nutrient solution these primary conidia may produce secondary or tertiary conidia. The process in this species differs from that of *U. cepulae* (1) in the length of the promycelium, and more especially (2) in the development of conidia.

In general, then, we may conclude that *U. cepulae* differs in its germination from the other species of Urocystis (except *U. Violae*) only in theshape of the promycelium which is here reduced to a nonseptate hemispherical vesicle. All other details of development appear to be identical.

Comparison with the Process as described by Thaxter.

As described and figured by Thaxter the spores germinate by a single long irregularly branched tube on the tips and lateral branches of which are borne small ellipsoidal to long ovoidal conidia. He does not mention a globose promycelium or whorl of branches such as the writer has always observed. The marked differences in the process as observed by the writer and as described by Thaxter are difficult to explain, unless they are due to contamination in the cultures used by the latter. He states that he was unable to obtain the material pure, and that "all the cultures swarmed with bacteria." The presence of these same bacteria might produce a difference in the development of the germination process. The writer in attempting to secure germination by Thaxter's method also failed to keep the spores free from bacteria and therefore changed to a different method.

III. Saprophytism.

The early botanists and mycologists believed that smut fungi were obligate parasites, *i.e.*, they developed only when in parasitic relation with host plants from the living cells of which they must take their nourishment. We now know, however, that at least most smut fungi have in their life cycle a saprophytic period during which they may develop extensively and propagate for a long time, deriving nourishment only from dead organic material in the soil or other substrata. Also most of them may be propagated indefinitely in artificial culture media of various compositions. Our knowledge of this stage began with the extensive investigations of Brefeld (3), and has been increased later by numerous smaller contributions from a large number of workers. *Urocystis cepulae* is no exception to the rule, and is very readily isolated and grown in a large number of culture media and on soil. It is probably able to exist and grow in the soil for years in entire absence of onions.

Isolation.

Two methods of isolation have been used by the writer. By the first method a germinating spore on an agar plate is located under the microscope by a ring of India ink, care being taken that this spore is far enough removed from all others to prevent confusion. When the mycelium from the germinating spore has increased to such an extent that it is visible to the naked eye as a tiny white speck it is transferred to an agar slant where it gradually spreads to the agar of the tube and can be grown for a long period. This method was used especially in the original isolations when it was necessary to know for certain that the resulting fungus originated from a single spore of *Urocystis cepulae.* In later work a more rapid method was used. A part of a cotyledon or young leaf containing a lesion which had not yet broken open was washed for a few minutes in mercuric chloride 1 to 1,000 and then in sterile water. The lesion was then cut into as many pieces as desirable and the pieces transferred to agar slants. One hundred per cent of pure cultures could be obtained in this way. Lesions of any age could be used, but the youngest were found to be most satisfactory.

Cultural Characters.

The range of media on which the fungus will develop is almost unlimited. Those which the writer has used are listed below along with a brief statement of the peculiarities exhibited by the organism on that particular medium.

Potato Agar.

The ordinary potato agar containing a boiled decoction from a large potato and 17 grams of agar to a liter of water. No sugar was added and the acidity was not determined. Growth very slow, reaching a diameter of 1 cm. in about ten days; very dense and compact like fine felt, snow white, dry, flat, but with considerable

aerial mycelium; margin very definite and even. After about ten days the mycelium shows more and more of a tendency to grow beneath the surface of the agar, and the edge has the appearance of gradually fading away into the surrounding agar. Growth may progress for several weeks, but is gradually checked by the drying out of the agar. Some of the cultures show indistinct zonation. With age the surface of the felt may become rugose.

Oat Agar.

Growth more luxuriant than on potato agar, showing denser zones of white mycelium. No change of color in mycelium or in the medium. Growth not sufficiently different from that on potato agar to have any diagnostic value.

Nutrient Beef Broth Agar.

The standard agar of bacteriological work. Growth scanty, much less than on potato agar, slimy, and taking on the color of the medium; never dry, very little aerial mycelium. A very poor medium for growing the organism.

Czapek's Agar.[1]

This was found to be a very favorable medium, the growth being more rapid and with a greater abundance of white, cottony aerial mycelium than on potato agar. After about two weeks the agar below the growth, especially in the upper part of the tube, turns maize yellow,[2] due to the suffusion of a pigment. After about four weeks the color becomes more intense — aniline yellow or citrine yellow. With age this darkens to orange citrine or to various shades of olive. Also the mycelium as seen from above loses its white color after three or four weeks, showing various shades of greenish yellow — citrine drab, olive lake, etc. These color changes on Czapek's agar offer one good diagnostic character.

Onion Decoction.

Prepared by boiling a sliced onion in a liter of distilled water and sterilizing the filtered product for one hour at 15 pounds' steam pressure. Growth very slow, resulting in development of little compact balls of mycelium; brown when in the bottom of the tubes or white when on the surface of the liquid. Growth continues for months very slowly, but the little balls of mycelium do not attain a diameter of over 1 cm

Onion Agar.

Prepared exactly like potato agar, but the onion decoction as described above is used instead of potato juice. This was found to be not only the best medium for culturing Urocystis, but also very much better than potato agar for growing many other fungi which the writer had occasion to try on it. It is very easily prepared, has a minimum of sediment even when not filtered, and altogether forms a very superior general purpose agar. Its only objectionable qualities are the obnoxious odor in the laboratory during preparation, and the fact that the growth of certain fungi is too luxuriant for some purposes. The growth starts with a dense white felt much like that on potato agar, but more rank. After about a week wrinkles begin to appear near the center, and these spread and become sharper and the irregular ridges more elevated with age, also at the same time the crests of the ridges become hygrophanous and gray. This appearance spreads until it involves

[1] For method of preparation see Soil Science, 2:113.

[2] All colors according to Ridgway's Color Standards.

the entire center or wrinkled part of the growth. The convoluted gray growth on onion agar is perhaps the best diagnostic cultural character of the species. It has been very constant in the many series of cultures which the writer has made with this agar. After a few weeks the color in reverse becomes darker, reaching cinnamon brown in about five weeks.

Sugar Potato Agar.

Prepared as potato agar with the addition of 3 per cent of saccharose. Growth is coarser in texture, more luxuriant and spreads more rapidly than on potato agar. The aerial mycelium is not snow white, but early assumes a cream color changing to cartridge buff after a few weeks.

Effect of Concentration of Sugar on Growth of the Mycelium in Culture. — In the series of cultures on different media it was observed that the best growth occurred on media containing considerable sugar, viz., Czapek's, sugar potato and onion agar. This led the writer to suspect that sugar is the essential element of nutrition both in culture media and on the host itself, since the onion contains a high percentage of saccharose. In order to determine the effect of sugar on the development of the organism, Czapek's synthetic agar was prepared first without any sugar and next with .5, 1, 2, 3, 5, 7 and 10 per cent of cane sugar. Five tubes of each were inoculated at the same time and accurate notes taken each day. No growth whatever occurred where no sugar was included. At the end of three weeks there was very little difference in the diameter of the growths on all the other concentrations, but those on the higher concentrates were a little more dense. The most apparent difference was in the color which was imparted to the agar. In the .5 per cent the culture was pure white in reverse, while in the 10 per cent it was bright yellow. The other concentrates formed a perfectly graded series between the two. The only other difference noticed was a wrinkling of the surface of the growth in some of the higher concentrates, and its entire absence from the cultures of low sugar content. Certain conclusions seem warranted from this experiment: (1) agar and inorganic salts alone do not furnish food for growth; (2) the yellow color in the agar is due to some reaction with the sugar; (3) the amount of growth (at least for three weeks) does not depend on the amount of sugar present. Any one of the concentrates apparently contained more than the maximum amount which the organism could utilize.

Substitution of Starch for Sugar. — In order to see whether the fungus can utilize starch as a source of carbon, agar tubes were prepared identical with Czapek's except for the substitution of soluble starch for saccharose. A scanty growth occurred, but even after four weeks it had not attained a diameter of 1 cm. and was very thin. Apparently, then, Urocystis can utilize starch, but it is a very poor source of carbon.

Soil Decoction Agar.

Prepared by adding 2 per cent of agar to the soil decoction described above. Growth was much less vigorous than on potato agar, and thin, but, on the other

hand, spread almost as rapidly over the surface for the first few weeks. There can be no question whatever but that soluble elements in the soil furnish sufficient food for the development of the mycelium.

Dung Decoction Agar.

Prepared by adding 2 per cent agar to the dung decoction previously mentioned. Growth much thicker than on the soil decoction agar, but not as heavy as on Czapek's, sugar potato, etc. Dense white aerial mycelium. The conclusion seems warranted that horse manure furnishes all the elements necessary for the growth of the fungus, and is more favorable medium than a good soil. Apparently a heavily manured soil would be more favorable for the propagation of smut than one which was not manured.

Tolerance of Acid. — Four series of cultures were made on onion agar, — the first series without lactic acid; second, with 1 drop of lactic acid per tube; third, with 2 drops per tube; fourth, with 3 drops. All were inoculated at the same time. Growth was rank and normal in the series in which no lactic acid was added; no growth whatever in the series in which 3 drops were added; a very slight growth where 2 drops were added; growth much retarded in the 1-drop series. This series was begun with the purpose of finding a method of excluding bacteria from cultures of the smut fungus, but the latter was apparently checked by acid just as much as the bacteria.

Effect of Freezing the Cultures.

Cultures on potato agar and on onion agar were kept out of doors for two months during the most severe winter weather of 1919–20. Transfers were then made to fresh agar tubes, and the mycelium grew luxuriantly and rapidly on the surface of the slants. In fact, the growth at first seemed to be even better than when transfers were made from cultures which had not been frozen. Accurate measurements on a second series showed a slight difference in favor of the transfers from frozen mycelium during the first few days, but it was not permanent. We may conclude, then, that freezing not only does not injure the mycelium, but possibly stimulates it to even better growth.

Microscopic Characters of the Mycelium in Culture.

The characters of the mycelium differ somewhat with the age of the culture. Microscopic examination of a culture a week old shows slender hyaline hyphæ of rather uniform diameter, about 2μ, with rather indistinct septa and homogeneous contents. Branches arise almost exclusively from the upper ends of the cells and diverge at a wide angle. The characters have not changed from the condition previously described under germination of the spores. Not all of the cells of the mycelium appear to be alive; some of them are empty and apparently dead; others are full of homogeneous protoplasm with no vacuoles. Under the oil immersion lens one notices certain very refractive granules scattered throughout the dense protoplasm (Fig. 2, A). The cells are easily broken apart, and when a

mount is made the hyphæ appear in segments as represented in the figure. At this early stage they show no constrictions at the septa. No conidia can be found. Clamp connections have not been observed.

If, however, cultures several weeks old are examined microscopically it will be observed that certain changes have taken place. The aerial mycelium may remain about the same as described, except that the cells appear vacuolated, but there will now be found a different kind of mycelium beneath the agar surface. These hyphæ are stouter, averaging 3.2μ in diameter, the cells are much shorter, the septa very distinct, and the hyphæ decidedly constricted at the septa, so much so that the hyphæ appear almost like strings of separate cells. A large proportion of the cells become shaped like dumb bells. When disturbed, as in mounting, the cells of the thread break apart very readily so that when one makes a mount of an old luxuriant culture, such as on onion agar, he hardly finds mycelium at all, but only these irregular separate units. Most of them are branched at the tip. A strand of this mycelium is represented in Fig. 2, B, with a young ordinary hypha for comparison. The appearance of the separate cells from an onion agar culture as seen floating about in the microscopic preparation is represented in Fig. 2, C.

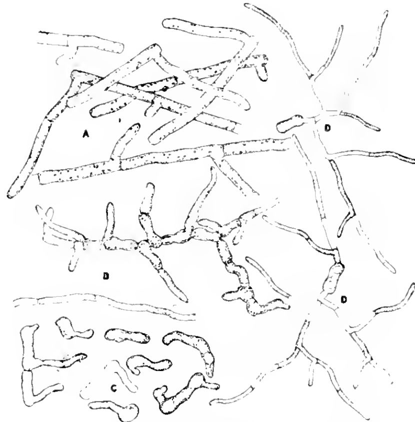


Fig. 2. — Details of hyphæ in culture. Detached hyphal cells at C and further development of same at D.

Fate and Function of the Detached Hyphal Cells.

Since these large detached cells appear so early in the development of a culture and in such large numbers, it does not seem probable that they represent merely a stage in the degeneration or breaking down of the mycelium. Apparently they have some rôle in the life history of the organism. In order to determine whether they are capable of further development, a culture was thoroughly shaken in water and the detached cells floated out on sterile agar plates as described previously for germination of

the spores. Within twenty-four hours slender tubes of about half the diameter of the original cells could be observed growing out from them. These tubes originate from one or from both ends of the cell, quickly become septate and branched, and within three days each is the center of a white mycelium which can be seen with the naked eye. The centrifugal emptying of the cells, the branching, and all other characters are the same as those of the growths from the chlamydospores. Practically 100 per cent germinated. No conidia could be found on them at any stage. The development of these cells is represented by Fig. 2, D.

Taken in connection with the fact that no true conidia have appeared in any of the cultures, the conclusion seems warranted that these cells detached by division of the vegetative hyphæ are analogous to and serve the same purpose as the sporidia (conidia) of other smut fungi in propagation and dissemination. In fact, almost any cell of the mycelium which retains its protoplasm is a potential spore, and may serve all the functions of the same. Since the cells are so easily detached and germinate so quickly and universally, their importance in the distribution of the disease can hardly be overestimated.

Life in the Soil.

There are at least two ways in which the organism may pass from the host into the soil; (1) when the spores are mature and the sorus is exposed by rupture of the enclosing host tissue, the spores fall out or are blown or shaken out by various agencies and fall to the ground; (2) as previously described, mycelium from any buried lesion may grow from the disintegrating tissues directly into the surrounding soil. It has also been indicated in cultures on soil extract media that the soil contains all the elements necessary to induce germination of the spores and to nourish the mycelium into further growth. In order to study further this period of development of the organism, pure cultures on soil were made by inoculating Ehrlenmeyer flasks of sterilized soil, some by placing a small portion of diseased cotyledon on the center of the surface of the soil, others by placing bits of mycelium from agar tubes in the same position. Within a few days the mycelium could be seen plainly with the naked eye passing from both into the soil and spreading over its surface. After four weeks it was isolated from all points of the soil surface. After more than a year it could still be isolated in pure culture. Microscopic examination of mycelium from the soil showed the same characters that are previously described for cultures and the same detached cells.

Summary of the Saprophytic Stage in the Natural Life History.

From all that has preceded concerning this stage we may draw some conclusions.

1. The fungus lives naturally in the soil, especially where there is an abundance of organic material.

2. It derives sufficient nutrient materials from the soil to grow and spread extensively during this stage.

3. It enters the soil either as spores or as mycelium from the buried parts of diseased onions.

4. No typical conidia (sporidia) are produced but it can be widely disseminated by the detached mycelial cells which may be carried about by water, wind, rain, tools, animals, workmen, etc.

5. It probably lives in the soil in this state for years without the presence of onions.

6. As will be shown later, infection may take place directly from this mycelium, and the presence of speres is not necessary.

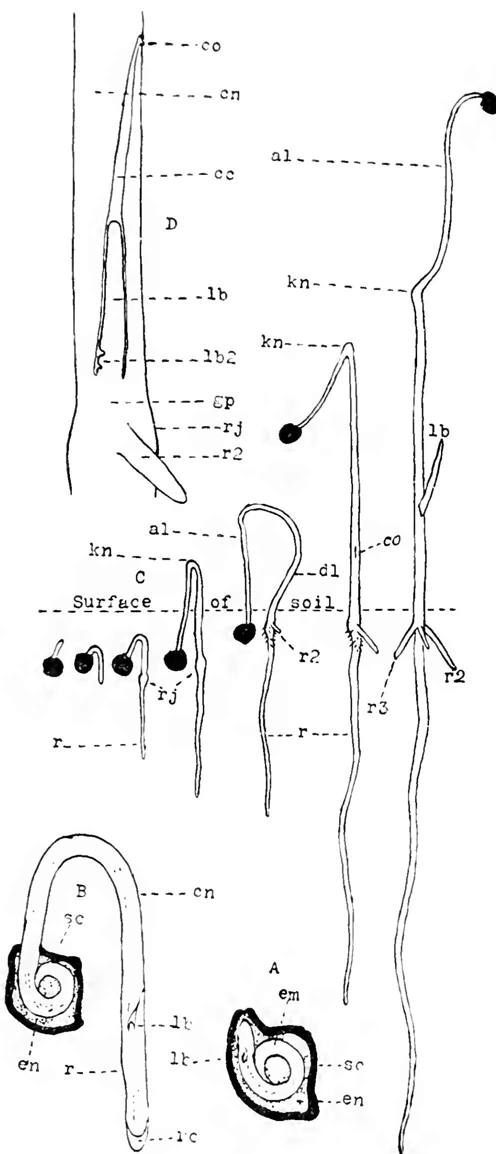
7. The number of years which must elapse before onions can be grown safely on an infested piece of land is not necessarily decided by the longevity of the chlamydospores, but in all probability by the length of time during which the mycelium can continue to live and develop saprophytically without having to pass again through a parasitic stage.

IV. Infection.

Very little has been published concerning infection except the bare fact that it occurs at an early period in the growth of the plant. Concerning the method and point of entrance, character of inoculum, etc., nothing has been previously ascertained.

Development of the Onion Seedling.

In order to understand the description of infection given below it is necessary that the reader should know something of the stages through which an onion seedling passes during the process of germination. The resting seed consists of a hard, black outer seed coat, a nutritive endosperm, and an embryo. The embryo is coiled like a snail within the endosperm (Fig. 3, A). The larger part of the coil represents the cotyledon; only a short portion of the free end is the radicle. In the lower part of the cotyledon, just above where it joins the radicle, there is, even at this early stage, a small cavity. A minute bud, *lb*, arises from the base of and projects into the cavity. This bud is the primordium of the first leaf, and the cavity in this and later stages is called the cotyledonary cavity, *cc*. Several layers of elongated cells throughout the length of the center of the embryo indicate the position which the fibrovascular bundle of the seedling will occupy. Germination begins with rapid elongation of the embryo, the radicle and lower part of the cotyledon being thus pushed through the micropyle, a small opening in the seed coat. This elongation is effected both by longitudinal stretching of the cells of the embryo and by cell division. Food and water for this activity are absorbed by the upper end of the cotyledon which remains attached in the endosperm. On the third day after planting, the projecting radicle is about 3 to 4 mm. long. The root usually points upward as it emerges, but geotropism soon causes it to turn downward and the cotyledon describes a sharp curve as indicated in Fig. 3, B and C. It will be noticed that the tip of the leaf bud now



A. Section through a resting seed.

B. Longisection of a seedling four days after planting.

C. Successive stages in the development from the third day to the twenty-fifth day.

D. Diagrammatic longisection through the growing zone at the end of two weeks. Symbols for parts are the same in all:

sc, seed coat.
en, endosperm.
em, embryo.
lb, leaf bud or primordium of first leaf.
rc, root cap.
r, radicle or first root.
cn, cotyledon.
rj, root joint.
kn, knee.
al, ascending leg.
dl, descending leg.
co, exterior opening of cotyledonary cavity.
r2 and *r3*, first and second secondary roots.
ec, cotyledonary cavity.
gp, growing zone, region of origin of all leaves and roots.
lb2, primordium of second leaf.

Fig. 3. — Development of an onion seedling.

points upward. At this early date the point of division between radicle and cotyledon is indicated by a slight swelling, the root joint, *rj*. As all the parts continue to elongate rapidly the curve in the cotyledon becomes a sharp knee, *kn*, the part between the knee and seed is the ascending leg, *al*, while that between the knee and the root joint is the descending leg, *dl*. The primary root grows down very rapidly and is soon several times as long as the cotyledon. From about the fifth day it will be noted that the descending leg elongates more rapidly than the ascending leg. The first part to appear above ground (seventh to tenth day) is the tip of the knee, and each part becomes green as soon as it has reached the light. The seed may still remain in the ground for a week or more after the knee has appeared, but since it is firmly attached, and since the descending leg continues to elongate more rapidly than the ascending leg, the seed is finally carried into the air (Fig. 3, C). The knee then has a tendency to straighten out, but its position is indicated as long as the cotyledon lives by a sharp kink. On about the ninth or tenth day the first secondary root, *r2*, may be seen pushing out from the swollen root joint, and this is followed later by others in rapid succession, *r3*. Meanwhile the first leaf bud has been elongating rapidly. The cotyledonary cavity elongates also in proportion (Fig. 3, D). It should not be understood that this cavity is absolutely included, without any opening to the outside; on the contrary, its upper narrowed apex communicates with the outside air through a small longitudinal slit in the side of the cotyledon (CO in Fig. 3, C and D). As the leaf bud pushes its way upward the sides of the cavity are distended, and finally from about the seventeenth to twenty-fifth day the tip passes through the slit and appears on the outside as the first leaf (*lb* in Fig. 3, C). But before this time the primordium of the second leaf, *lb2*, has appeared in a depression at the base of the first, and successive leaves follow rapidly, each starting from the base of the next preceding at a very early stage. The successive secondary roots also start from the same region. This very active meristematic region, the growing point, *gp*, is very restricted, and remains stationary in the onion until after the bulb is formed. The limited size and stationary position of the growing point from which all new organs, roots or leaves, originate are characters of prime importance in the spread of the smut fungus within the host plant.

Period of Susceptibility.

It is a well-known fact that onions are susceptible only in the seedling stage, and are immune after a certain stage of maturity is reached. But we have no exact knowledge of the duration of this period of susceptibility, the exact stage or time at which infection first occurs, or the stage or time at which it ceases. The establishment of two points is thus necessary: (1) the first day on which infection takes place, and (2) the last day during which the plant can be infected. The latter of these two points was established by the following experiment. Seed was planted in a flat of sterilized soil. Beginning with the third day, when the radicle on the

most advanced was less than $\frac{1}{2}$ cm. long, and had not even started in many of them, 50 plants were transferred each day to soil which was badly infested and which could be depended on to produce almost 100 per cent of infection. Notes were made on the stage of development of the seedlings each day, and a careful record was kept of all the plants which became smutted. After six weeks, when the plants were mostly in the fourth leaf (after which infection never starts), all of them were pulled, and the following table compiled to show the complete results of the experiment: —

Days between Planting and Transplanting.	Percentage of Infection.
3	100
4	100
5	95
6	100
7	100
8	98
10	87
11	87
12	70
13	59
14	15
17	6
18	–
19	–
Check (left in original sterile sand)	–

The following conclusion may be drawn from this experiment: Under greenhouse conditions the greater part of the infection occurs within two weeks after planting, and the plants are no longer susceptible after the seventeenth day. Since it seems probable that the period of susceptibility is not limited by the number of days during which the seeds have been in the soil, but by the length of time required for the seedling to pass through certain stages of development, we may express this first conclusion by stating that susceptibility begins to diminish from the time that the knees emerge from the ground, and that little if any infection occurs after the first leaf has emerged from the side of the cotyledon. In a large number of experiments in the greenhouse at all times of the year it has been found that the knees begin to appear above ground in seven to twelve days. In one experiment, where the house was very cool, it required over two weeks, and in this case the percentage of infection was 100, and the individual plants were more thoroughly smutted than in any other experiment tried. Since, then, the period of susceptibility might be increased

by the length of time required for the seedlings to reach a certain stage, it is well to inquire how the rate of growth in the greenhouse compares with that in the field. During the spring of 1920, when the spring was late and cold, onions in the field did not come up for over two weeks in most cases, but growers have frequently told the writer that they have had fields which came up within eight days. Apparently weather and soil conditions may materially affect the length of this period. Depth of planting might also influence slightly the length of the period and also the chances of infection. The experiment reported above, however, gives us no information as to the date when infection begins, but only indicates that it ends with about the seventeenth day.

In order to determine the stage at which the earliest infection starts, — and at the same time to work out other points in the early life history, — another bed of onions was started in the greenhouse with soil known to give 100 per cent of smut infection. Beginning with the third day, a certain number of plants was dug up each day, fixed in Flemming's weaker solution, run up into paraffin, sectioned, mounted serially and stained with triple stain. No mycelium was found in the tissues of those which were fixed on the third and fourth days. The first infection was found in a plant which was dug up on the fifth day after planting, and was apparently a very young infection because it had at no point penetrated more than to the fifth layer of cells below the epidermis, and at its furthest point was not more than 150μ from the point of infection. Fifteen other plants dug at the same time were carefully searched under high power through every section of 92 slides, but no other trace of mycelium was found. It is probable, therefore, that only rarely, if ever, has the mycelium entered the tissues of the plant on the fifth day after planting (second day after germination has started). Since cultural experiments with the smut fungus have shown it to be of very slow growth, at least in the saprophytic condition, it seems hardly possible that it could have succeeded in entering the tissues before the second day after germination of the seed starts.

It may be concluded from everything which has been learned up to the present in regard to the period of susceptibility that *infection may take place at any time between about the second day after the seed starts to germinate until the seedling is in the first leaf* (a period of about twelve days in the greenhouse).

Point of Infection.

In the study of the plants fixed and stained as mentioned above, many very young infections were found where it was possible to determine the point of entrance for the mycelium. Infections were found at the knee above, at the root joint below, and at various points between, also at least one through the interior wall of the cotyledonary cavity. The conclusion is, therefore, that all points of the epidermis at least between the root joint and the knee are susceptible to penetration by the smut tubes. Infection was never found taking place in the roots proper or between the

seed and knee. From observation of mature sori, however, it seems probable that infection sometimes occurs above the knee. Mycelium in various quantities has been found in the cotyledonary cavity of many plants, even in the youngest stages, and by tracing it to the opening of this cavity it can be seen that it comes in from the outside through the natural opening, but in most cases it has been impossible to trace a direct connection between this mycelium and any hyphæ inside the tissues between the cells. This mycelium has the size and all the other distinctive characters of smut mycelium, but it is not possible to prove that it is such. It was thought at first that this was the usual infection court, but after it was demonstrated beyond any question that in a large number of cases young infections could have no connection whatever with this cavity, the conclusion was reached that only a small part of the infection could be accounted for in this way. It is still doubtful whether the mycelium which was found in the cavity was always that of Urocystis, or whether it may have been that of another soil fungus.

It is probable that *all infection takes place through the cotyledon*. A case was never noted where the leaf became smutted while the cotyledon remained healthy. More careful experiments on this point, however, might show that the leaf does sometimes become infected first. It is probable that all infection takes place beneath the surface of the ground.

Character of the Inoculum.

In all literature on onion smut it has been assumed that the spores of the organism must be present in close proximity to the seedling in order that infection may occur. The possibility that the mycelium might be present and growing saprophytically and indefinitely in the soil, and might infect without the immediate presence of spores, has been left out of consideration. In order to determine the ability of saprophytic mycelium to produce infection, onion seeds were germinated beneath the surface of agar cultures in test tubes in such a way that the developing seedling as it elongated must pass through the mat of mycelium. Over 50 per cent of the seedlings became infected, although no smut spores could have been present. In the stained sections which were studied, in a few cases mycelium was found outside the walls of the epidermal cells where infection has occurred. Only in one case were spores found in these sections, and at that time there was no infection beneath them. It is probable, however, that spores would usually be removed by the washing process, and this could hardly be adduced as conclusive evidence against the necessity of spores for infection. It is probable that *either spores or saprophytic mycelium in the soil can serve as the inoculum.*

Method of Entrance.

The infecting hypha enters the epidermal cell by boring directly through the outer wall. Since in the younger infections the stomates are not yet open, and mechanical wounds have not been found, there is no other route

by which it could make its way into the interior tissues of the plant. A stage of infection has not yet been found so young that the tube has just entered the epidermal cell and has not progressed further.

Passage through the Epidermal Cells.

In the youngest infections observed, the mycelium had already grown through the epidermal cells, and its tips could be found in the intercellular spaces at a depth of two or more layers below. In some cases a piece of the infecting hypha still remained on the exterior of the cuticle, but was always devoid of contents and consisted only of somewhat crumpled walls

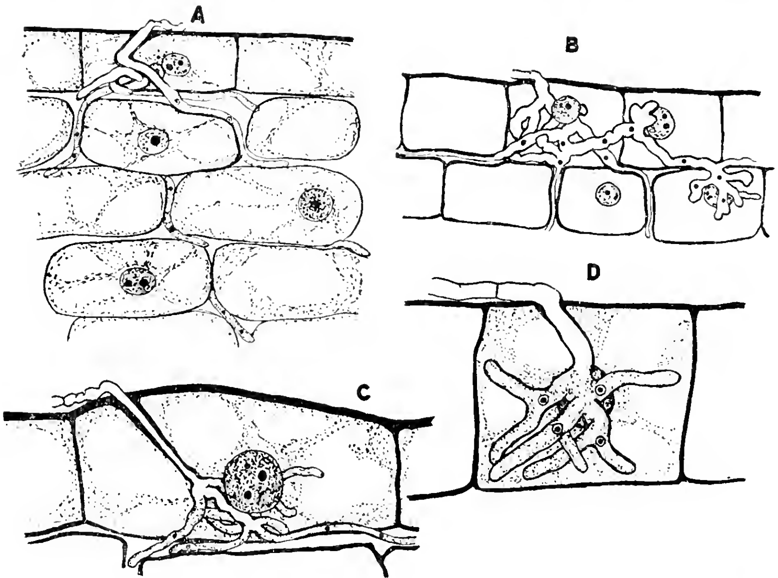


Fig. 4. — Infection through the epidermal cells; A, B, C from outside the cotyledon, D from the cotyledonary cavity.

(Fig. 4). A broad clear canal passes inward from the outer wall usually directed toward the cell nucleus. The wall of the canal appears to be continuous with the cell wall as if merely an inward extension of the same. Commonly it is much thicker at the point of entrance, and resembles a slender funnel or trumpet in shape. It was not found possible to determine whether part of the wall of the canal is an inward growing sheath of the same substance as the cell wall, or whether it is merely a thickened wall of the hypha. In all the cases observed, the canal was empty at the point of entrance. The host nucleus appears to exert an attractive influence. When the tube has reached the depth of the nucleus, it branches to form a tangle of stout, swollen, gnarled, hyphæ which may be confined to the region immediately about the nucleus, or may reach to all parts of the lumen of the host cell (Fig. 4). They may be entirely devoid of con-

tents or — depending on the stage at which one finds them — may contain protoplasm and bright red nuclei scattered singly or in pairs. The hyphal tangle may be confined to the lower (inner) part of the cell, and is always more dense there (Fig. 4, C). Its windings are difficult to follow. These intracellular windings stain red with the triple stain. There is a marked contrast between the large, swollen winding intracellular hyphæ and the trim, slender, straight intercellular hyphæ between the cells below, which stain violet and are of only about one-half the diameter of the former. Usually the tangle is confined to one epidermal cell, but sometimes the adjacent cells may be invaded (Fig. 4, B). The attacked epidermal cells do not collapse, and, in fact, appear practically normal. Hyphæ pass down from the tangle through the inner wall of the epidermis into the intercellular spaces immediately beneath.

Multiple Infection.

The same plant may suffer from a number of infections. In one plant fixed eight days after planting, the mycelium was found passing in through the epidermis at six points on a piece of the cotyledon less than a centimeter in length. In young stages it is not difficult to trace each mycelium to its limits between the cells, and in this case no one of the six had come into contact with another. It is not unusual to find seedlings which show five or six sori on the same cotyledon. Microscopic examination indicates that these are not the results of a single infection, but that for each sorus there is at least one infection thread which penetrated the epidermis from the outside. This statement, however, does not apply to the sori which appear later on the true leaves.

V. Incubation Period.

The incubation period is the time which elapses between infection and the first externally visible symptom of disease. Since the first external symptoms appear at approximately the same time that the spores are forming, we may say that the incubation period is that segment of the life cycle between infection and sporogenesis. In the greenhouse the first symptom, a slight curving and thickening of the cotyledon, has been observed here on the tenth day. Since, as previously stated, infection may take place as early as the fifth day, we may consider that this period occupies a space of about five days under favorable conditions in the greenhouse. It may be longer outside, but, at most, is a comparatively short period. During this period the parasite grows rapidly, spreads inside the host and prepares to form spores.

Young Hyphæ in the Intercellular Spaces.

After passing through the epidermis the hyphæ are intercellular during the remainder of their development. Just below the inner epidermal wall they spread in all directions. They are long, slender, and, as they pass

along the longitudinal walls, appear very straight. They appear to progress somewhat more rapidly up and down the cotyledon than in a radial direction inward. In the young stages they do not occur in strands or bunches between the cells, but one finds them running singly (Figs. 4, A and 5, D). They do not appear to be going toward any definite point, but are spreading more or less in all directions. They are undoubtedly septate, but the septa in the very young hyphæ are difficult to distinguish. The protoplasm passes to the growing tips, and leaves empty the cells behind it. These tip cells stain deep violet with the triple stain, while those cells behind them take less and less stain until only the thin line of the walls can be seen. The nuclei stain bright red and are very prominent, especially back of the deep violet tip cells. These nuclei may occur singly or in pairs distributed along the hyphæ. At this stage it is not always possible to tell whether the two nuclei of a pair are in the same or different cells, but by a comparison with what is found in hyphæ somewhat older, it is probable that here also the cells may be either uninucleate or binucleate. The contents of the hyphal cells appear homogeneous, and at this stage there are no vacuoles or oil drops. The hyphæ seem to be mostly tightly pressed against the walls of the cells, but at places can be seen passing from the wall of one cell to that of another across the open spaces. The cells are long and the branching not close as in the later stages. The branches always arise monopodially from just below the septum, as previously described.

Haustoria.

These absorbing organs are not numerous, but are not uncommon. In some infections none could be found, while in others they are fairly common. They are of various sizes and of very irregular shape (Fig. 5, A–E). They are not much different from the haustoria of other smuts as described by various writers. They are always very much branched, but the branches may be reduced to mere knobs or short stubs which are frequently bifid at the apices (Fig. 5, A). In the larger haustoria, however, the branches are longer and more lax, and may go to all parts of the cell (Fig. 5, B and C). The branches of these larger haustoria are usually — but not always — imbedded in the protoplasm about the nucleus. In some cases they seem to be tightly gripping the nucleus, and the latter appears indented by the pressure. Their shape and size can be best understood by reference to the figures. In many of them an appressorium-like expansion of the hypha can be seen flattened against the outside of the cell wall, and from the lower side of this expansion a narrow neck passes through the wall (Fig. 5, A, E). It is not certain, however, that this appressorium is always present. In the larger haustoria, red nuclei can be distinguished in varying numbers, but in smaller ones, and, in fact, in many of the larger ones, no nuclei can be seen. In some, the position of the nucleus in the stalk of the haustorium is evident (Fig. 5, C) but

apparently there is no uniformity either in the position or number of nuclei. The haustoria usually stain yellowish brown with the orange G of the triple stain.

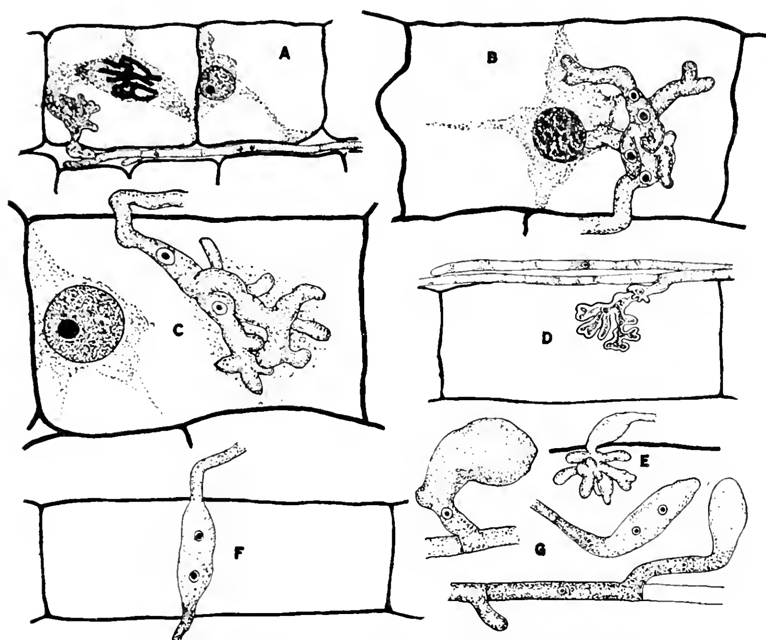


Fig. 5. — Haustoria (A–E) and absorptive hyphal expansions (F, G).

Absorptive Hyphal Expansions.

Frequently during the incubation period one finds the tips, especially of short lateral branches, flattened out like spatulas against the cells of the host. In some sections, just before sporogenesis, these structures may be found in great numbers. Usually they are terminal (Fig. 5, G), but not infrequently they may be found intercalary within the ordinary course of a hypha which, beyond the expansion, continues in its normal size and shape (Fig. 5, F). They resemble the appressoria previously mentioned as the bases from which the haustoria arise, but their number is out of all proportion to the number of haustoria which one finds in the same sections. No description of these organs has been given elsewhere, and their function or meaning is not clear. One can only conjecture that their purpose is to present a broad absorbing surface for securing more nourishment from the host cells. It seems doubtful whether haustoria are really necessary in this connection, because many infections have been studied under the microscope in which no haustoria could be found.

Progressive Infection of New Leaves.

It is a common belief, supported by statements in the literature of the disease, that when a seedling once becomes infected it never recovers. Such, however, is not the case. The writer has watched the development of many seedlings which had infected cotyledons, but which developed into healthy onions. On the other hand, he has not seen an onion, in which the *first leaf* was affected, which produced a healthy bulb. Usually each successive leaf will show smut sori, and they are not always in any apparent relation to the sori on older leaves. As previously stated, all infections come through the cotyledon, but the fate of the plant depends on the point in the cotyledon at which infection takes place. If it occurs only high up toward the knee, or above it, there is a pretty good chance that the host tissue will have become mature or dead and no longer suitable for spread of the mycelium before the latter has reached the growing zone, and the bulb will develop normally. But if infection occurs at or very near the root joint, the mycelium quickly penetrates tc the growing zone from which all future leaves arise. This meristematic tissue furnishes the ideal condition for continuous vegetation of the pathogene, and as each new leaf pushes out from this restricted stationary zone it contains filaments from which the new sori of the successive leaves develop. When the parasite is once established in this growing point, the host seems never to be able to shake off its grip, and is doomed. It is not quite so clear why the mycelium does not enter the tissues of the developing roots in the same way, but the writer has never been able to find it in these organs.

VI. Sporogenesis.

The approach of spore formation is first indicated by massing of the mycelium between the cells. Up to this time only long straight slender hyphæ are found spreading singly, or at most not more than two or three together, between the cells. The period during which the pathogene appears to be spreading as widely and rapidly as possible between the cells has just been described as the incubation stage. The distributive hyphæ now begin to branch profusely, and the branches are not straight and parallel to the main hyphæ, but become twisted and interwoven into dense tangles which push the cells apart and increase the area of intercellular spaces within which the spores are to be formed. The hyphæ now become highly vacuolated, and the protoplasm between the colorless vacuoles stains densely blue with the triple stain, while the old cells from which the protoplasm has passed take the orange stain. The beaded appearance of the alternating vacuoles and densely staining cytoplasm is the surest indication of approaching sporogenesis.

These spore nests or sori always occur between the cells of the mesophyll anywhere between the epidermis and the bundles, but have not been found inside the bundles. They are extended in the direction of the length of the leaf or cotyledon.

Observation of the exact course of events in the formation of a spore is rendered difficult by the denseness of the mass of developing spores, and by the fact that in the young stages all the developing parts stain so deeply on account of their very active protoplasm that the nuclei and septa can hardly be made out. In all cases which have been observed, the spore begins as a lateral or terminal branch which curves back on itself in the form of a crozier (Fig. 6, A–I). These hook-like croziers may be seen in enormous numbers in the mycelial tangle at the initiation of sporo-

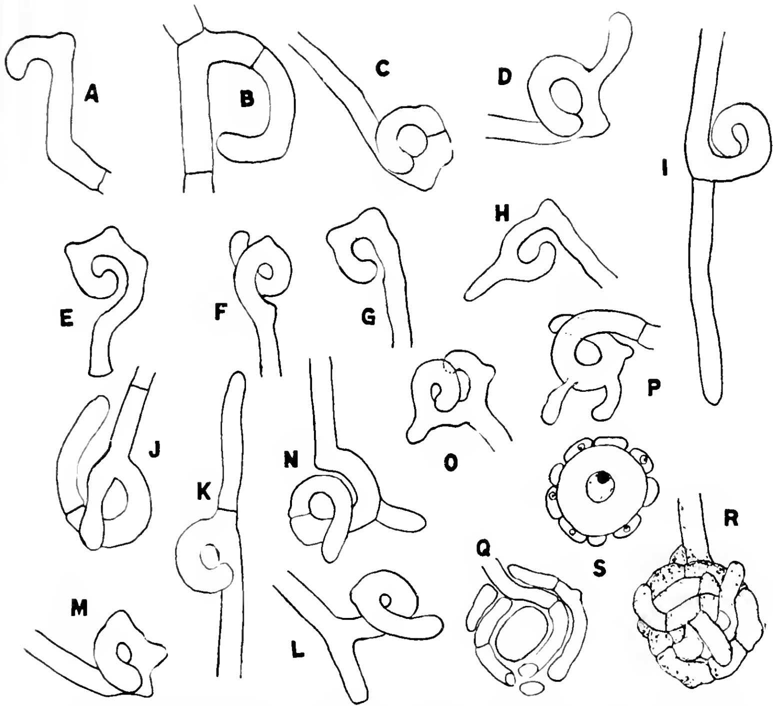


Fig. 6. — Stages of sporogenesis. A–P, development of the crozier and origin of the enveloping hyphæ; Q, section through young spore which is shown in surface view at R; S, section through mature spore.

genesis. Even after the spores at the center of a sorus are fully formed, one may still find various stages of development extending as far back as the crozier, as he passes from the center toward the periphery of the tangle. The croziers remind one of those from which the asci of the Ascomycetes are developed. They stain very deeply, and apparently the protoplasm from the other cells of the hyphæ passes into them. The various shapes which they may assume are best understood by consulting Fig. 6. By growth from the apex of the crozier a complete circle is soon formed and then a spiral if further terminal elongation occurs (Fig. 6, F, L, N, P). At about this time the crozier or spiral begins to appear angular and

irregular (Fig. 6, M), due to protuberances which mark the origin of short lateral outgrowths which soon curve inward along the surface of the developing ball (Fig. 6, P). The whole structure becomes so complicated at this time that it is not always possible to make certain of the exact course of events. The surface view now shows a dense ball of interwoven hyphæ (Fig. 6, R). A cross section (Fig. 6, Q) shows that at the center there is a larger cell which represents what will later be the fertile cell of the spore. This cell appears to be the enlarged terminal cell of the crozier, though it is not certain that this is always its origin. Also it is not entirely certain that all the branches which form the outside of the tangled mass arise directly from the surface of the crozier. In some cases one gets the impression that other hyphæ may be involved, or that branches arise from below the crozier on the same hypha. The transformation from the stage represented in Fig. 6, Q, R, to the mature spore is very rapid. The central cell enlarges while the cells of the surrounding hyphæ become pressed tightly against and united with it. The union between the central cell and the cells of the enclosing hyphæ appears to be stronger than that between the cells of a single hypha of the latter; at any rate, the hyphæ now break up and their elements no longer appear as cells of individual hyphæ, but as scattered conical cells whose flattened bases are firmly attached to the surface of the central cell (Fig. 6, I). This involves a decided change in shape as well as orientation. Nothing has been seen in this process which could be called a gelatinization of cells, such as has been described so often as occurring during sporogenesis in the Ustilaginales.

Approximately at the center of the fertile cell of each fully developed spore there is a nucleus which stains very prominently at this stage of development (Fig. 6, S). In thousands of beautifully stained spores examined by the writer, more than a single nucleus has never been found. It is 3 to 4μ in diameter, with a prominent very red single nucleolus of about .6μ diameter, usually in contact with the nuclear membrane. The membrane is very plain, but the nuclear content, with the exception of the nucleolus, appears only as a few fine granules of cromatin aggregated about the nucleolus or around the inside of the membrane. In each accessory cell there is a single small nucleus of about the diameter of the nucleolus of the fertile cell. In *Urocystis Violae*, Dangeard reported that there were no nuclei in the accessory cells. With the staining methods used it was impossible to determine whether the nucleus of the mature spore results from the fusion of two nuclei. In *U. Anemones* (Pers.) Wint., Lutman found that the cells of the vegetative hyphæ are binucleate and remain so until after the formation of the spore ball, and that the large nucleus of the mature fertile cell results from fusion of the two nuclei. Such might well be the case here, because in the vegetative hyphæ, as previously mentioned, about half of the cells are binucleate, while in the mature spores all cells are uninucleate.

With the full development of the sorus, the host tissue above it dries out and may split open and permit the escape of the dry powdery mass of

spores. In the larger leaves the opening of the sorus may first occur on the interior of the hollow leaves. Under moist conditions other fungi, such as Fusarium, may cause the tissue to decay more rapidly, and thus aid in the liberation of the spores.

The first outward indication of disease in a young seedling is a slight curvature of the cotyledon accompanied by some enlargement of the affected part. In the greenhouse I have found these symptoms as early as the tenth day after planting. Within another day or two, when an affected seedling is held so that the light will shine through it, the lesions may be located by the darker appearance. As soon as the spores are mature the dark sorus can be seen through the tissue without holding it up to the light. The length of time which elapses before it splits open and permits the escape of spores varies greatly with the weather, age of leaf, and other factors.

VII. Summary.

1. Spores as soon as mature germinate in the laboratory in onion decoction, sugar solutions, onion decoction agar, soil agar, manure decoction agar and various agars containing sugar.

2. They do not germinate in tap water, distilled water or soil water.

3. The presence of the onion or any substance from the onion is not necessary.

4. Freezing does not increase or hasten germination, but when spores are frozen in the ground they are not killed.

5. Free access to air increases the percentage of germination.

6. A period of rest in damp soil increases the percentage of germination, but is not necessary.

7. In the soil the spores do not all germinate at once, but become progressively prepared for germination. They do not wait until a host plant starts to grow near them.

8. Germination begins in three to six days after the spores are brought under favorable conditions.

9. A short hemispherical promycelium is first developed, and from this a whorl of branches grows out.

10. The branches grow as mycelium indefinitely without producing conidia (sporidia). The older cells become devoid of their protoplasm progressively.

11. The germination process is very similar to the same process in other species of Urocystis, being almost identical with that of *Urocystis Anemones*. Of the investigated species of this genus, only *U. Violae* produces sporidia.

12. *Urocystis cepulae* lives and grows as a saprophyte indefinitely in the soil, its growth being favored by manure.

13. It may be grown in pure culture on a wide range of culture media, and shows cultural peculiarities by which it may be distinguished from other fungi.

14. Sugar in the media greatly increases the growth. The same substance probably accounts for its rapid growth in the host.

15. Starch furnishes a very poor source of carbon.

16. Decoctions from soil or manure furnish all the essentials for growth.

17. A small amount of acid checks its growth.

18. Freezing does not kill the mycelium.

19. No sporidia (conidia) have been found by the writer in pure cultures or in soil.

20. The mycelium at an early stage breaks up into short plump cells which have all the functions of sporidia and are probably of great importance in dissemination.

21. The organism gets into the soil either by means of spores when the sorus is broken up, or as mycelium which grows from the lesions when in contact with moist soil.

22. Infection occurs during the time from the second day after the seed germinates until about the time that the first leaf appears on the side of the cotyledon, after which the plant is immune.

23. Infection occurs only through the cotyledon, and any part of its epidermis may serve as the point of infection.

24. The infecting hypha bores directly through the outer wall of the epidermal cell, forms a hyphal gnarl inside the cell, and then passes through the inner wall into the intercellular spaces where it grows during the rest of its development.

25. Many infections may occur on the same cotyledon.

26. The incubation period is less than a week.

27. Large complicated haustoria are formed within the host cells.

28. An infected plant recovers if the fungus fails to reach the growing zone; but if it once becomes established in this zone, the plant never recovers, and most if not all the leaves will contain lesions.

29. At the close of the incubation period the mycelium is in dense masses between the cells, and from this the spores develop in sori.

30. The spore begins as a recurved lateral or terminal branch, forming a crozier, circle or short spiral.

31. Branches arising from the circle (crozier) form a close covering about the terminal (fertile) cell.

32. By adhesion of the cells of the covering hyphæ and rapid expansion of the fertile cell the enclosing hyphæ are separated into the scattered elements which appear as the sterile cells of the mature spore.

33. The fertile cell contains a single, large nucleus, and each sterile cell a single small nucleus. Probably the large nucleus is a result of fusion.

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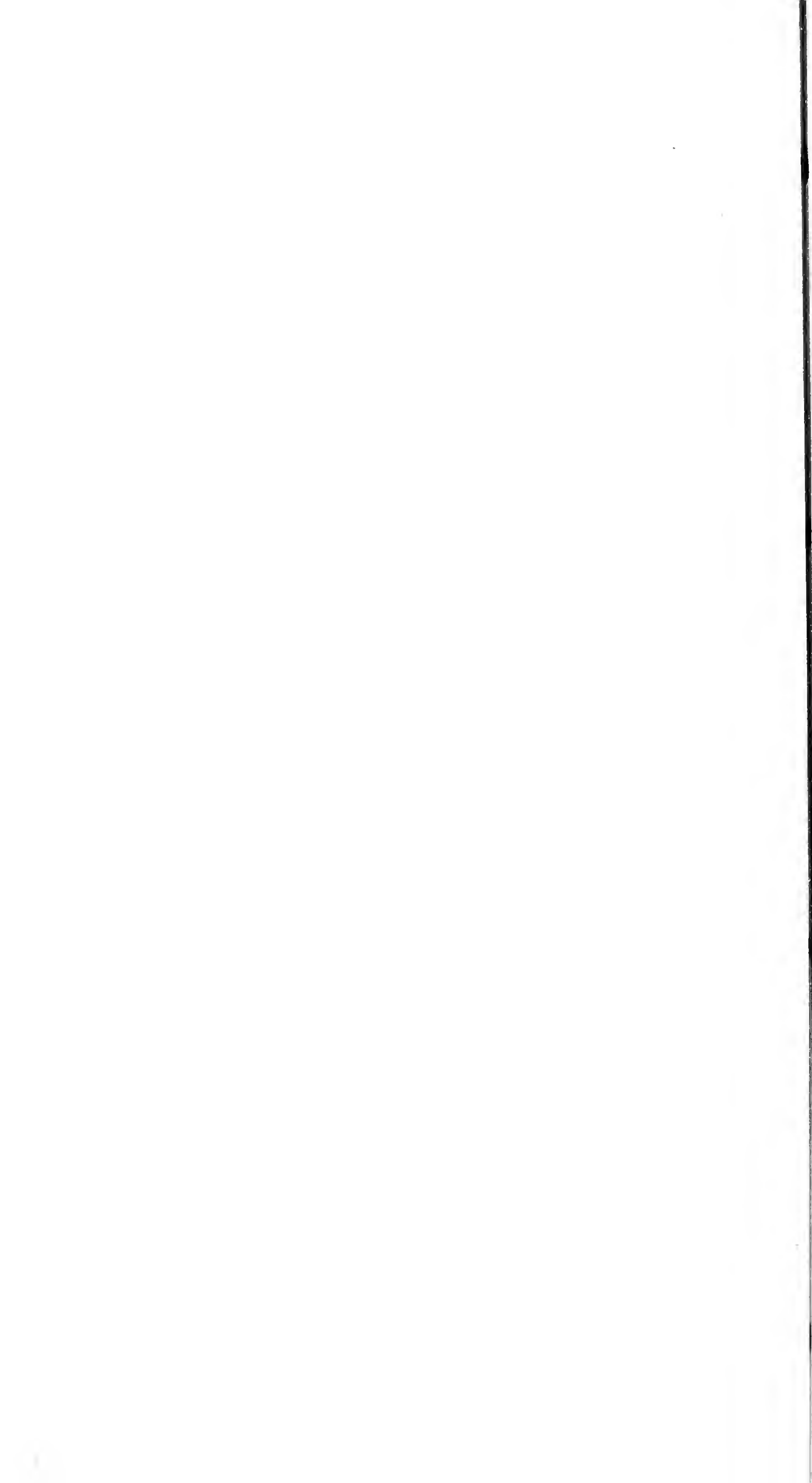

AMHERST, MASS.

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AGRICULTURAL EXPERIMENT STATION

TECHNICAL BULLETIN No. 5 AUGUST, 1922

Concerning the Diagnosis of Bacterium Pullorum Infection in the Domestic Fowl

By GEORGE EDWARD GAGE

For several years the Experiment Station has been conducting studies of *Bacterium pullorum*, the object being to determine factors which aid in accuracy of diagnosis. The data obtained are recorded in this bulletin, and indicate that there are two forms of *Bacterium pullorum*, both of which are distinct from *Bacterium sanguinarium* and can be distinguished from it by certain biochemical tests; that *Bacterium sanguinarium* is not widely distributed in Massachusetts; that neither *Bacterium pullorum* nor *Bacterium sanguinarium* is the cause of the so-called "paralysis" common in Massachusetts at certain seasons of the year; and that the agglutination test, when carefully controlled through epidemiological work, is the best method now available for locating *Bacterium pullorum* infection and furnishing to poultrymen a starting point for its elimination.

Requests for Bulletins should be addressed to the

AGRICULTURAL EXPERIMENT STATION

AMHERST, MASS.

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Supervisor of Administration.

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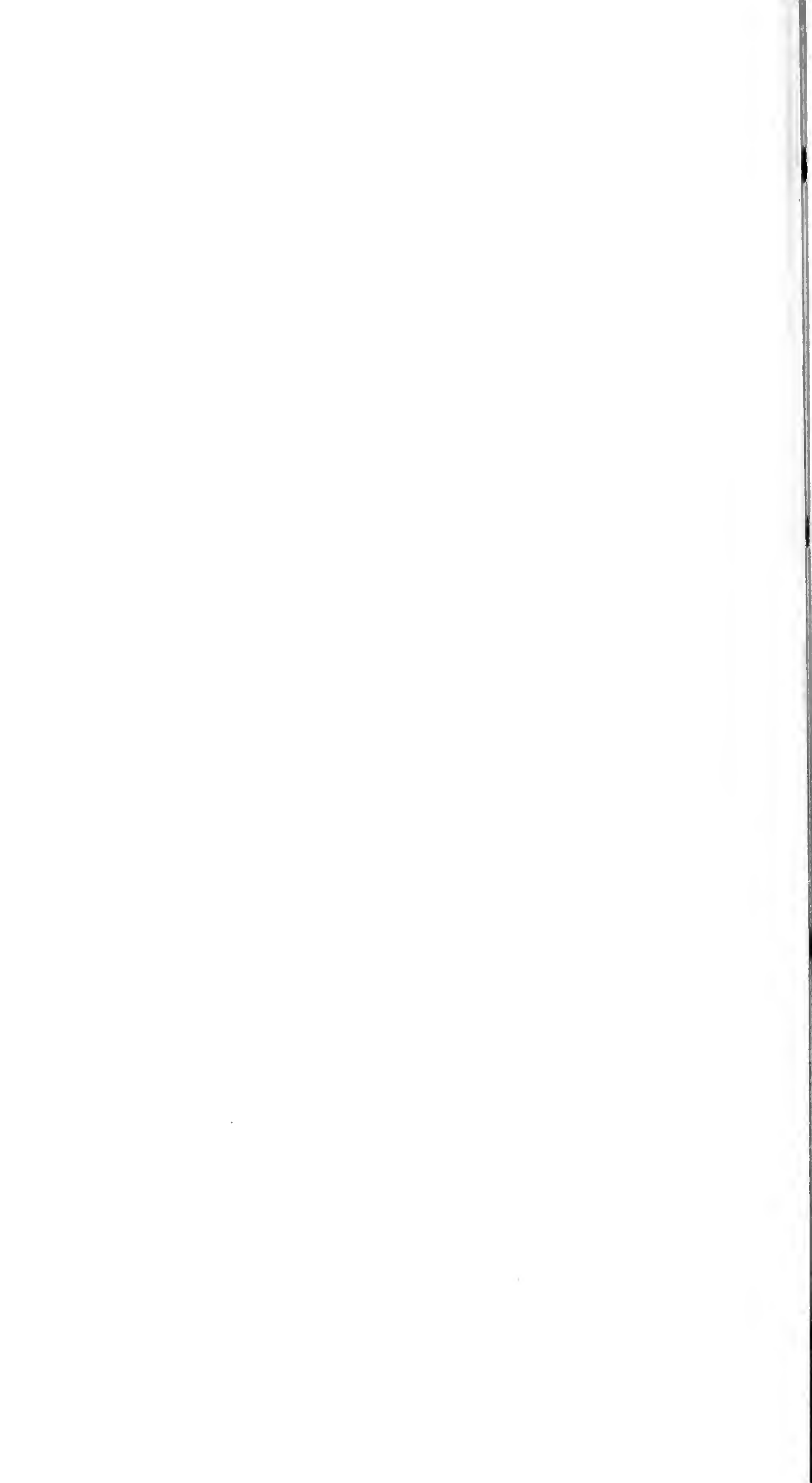

TECHNICAL BULLETIN No. 5.

DEPARTMENT OF VETERINARY SCIENCE.

CONCERNING THE DIAGNOSIS OF BACTERIUM PULLORUM INFECTION IN THE DOMESTIC FOWL.

BY GEORGE EDWARD GAGE.

During the years 1916, 1917, 1919 and 1920 special studies have been conducted in this department concerning the diagnosis of *Bact. pullorum* infection in chicks and adult birds. The object in view has been to determine factors which aid in accuracy of diagnosis. Therefore the plan here is to set forth the data obtained which may be of some value in substantiating the work of others, and to add any data from experimental studies and routine which may assist those who have to do with the pullorum problem.

Among the points to be considered by the laboratory and field worker in the *Bact. pullorum* problem, the following are of interest: —

1. Are there a *Bacterium pullorum* A and a *Bacterium pullorum* B?
2. Can infections with *Bacterium pullorum* and *Bacterium sanguinarium* be differentiated?
3. Is *Bacterium sanguinarium* (fowl typhoid) widely distributed in Massachusetts?
4. Is it necessary to submit suspicious *Bacterium pullorum* cultures to biochemical tests before a diagnosis is justified?
5. Is either *Bacterium pullorum* or *Bacterium sanguinarium* related to the so-called "paralysis" so widely distributed at certain periods of the year in Massachusetts?
6. Is *Bacterium sanguinarium* of any significance as the cause of epidemic disease in very young chicks?
7. What is the present status of the specificity of the agglutination test as a means of control of *Bacterium pullorum* infection in young chicks?

Historical.

The presence of cholera-like or typhoid-like epidemics in domestic birds dates back many years, but careful study extends only from the last quarter of a century. For a most excellent historical résumé of these studies from 1789 to 1913, the reader is referred to Hadley (1).

Since 1913 several investigators have added much to our knowledge concerning the biology of *Bact. pullorum*. Smith and Ten Broeck (2),

carrying out five sets of experiments in which serum of rabbits immunized with heated cultures of human typhoid, fowl typhoid and *Bact. pullorum*, considered that the agglutination tests were sufficiently definite to enable them to group the fowl typhoid and pullorum types together, both demonstrating the same intimate relation to typhoid bacilli. Again, in another paper (3), these writers demonstrated that fowl typhoid has many diagnostic features in common with the human typhoid bacillus, namely, the behavior toward carbohydrates and the agglutination reactions.

Rettger and Koser (4) carried out agglutination tests using reacting sera from rabbits immunized by subcutaneous injections, first of killed suspensions and later of living suspensions of *Bact. pullorum* and *Bact. sanguinarium*. Five days after the injections of heated vaccine, the rabbits were bled and the agglutinative power of the sera tested against definite suspensions of both *Bact. pullorum* and *Bact. sanguinarium*. No difference in agglutination properties was manifested. Attempts were made to increase the agglutination titre by the injection of living organisms. The titre remained the same and no change in the agglutinative ability of the two sera was manifested. Although these organisms have several characters in common, and particularly the serological reactions, they constitute two separate and distinct types, each bearing a specific relationship to the disease with which it has been associated, namely, either bacillary white diarrhœa or fowl typhoid. Taylor (5) concludes from his studies on fowl typhoid that the lesions produced in fowls which are infected with *Bact. sanguinarium* resemble in many respects those produced by *Bact. pullorum*, but, although there is a still closer resemblance in the biological characters of the two organisms, there is enough difference to warrant the conclusion that they are distinctly different diseases. Ward and Gallagher (6), studying forty-seven birds for comparison of agglutination and intradermal tests on naturally infected birds, report the absolute failure of each test as judged by the other test and by an autopsy, findings being similar in amount. Field tests on two hundred and thirty-one birds made simultaneously with the agglutination test at thirty-eight hours failed to detect one case reported positive to the other test.

Pfeiler and Rehse (7) present the clearest description of an epidemic in fowls due to the fowl typhoid bacillus. The fermentative reaction showed the organism to be similar to the human typhoid bacillus. According to Goldberg (8) the principal differences between the strains of *Bact. pullorum* and *Bact. sanguinarium* studied lie in the fact that *Bact. pullorum* produces gas in various carbohydrates while *Bact. sanguinarium* lacks this power in any of the carbohydrates he used, which included sugar-free media containing dextrose, lactose, saccharose, mannite, dextrine, inuline, galactose, levulose, raffinose, amygdalin, arabinose, adonite, dulcite, xylose, salicin, isodulcite, mannose, starch, glycerine, erythrol. The difference in gas production, as well as in their actions on milk, maltose, dulcite, dextrine, and isodulcite seems to indicate that these two organisms are distinct species of bacteria.

Hadley (1) concludes from his studies on the colon-typhoid intermediates that in carbohydrate media used known types of *Bact. pullorum*, *B. gallinarum*, *B. avisepticus*, *B. paratyphosus* A and B, manifest definite fermentative differences which justify regarding them as distinct species. Since paratyphoid A does not ferment xylose, a close relationship is shown between the types from poultry (pullorum and gallinarum) and paratyphoid B. The data presented indicate that pullorum is much less active than gallinarum on xylose. Aside from gas production there is a closer fermentative relation between *B. gallinarum* and the paratyphoids than between *Bact. pullorum* and the paratyphoids; this is due to the fact that pullorum is maltose-dextrine-dulcite negative. Hadley also finds that all the maltose-dextrine-dulcite negative strains isolated from chicks have been aerogenic, while all of the maltose-dextrine-dulcite negative strains isolated from adult birds were anaerogenic. The author has been able to isolate from the eggs of fowls experiencing infections with the maltose-dextrine-dulcite negative anaerogenic strains both aerogenic and anaerogenic forms. The gas production may vary quantitatively within wide limits. The writer has found that no one of the many original aerogenic pullorum strains, cultivated for years in artificial media, has lost its aerogenic power when placed under favorable conditions for growth; and none (either pullorum or gallinarum) that originally lacked this power ever attained it. According to these data one may conclude that if a strain, possessing otherwise the characteristics of pullorum or of gallinarum, is aerogenic it is not *B. gallinarum*, while if it is anaerogenic it may be either *Bact. pullorum* B or *B. gallinarum*. This indicates that it is necessary to make use of the maltose-dextrine-dulcite fermentation tests only when the strain in question is anaerogenic. In another paper (9) this same author concludes from his data that gas production by *Bact. pullorum* may depend upon whether the cultures are grown in glucose extract or glucose infusion broth. Propagating cultures for many years on artificial media does not cause them to lose their gas-producing ability. *Bact. pullorum* isolated from epidemics of bacillary white diarrhœa in young chicks or from infected eggs is aerogenic; there exist also anaerogenic strains which, in all the cases in which they have been observed, have been isolated from adult fowls experiencing acute or subacute infections simulating fowl typhoid in both clinical symptoms and pathological alterations of tissues. Therefore the writer proposes tentatively to postulate for *Bact. pullorum:* (1) *Bact. pullorum* A, aerogenic; and *Bact. pullorum* B, anaerogenic, pathogenic for adult stock only.

Hadley (10) suggests that *Bact. pullorum* appears to stand as a borderline group in the colon-typhoid intermediates, separating the actual paratyphoids from the actual paracolons; and further suggests that, in order to facilitate bringing about some degree of order in the group of colon-typhoid intermediates, gas-forming strains be referred to the paracolon group which should be revived; and that anaerogenic forms only be referred to the paratyphoid group, in which *B. gallinarum* (Klein) might stand as the type species.

Rettger and Koser (4) present data which indicate that dextrine, maltose and dulcite are attacked by *Bact. sanguinarium* with the production of acid but no gas. *Bact. pullorum* produces, on the other hand, no visible change of media containing these agents except slight alkali production. *Bact. pullorum* acts upon dextrose and mannite with evolution of appreciable amounts of gas, while *Bact. sanguinarium*, whether recently isolated or artificially cultivated for many years, does not produce gas in any of the carbohydrate media. Prolonged cultivation of *Bact. pullorum* in the laboratory does not cause this organism to lose its power of producing gas in dextrose and mannite broth. These authors conclude that *Bact. pullorum* manifests itself only as the cause of natural epidemic infection in young chicks. They further maintain that *Bact. sanguinarium* attacks fowls of different ages, and is of relatively little, if indeed any, significance as the cause of epidemic disease in very young chicks.

Mulsow (11) concludes from his studies that *B. avisepticus* may generally be distinguished from *Bact. sanguinarium* by its action in milk, indol production, fermentation of carbohydrates, agglutination reaction and pathogenesis. *Bact. pullorum* and *Bact. sanguinarium* do not produce indol, generally form hydrogen sulphid in lead acetate medium, and produce a temporary acidity in milk, but later alkalinity. As regards fermentation, *Bact. pullorum* produces acid and generally gas in the same carbohydrates, and in addition produces acid in dulcite and maltose. According to this author, *Bact. pullorum* may be distinguished from *Bact. sanguinarium* by the inability of the former to ferment dulcite, while the latter ferments this carbohydrate. *Bact. sanguinarium* generally produces acid promptly in maltose, and does not produce gas in any of the carbohydrates. Rhamnose is fermented promptly by *Bact. pullorum*, while *Bact. sanguinarium* produces acid only after forty-eight hours' incubation. It appears that there are sufficient differences, reported in this paper by Mulsow, between *Bact. sanguinarium* and *Bact. pullorum* to regard these as separate types.

Krumwiede and Kohn (12) report results which indicate that the essential characteristic of the paratyphoid-enteritidis group is the ability of its members to produce acid from rhamnose, differentiating both the aerogenic and anaerogenic members from *B. typhosus*. They point out that, without due regard to low and latent avidity for carbohydrates in relation to variability and practical differentiation, erroneous differential significance might easily be given to variation even among members of the fixed groups.

Experimental.

In the experiments presented, a study has been made of 112 different strains of *Bact. pullorum* isolated from diseased materials from poultry plants in various parts of Massachusetts, to determine, if possible, biochemical and cultural details which are constant enough to warrant their recommendation as a part of the procedure in diagnosis. The following organisms, listed in Table 1, have been isolated from cases of chick disease,

clinically white diarrhœa, and these conformed morphologically, biochemically and serologically to this group of organisms. It was further decided to study the uniformity of these 112 cultures biochemically and serologically, and to determine how many of them gave reactions which were similar to the reactions of its close relative, the fowl typhoid organism (*Bact. sanguinarium*). The cultures of *Bact. sanguinarium* were isolated from birds sent here for diagnosis, and the Smith, Cornell and Gage strains. There were five strains in this list. The two other than the three mentioned appeared typical of sanguinarium, were isolated during the early part of 1920, and designated the Humphrey and Massachusetts strains, respectively.

The following table lists the cultures of *Bact. pullorum* isolated and studied during the course of this work: —

TABLE 1. — *Strains of Bacterium Pullorum studied in this Investigation.*

Bacterium Pullorum.	Source of Culture.	When Isolated and Studied.
Strain No. 1 . . .	M. A. C. Amherst, Mass. Isolated March, 1914, from M. A. C. chick. Used in summer of 1914 as Strain A.	March, 1914
Strain No. 2 . . .	Experimental material from this laboratory. From unabsorbed yolk of chick inoculated summer of 1913 with S_3 (S_3 from Cutler egg). Used in summer of 1914 as Strain B.	
Strain No. 3 . . .	Isolated from material sent to laboratory. Used as Strain C in summer of 1914.	
Strain No. 4 . . .	Bridgewater, Mass. Isolated from Cutler chick. Used as S_2 in 1913. Used as Strain D in 1914.	
Strain No. 5 . . .	Maryland. Used at Maryland Experiment Station in 1911.	
Strain No. 6 . . .	Sterling, Mass. Isolated 1914. Trask Strain. Used as Strain F in summer of 1914.	May 1, 1914
Strain No. 7 . . .	Holliston, Mass. Isolated from chicks sent by C. E. Cristman, Silverwood Farm, Holliston, Mass. These chicks were bought of A. B. H. Arnold, Holliston, Mass.	Feb. 20, 1915
Strain No. 8 . . .	M. A. C. Amherst, Mass. No. 231 (2703) from unabsorbed yolk (chick).	Mar. 31, 1915
Strain No. 9 . . .	Holliston, Mass. Isolated from unabsorbed yolk of chick. Isolated from liver of chick.	
Strain No. 10 . . .	Northborough, Mass. Isolated from liver of chick .	Apr. 1, 1915
Strain No. 11 . . .	Franklin, Mass. 11-1 isolated from unabsorbed yolk of chick No. 2; 11-2 isolated from liver of chick No. 5.	
Strain No. 12 . . .	North Hadley, Mass. 12-1 from unabsorbed yolk of chick No. 1; 12-2 from unabsorbed yolk of chick No. 4; 12-3 from unabsorbed yolk of chick No. 9.	Apr. 5, 1915
Strain No. 13 . . .	Kingston, Mass. Isolated from unabsorbed yolk of chick No. 2.	Apr. 5, 1915
Strain No. 14 . . .	Center Marshfield, Mass. Isolated from unabsorbed yolk of chick No. 4.	Apr. 6, 1915
Strain No. 15 . . .	Brookline, Mass. Isolated from unabsorbed yolk of chick No. 1.	Apr. 7, 1915
Strain No. 16 . . .	Amherst, Mass. Isolated from liver of chick No. 1; 16-2 isolated from unabsorbed yolk of chick No. 1; 16-3 isolated from liver of chick No. 2.	Apr. 12, 1915
Strain No. 17 . . .	Southborough, Mass. 17-1 isolated from liver of chick No. 1; 17-2 isolated from heart of chick No. 2; 17-3 isolated from heart of chick No. 3; 17-4 isolated from unabsorbed yolk of chick No. 4; 17-5 isolated from unabsorbed yolk of chick No. 5; 17-6 isolated from unabsorbed yolk of chick No. 6.	Apr. 16, 1915
Strain No. 18 . . .	Littleton, Mass. 18-1 isolated from heart of chick No. 1; 18-2 isolated from liver of chick No. 1.	Apr. 17, 1915
Strain No. 19 . . .	Andover, Mass. Isolated from unabsorbed yolk of chick No. 1.	Apr. 22, 1915

TABLE 1. — *Strains of Bacterium Pullorum studied in this Investigation* — Continued.

Bacterium Pullorum.	Source of Culture.	When Isolated and Studied.
Strain No. 20 . . .	Westborough, Mass. Isolated from unabsorbed yolk of chick No. 2.	Apr. 23, 1915
Strain No. 21 . . .	Amherst, Mass. Chicks hatched from eggs bought at Hickory Farm, Ludlow, Mass. 21-1 isolated from heart of chick; 21-2 isolated from liver of chick.	May 15, 1915
Strain No. 22 . . .	Shrewsbury, Mass. Isolated from unabsorbed yolk of chick No. 1.	May 13, 1915
Strain No. 23 . . .	Natick, Mass. Isolated from liver of chick No. 1 .	May 14, 1915
Strain No. 24 . . .	Lowell, Mass. 24-1 isolated from unabsorbed yolk of chick No. 1; 24-2 isolated from unabsorbed yolk of chick No. 2.	May 15, 1915
Strain No. 25 . . .	South Hadley, Mass. 25-1 isolated from liver of chick No. 1; 25-2 isolated from unabsorbed yolk of chick No. 2.	June 2, 1915
Strain No. 26 . . .	Amherst, Mass. 26-1 isolated from liver of chick No. 1; 26-2 isolated from liver of chick No 2.	June 2, 1915
Strain No. 27 . . .	Dedham, Mass. 27-1 isolated from liver of chick No. 1; 27-2 isolated from liver of chick No. 2.	June 2, 1915
Strain No. 28 . . .	Belchertown, Mass. Isolated from liver and unabsorbed yolk of chick.	May 2, 1916
Strain No. 29 . . .	Nobscot, Mass. 29-1 isolated from liver and unabsorbed yolk of chick; 29-2 isolated from liver and unabsorbed yolk of chick; 29-3 isolated from liver and unabsorbed yolk of chick; 29-4 isolated from liver and unabsorbed yolk of chick.	July 28, 1916
Strain No. 30 . . .	Concord, Mass. 30-1 isolated from liver and unabsorbed yolk of chick; 30-2 isolated from liver and unabsorbed yolk of chick; 30-3 isolated from liver and unabsorbed yolk of chick; 30-4 isolated from liver and unabsorbed yolk of chick; 30-5 isolated from liver and unabsorbed yolk of chick; 30-6 isolated from liver and unabsorbed yolk of chick.	Mar. 24, 1916
Strain No. 31 . . .	Holliston, Mass. 31-1 isolated from unabsorbed yolk of chick; 31-2 isolated from liver of chick; 31-3 isolated from unabsorbed yolk of chick.	May 2, 1917
Strain No. 32 . . .	Shrewsbury, Mass. Isolated from unabsorbed yolk of chick.	Feb. 28, 1917
Strain No. 33 . . .	Morrisville, N. Y. 33-1 isolated from unabsorbed yolk of chick; 33-2 isolated from unabsorbed yolk of chick.	Mar. 28, 1917
Strain No. 34 . . .	Egypt, Mass. Isolated from unabsorbed yolk of chick.	Mar. 16, 1917
Strain No. 35 . . .	Plainville, Mass. Isolated from unabsorbed yolk of chick.	Apr. 15, 1917
Strain No. 36 . . .	Fitchburg, Mass. 36-1 isolated from liver of chick; 36-2 isolated from liver of chick.	Apr. 13, 1917
Strain No. 37 . . .	Lunenburg, Mass. Isolated from liver of chick; 37-2 isolated from liver of chick.	Apr. 13, 1917
Strain No. 38 . . .	Sutton, Mass. 38-1 isolated from unabsorbed yolk of chick; 38-2 isolated from liver of chick.	Apr. 13, 1917
Strain No. 39 . . .	Southborough, Mass. Isolated from liver of chick	Apr. 16, 1917
Strain No. 40 . . .	Cohasset, Mass. Isolated from unabsorbed yolk of chick.	Apr. 16, 1917
Strain No. 41 . . .	Amherst, Mass. 41-1 isolated from unabsorbed yolk of chick; 41-2 isolated from unabsorbed yolk of chick; 41-3 isolated from unabsorbed yolk of chick; 41-4 isolated from unabsorbed yolk of chick.	Apr. 15, 1917
Strain No. 42 . . .	Shirley, Mass. 42-1 isolated from unabsorbed yolk of chick; 48-2 isolated from unabsorbed yolk of chick.	Apr. 18, 1917
Strain No. 43 . . .	Middleton, Mass. 43-1 isolated from ovary of chick; 43-2 isolated from ovary of chick.	Apr. 21, 1917
Strain No. 44 . . .	Spencer, Mass. Isolated from liver of chick . .	May 2, 1917
Strain No. 45 . . .	Greenfield, Mass. 45-1 isolated from liver of chick; 45-2 isolated from liver of chick.	May 3, 1917
Strain No. 46 . . .	Winchendon, Mass. 46-1 isolated from liver of chick; 46-2 isolated from liver of chick.	May 8, 1917

TABLE 1. — *Strains of Bacterium Pullorum studied in this Investigation* — Continued.

BACTERIUM PULLORUM.	Source of Culture.	When Isloated and Studied.
Strain No. 47 . . .	Pittsfield, Mass. Isolated from liver of chick . .	May 7, 1917
Strain No. 48 . . .	Peabody, Mass. 48-1 isolated from unabsorbed yolk of chick; 48-2 isolated from unabsorbed yolk of chick.	May 24, 1917
Strain No. 49 . . .	Weymouth, Mass. 49-1 isolated from unabsorbed yolk of chick; 49-2 isolated from unabsorbed yolk of chick; 49-3 isolated from unabsorbed yolk of chick; 49-4 isolated from unabsorbed yolk of chick.	Apr. 10, 1917
Strain No. 50 . . .	Westfield, Mass. Isolated from unabsorbed yolk of chick.	May 24, 1917
Strain No. 51 . . .	Methuen, Mass. Isolated from liver of chick . .	Mar. 7, 1920
Strain No. 52 . . .	Methuen, Mass. Isolated from unabsorbed yolk of chick.	Mar. 7, 1920
Strain No. 53 . . .	Methuen, Mass. Isolated from unabsorbed yolk of chick.	Mar. 7, 1920
Strain No. 54 . . .	Methuen, Mass. Isolated from heart of chick . .	Mar. 7, 1920
Strain No. 55 . . .	Webster, Mass. Isolated from unabsorbed yolk of chick.	Mar. 15, 1920
Strain No. 56 . . .	Webster, Mass. Isolated from heart of chick . .	Mar. 15, 1920
Strain No. 57 . . .	Webster, Mass. Isolated from unabsorbed yolk of chick.	Mar. 15, 1920
Strain No. 58 . . .	Andover, Mass. Isolated from unabsorbed yolk of chick.	Mar. 19, 1920
Strain No. 59 . . .	Andover, Mass. Isolated from liver of chick . .	Mar. 19, 1920
Strain No. 60 . . .	Natick, Mass. Isolated from unabsorbed yolk of chick.	Mar. 19, 1920
Strain No. 61 . . .	Natick, Mass. Isolated from unabsorbed yolk of chick.	Mar. 19, 1920
Strain No. 62 . . .	Natick, Mass. Isolated from heart of chick . .	Mar. 19, 1920
Strain No. 63 . . .	Natick, Mass. Isolated from unabsorbed yolk of chick.	Mar. 19, 1920
Strain No. 64 . . .	Hubbardston, Mass. Isolated from liver of chick .	Mar. 23, 1920
Strain No. 65 . . .	Hubbardston, Mass. Isolated from liver of chick .	Mar. 23, 1920
Strain No. 66 . . .	Hubbardston, Mass. Isolated from unabsorbed yolk of chick.	Mar. 23, 1920
Strain No. 67 . . .	Hubbardston, Mass. Isolated from liver of chick .	Mar. 23, 1920
Strain No. 68 . . .	Lexington, Mass. Isolated from heart of chick .	Apr. 8, 1920
Strain No. 69 . . .	Lexington, Mass. Isolated from liver of chick .	Apr. 8, 1920
Strain No. 70 . . .	Lexington, Mass. Isolated from liver of chick .	Apr. 8, 1920
Strain No. 71 . . .	Lexington, Mass. Isolated from heart of chick .	Apr. 8, 1920
Strain No. 72 . . .	Longmeadow, Mass. Isolated from liver of chick .	Apr. 3, 1920
Strain No. 73 . . .	Plymouth, Mass. Isolated from liver of chick .	Apr. 3, 1920
Strain No. 74 . . .	Essex, Mass. Isolated from heart of chick . .	Apr. 9, 1920
Strain No. 75 . . .	Worcester, Mass. Isolated from unabsorbed yolk of chick.	Apr. 9, 1920
Strain No. 76 . . .	Worcester, Mass. Isolated from unabsorbed yolk of chick.	Apr. 9, 1920
Strain No. 77 . . .	Belchertown, Mass. Isolated from unabsorbed yolk of chick.	Apr. 9, 1920
Strain No. 78 . . .	Bridgewater, Mass. Isolated from liver of chick .	Apr. 12, 1920
Strain No. 79 . . .	Bridgewater, Mass. Isolated from unabsorbed yolk of chick.	Apr. 12, 1920
Strain No. 80 . . .	Wellesley, Mass. Isolated from unabsorbed yolk of chick.	Apr. 14, 1920
Strain No. 81 . . .	East Braintree, Mass. Isolated from liver of chick	Apr. 14, 1920

TABLE 1. — *Strains of Bacterium Pullorum studied in this Investigation* — Concluded.

BACTERIUM PULLORUM.	Source of Culture.	When Isolated and Studied.
Strain No. 82 . . .	M. A. C. Amherst, Mass. Isolated from liver of chick.	Apr. 20, 1920
Strain No. 83 . . .	M. A. C. Amherst, Mass. Isolated from unabsorbed yolk of chick.	Apr. 20, 1920
Strain No. 84 . . .	M. A. C. Amherst, Mass. Isolated from unabsorbed yolk of chick.	Apr. 20, 1920
Strain No. 85 . . .	Chester, Mass. Isolated from unabsorbed yolk of chick.	Apr. 21, 1920
Strain No. 86 . . .	Chester, Mass. Isolated from liver of chick . .	Apr. 21, 1920
Strain No. 87 . . .	Chester, Mass. Isolated from liver of chick . .	Apr. 21, 1920
Strain No. 88 . . .	Boston, Mass. Isolated from liver of chick . .	Apr. 21, 1920
Strain No. 89 . . .	Leominster, Mass. Isolated from liver of chick .	Apr. 21, 1920
Strain No. 90 . . .	Medway, Mass. Isolated from liver of chick . .	Apr. 27, 1920
Strain No. 91 . . .	Medway, Mass. Isolated from liver of chick . .	Apr. 27, 1920
Strain No. 92 . . .	Wakefield, Mass. Isolated from liver of chick . .	Apr. 27, 1920
Strain No. 93 . . .	Wakefield, Mass. Isolated from liver of chick .	Apr. 27, 1920
Strain No. 94 . . .	M. A. C. Amherst, Mass. Isolated from unabsorbed yolk of chick.	Apr. 27, 1920
Strain No. 95 . . .	M. A. C. Amherst, Mass. Isolated from liver of chick.	Apr. 27, 1920
Strain No. 96 . . .	Littleton, Mass. Isolated from heart of chick . .	Apr. 30, 1920
Strain No. 97 . . .	Bedford, Mass. Isolated from liver of chick . .	Apr. 30, 1920
Strain No. 98 . . .	Bedford, Mass. Isolated from liver of chick . .	Apr. 30, 1920
Strain No. 99 . . .	Worcester, Mass. Isolated from liver of chick . .	May 4, 1920
Strain No. 100 . . .	Worcester, Mass. Isolated from liver of chick . .	May 4, 1920
Strain No. 101 . . .	West Acton, Mass. Isolated from liver of chick .	May 7, 1920
Strain No. 102 . . .	West Acton, Mass. Isolated from liver of chick .	May 7, 1920
Strain No. 103 . . .	Woonsocket, R. I. Isolated from liver of chick .	May 11, 1920
Strain No. 104 . . .	Woonsocket, R. I. Isolated from liver of chick .	May 11, 1920
Strain No. 105 . . .	Woonsocket, R. I. Isolated from liver of chick .	May 11, 1920
Strain No. 106 . . .	Belchertown, Mass. Isolated from unabsorbed yolk of chick.	May 14, 1920
Strain No. 107 . . .	Segreganset, Mass. Isolated from liver of chick .	May 18, 1920
Strain No. 108 . . .	Waltham, Mass. Isolated from liver of chick . .	May 21, 1920
Strain No. 109 . . .	Charlemont, Mass. Isolated from unabsorbed yolk of chick.	May 28, 1920
Strain No. 110 . . .	Hampton Falls, N. H. Isolated from liver of chick	May 29, 1920
Strain No. 111 . . .	Southwick, Mass. Isolated from liver of chick .	May 19, 1920
Strain No. 112 . . .	Hudson, Mass. Isolated from unabsorbed yolk of chick.	June 3, 1920

Change of Reaction in Carbohydrate Media by the 112 Strains of Bacterium Pullorum.

The cultures of *Bact. pullorum* were grown in test tubes of uniform length and caliber and in standard beef extract bouillon containing 1 per cent of the carbohydrate. These results were somewhat lower than those obtained by Goldberg (8), who found by using infusion broth that the percentage was higher. According to Hadley (10), on an average 0.7 per cent more acid is produced in sugar-infusion broth than in sugar-extract broth. Two drops of a bouillon suspension of each strain were used as the inoculum for a test, triplicate titrations made, and the average percentage acidity noted at the end of the fifth day. It appeared from our work in relation to time of acid production that the maximum occurred between the fifth and tenth day. Therefore the tables and curves represent the amount of acid at the end of a five-day period, at 37.5° C., expressed in percentage normal acid. All titrations were made in the cold, using $\frac{N}{20}$ NaOH and $\frac{N}{20}$ HCl and phenolphthalein as the indicator. Gas production was determined in dextrose, galactose, mannite, levulose, arabinose, salicin, mannose, xylose, adonite, erythrol, saccharose, dulcite, dextrine, lactose, raffinose, inulin, maltose and glycerine. Durham double-barreled fermentation tubes were employed, and the percentage of gas in the inner tube read off on the Frost gasometer chart at the end of five days' incubation at 37.5° C.

Dextrose. — This sugar was fermented by all the 112 strains. The lowest amount of acidity was 0.6 per cent and the highest 1.8 per cent, the mean of 108 determinations being 1.4 per cent acid. Gas was produced in this carbohydrate by all strains, ranging in quantity from a bubble to 55 per cent, the average for all the 112 strains being 20 per cent.

Mannite. — The acid production in mannite was greater than in dextrose and much more variable. After five days' growth the 112 strains had produced an average of 1.0 per cent acidity. The exceptions to this average were strains 23, 46 and 72 which produced 2.0 per cent, 2.2 per cent, and 1.7 per cent, respectively. Gas was produced by all strains, ranging in quantity from 20 to 50 per cent, with an average for the 112 strains of 30 per cent.

Galactose. — This sugar was fermented by all strains, being very much like mannite and dextrose. The acidity ranged from 0.1 to 2.1 per cent, the average for all cultures being 0.9 per cent. There were four exceptions which make a wide variation in the curve, — strains 29, 33, 42 and 49, which produced 0.1, 1.9, 2.0 and 2.1 per cent, respectively.

Levulose. — This sugar was fermented easily by all strains of *Bact. pullorum*, and the changes in reaction here correspond with those in dextrose, mannite and galactose, the acidity ranging from 0.2 to 2.0 per cent, the average for the 112 strains being 0.9 per cent. The exceptions were strains 63, 72 and 73, which produced 2.0, 1.9 and 1.5 per cent acidity, respectively.

Arabinose. — All strains fermented this carbohydrate, the acidity ranging from 0.5 to 1.0 per cent, with an average for the 112 strains of 0.7 per cent. This carbohydrate was fermented in a very variable manner.

Salicin. — None of the 112 strains fermented salicin. On the fifth day there was marked alkaline reaction in some strains. The average acidity for the 112 strains was 0.1 per cent.

Mannose. — This sugar was fermented by all the strains. The minimum acidity by any strain was 0.6 and the maximum 1.3 per cent. The average for the 112 strains was 0.9 per cent acid.

Xylose. — This sugar was fermented by all the strains, but none produced marked quantities of acid. The minimum produced by any strain was 0.1 and the maximum 0.4 per cent, with a mean of 0.25 per cent for the 112 strains. Therefore it may be said that these pullorum strains are not strongly xylose positive.

Adonite. — For the most part the initial acidity was not greatly changed. The minimum figure observed was an alkalinity of 0.1 per cent and the maximum an acidity of 0.1 per cent. As a group these strains were adonite-negative, the curve of results from the 112 strains running close to the line of initial acidity.

Erythrol. — This carbohydrate was not fermented significantly by any of the cultures of *Bact. pullorum* studied. All strains gave a reduction of the initial acidity. The acidity ranged from a minimum of —0.4 per cent to a figure which represented no change from original acidity. Therefore these 112 strains are erythrol negative.

Saccharose. — There was no appreciable amount of acid produced in this carbohydrate. The minimum reading was —0.2 per cent and a few readings showed no change from the initial acidity. The average acidity determination for the 112 strains was —0.2 per cent. There were two exceptions, strains 67 and 84, which showed a determination of —0.4 and —0.5 per cent for acidity. Therefore in saccharose there is no acid formed by *Bact. pullorum.*

Dulcite. — All the 112 strains of *Bact. pullorum* showed a marked reduction of acidity. A few strains did not change the initial acidity, the range being between no change of acidity and —0.4 per cent. There were three exceptions, however, cultures 32, 46 and 47, which produced the following results: —0.6, —0.5 and —0.5 per cent, respectively. Therefore it may be said that the results from these determinations indicate that *Bact. pullorum* is dulcite negative.

Dextrine. — The initial acidity was readily reduced by all strains studied. The readings ranged from no change in acidity to —0.3 per cent. There were no exceptions, all cultures demonstrating this reduction.

Lactose. — The initial acidity was reduced by all strains. The readings ranged from no change in acidity to —0.4 per cent, the mean reading being —0.12 per cent. *Bact. pullorum* may be considered, consequently, lactose-negative as regards acid production. Two strains, 93 and 109, were unusually prompt in this particular. Both strains gave a reading of —0.4.

Raffinose. — The acidity was reduced by all the pullorum strains. The average reading for the 112 cultures was —0.2 per cent. Strain 48 was capable of greater alkaline production than the others, giving a result of —0.5 per cent.

Inulin. — All strains of *Bact. pullorum* were negative in this carbohydrate, the mean reading being —0.19 per cent. There was a prompt reduction in initial acidity, only one culture of the 112 showing no change in the initial acidity.

Maltose. — None of the 112 strains produced any acid. The change was usually marked in all tubes on the fifth day. There was an average reduction of acidity of —0.18 per cent.

Glycerine. — None of the 112 strains produced any acid in glycerine. The determination on the fifth day showed a reduction in the final acidity, averaging —0.1 per cent.

Conclusions from the Fermentation Tests.

From the tests reported concerning the fermentation of the 112 strains of *Bact. pullorum*, it appears that this organism is positive in dextrose, galactose, mannose, mannite, levulose, xylose and arabinose; and negative in glycerine, maltose, adonite, dulcite, lactose, dextrine, saccharose, inulin, erythrol and raffinose. In salicin there is a slight indication of fermentation, at least a slight acidity in a large percentage of the strains. All strains of this organism studied showed a marked tendency to produce gas in

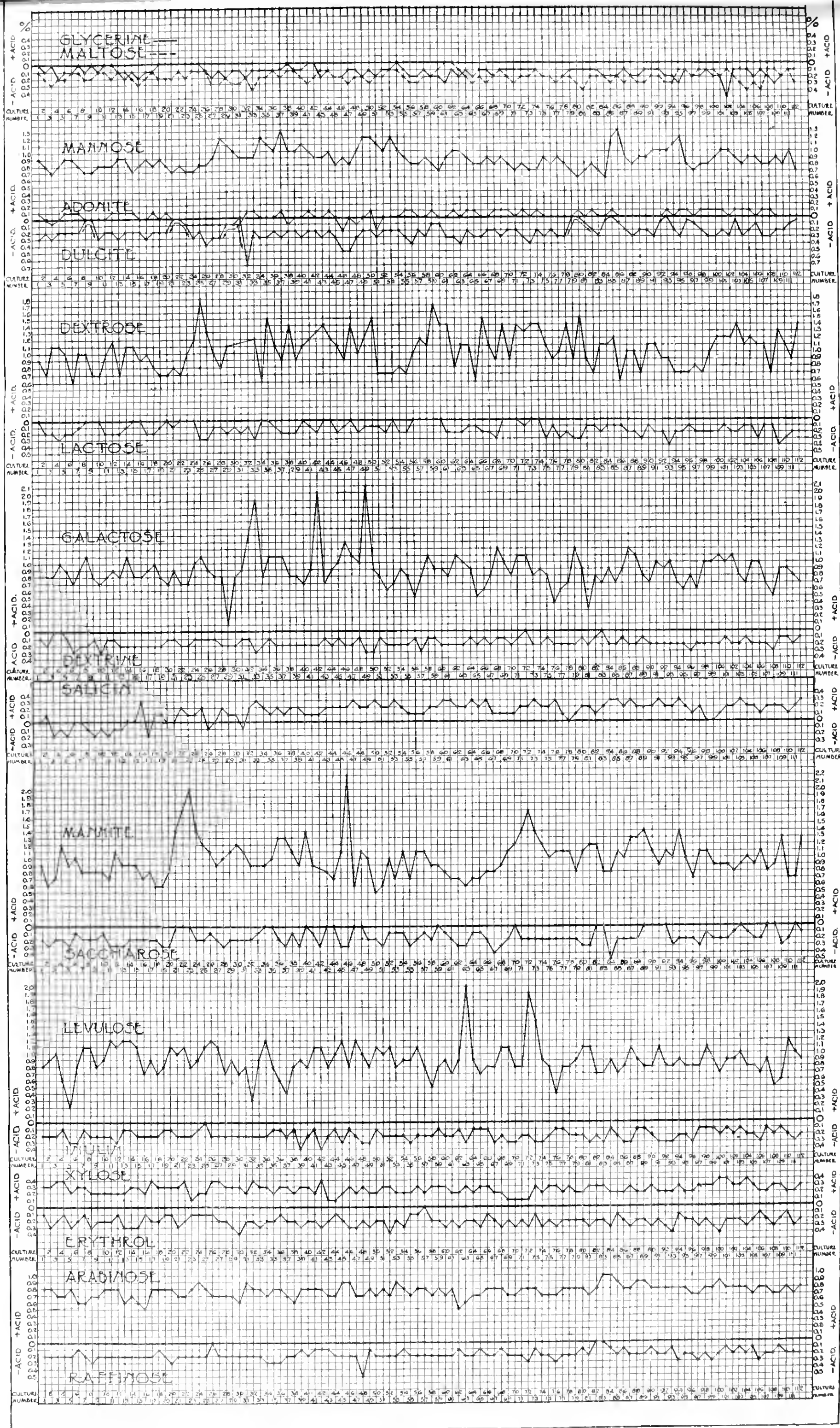

FIG. 1.—Curves showing change of reaction in carbohydrate media by 112 different cultures of *Bact. pullorum*. Percentage of acid produced at end of five-day period. Titration of 5 c c samples in the cold, using $\frac{N}{20}$ NaOH and $\frac{N}{20}$ HCl

Mann
by any
strains
Xylos
quantiti
0.4 per
said tha
Adon
minimu
acidity
of resul
Eryth
cultures
The aci
no char
Sacch
hydrate
change
strains
showed
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acidity
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—0.5 p
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Raff
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Ma
marke
—0.18
Gly
detern
—0.1

Fro
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dextrose. This aerogenic property of the pullorum strains is persistent. Cultures of pullorum carried for fourteen months in France during the war, and kept under adverse conditions, when planted again under favorable conditions regained their aerogenic properties, and the activities in this direction were as marked as in the original cultures. The 112 strains of *Bact. pullorum* studied, even after being transferred eighteen times, still retain active gas production in dextrose and mannite. In one exception, culture No. 44, there has never developed more than a bubble of gas in the dextrose. This is recorded in the table in the dextrose column as B, meaning bubble. All strains are methyl red negative. Therefore from previous morphological and cultural tests, linked with these biochemical findings, it may be concluded that the organism classed to-day as *Bact. pullorum* A should be a slender, non-motile, non-liquefying, gram-negative bacillus. It does not coagulate or peptonize milk. It produces gas in dextrose and mannite, forms H_2S in lead acetate medium, does not produce indol, and does not reduce nitrates.

Fermentation Tests with Bacterium Sanguinarium.

Dextrose. — This sugar was fermented by all the five strains, 0.8 per cent being the highest amount and 0.7 per cent the lowest, the mean being 0.7 per cent.

Mannite. — All cultures of *Bact. sanguinarium* produced about the same quantity of acidity, 0.8 per cent.

Galactose. — Fermented by *Bact. sanguinarium*, the percentage acidity being 0.7, 0.7, 0.6, 0.8 and 0.7 per cent, respectively.

Levulose. — Fermented more variably than galactose, 0.6 per cent being the lowest figure, and 0.9 per cent the highest.

Arabinose. — All strains fermented this carbohydrate, the readings being between 0.6 and 0.8 per cent acid.

Salicin. — Not fermented by the five strains.

Mannose. — This carbohydrate was fermented by *Bact. sanguinarium* about the same as mannite.

Xylose. — Fermented less actively in this carbohydrate, the readings being 0.5, 0.3, 0.2, 0.5 and 0.4 per cent acidity, respectively.

Adonite. — Not appreciably fermented by *Bact. sanguinarium.* The maximum figure obtained was 0.1 per cent acidity.

Erythrol. — Not fermented significantly by any of the five strains of *Bac. sanguinarium.*

Saccharose. — Not fermented by *Bact. sanguinarium.* There was increased alkalinity.

Dulcite. — In this carbohydrate the initial acidity was increased, 0.4 per cent being the maximum amount determined in any of the five cultures.

Dextrine. — There was a marked increase in acidity, four of the five strains of *Bact. sanguinarium* showing 0.6 per cent.

Lactose. — There was no increase in acidity by *Bact. sanguinarium.* There was a marked production of alkalinity.

Raffinose. — There was no increase in acidity in this carbohydrate; the initial acidity was markedly reduced.

Inulin. — There was no increase in acidity in this carbohydrate; the initial acidity was markedly reduced.

Maltose. — Large increase in acid was noted by all strains of *Bact. sanguinarium* in this carbohydrate.

Glycerine. — None of the strains of *Bact. sanguinarium* produced any acid in glycerine. The determination on the fifth day showed a reduction in initial acidity.

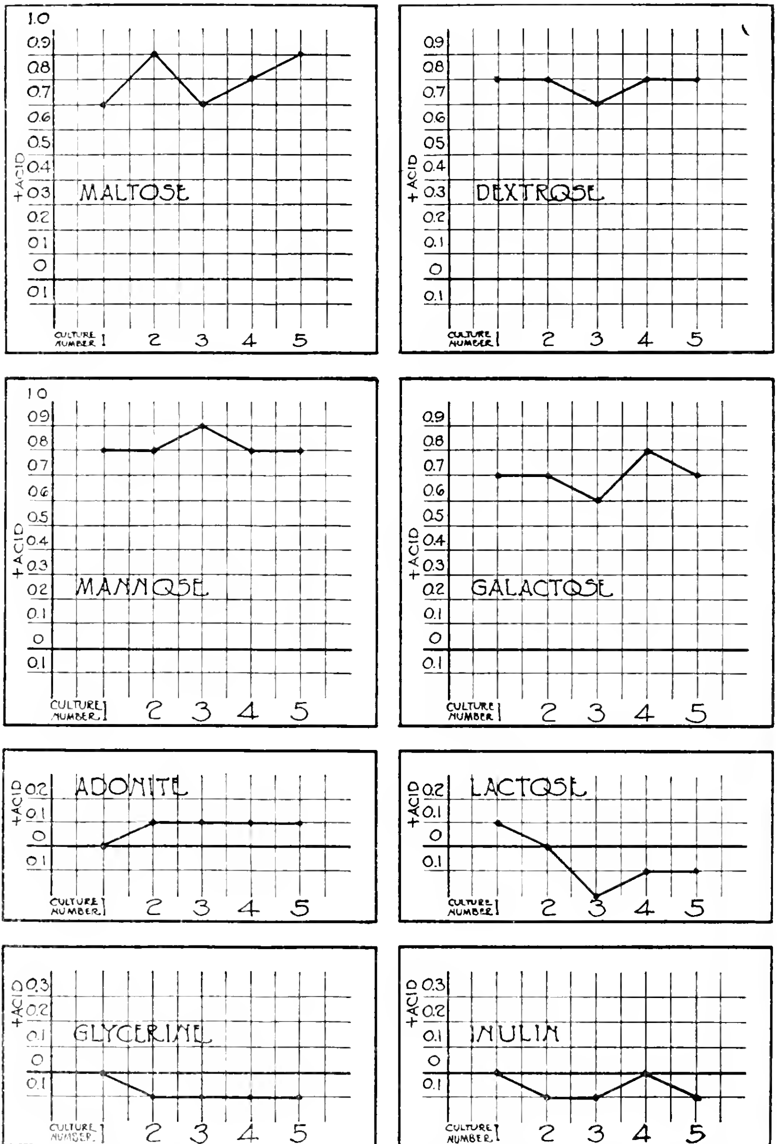

FIG. 2. — Curves showing change of reaction in carbohydrate media by cultures of *Bacterium sanguinarium*. Percentage of acid produced at end of five-day period. Titration of 5 c.c. samples in the cold, using $\frac{N}{20}$ NaOH and $\frac{N}{20}$ HCl.

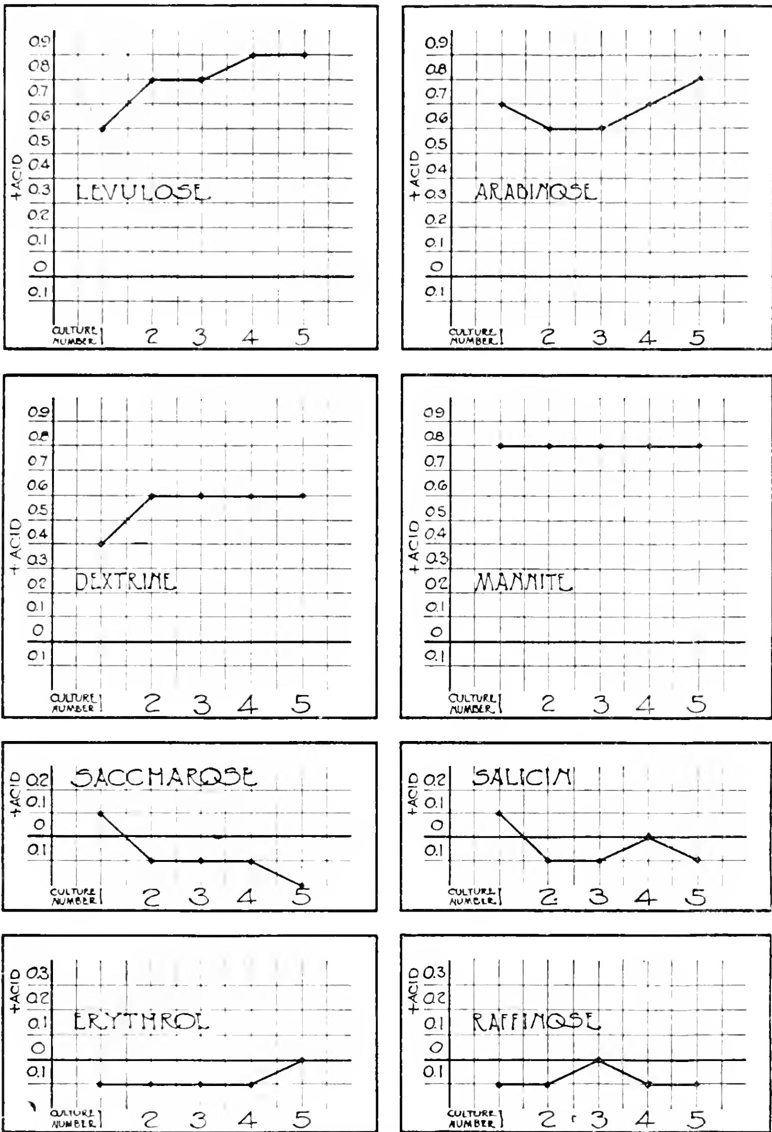

Fig. 2. — Curves showing change of reaction in carbohydrate media by cultures of *Bacterium sanguinarium* — *Continued*.

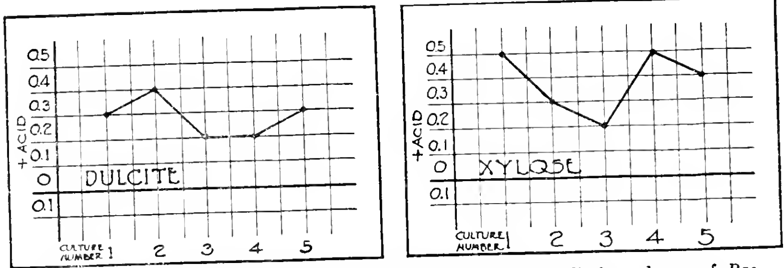

FIG. 2. — Curves showing change of reaction in carbohydrate media by cultures of *Bacterium sanguinarium* — *Concluded.*

TABLE 2. — *Gas Production of the 112 Strains of Bacterium pullorum in Carbohydrate Broth.*

[Percentage of gas in closed arm of fermentation tube.]

Culture Number.	Dextrose.	Mannite.	Galactose.	Levulose.	Arabinose.	Salicin.	Mannose.	Xylose.	Dulcite.	Culture Number.	Dextrose.	Mannite.	Galactose.	Levulose.	Arabinose.	Salicin.	Mannose.	Xylose.	Dulcite.
1	17	20	0	0	0	0	26	0	0	57	20	45	0	0	0	0	25	0	0
2	33	50	0	0	0	0	30	0	0	58	15	30	0	0	0	0	30	0	0
3	37	35	0	B	4	43	30	5	25	59	18	25	0	0	0	0	20	0	0
4	43	30	0	0	0	25	0	0	0	60	17	40	0	0	0	0	20	0	0
5	25	25	0	B	0	0	20	0	0	61	10	25	0	0	0	0	25	0	0
6	30	30	5	0	0	0	20	0	0	62	25	45	5	B	0	0	30	0	0
7	55	25	0	0	0	0	30	0	0	63	18	45	0	0	0	0	22	0	0
8	12	20	0	0	0	0	20	0	0	64	22	40	0	0	0	0	20	0	0
9	15	30	0	0	0	0	30	0	0	65	10	35	0	0	0	0	25	0	0
10	10	25	0	0	0	0	20	0	0	66	12	30	0	0	0	0	30	0	0
11	16	30	0	0	0	0	B	0	0	67	19	25	0	0	0	0	30	0	0
12	16	25	0	0	0	0	B	0	0	68	22	25	0	0	0	0	B	0	0
13	10	25	0	B	0	0	B	0	0	69	23	45	0	0	0	0	20	0	0
14	22	25	0	0	0	0	B	0	0	70	16	35	0	0	0	0	25	0	0
15	10	25	0	0	0	0	25	0	0	71	22	30	0	0	0	0	20	0	0
16	17	30	0	0	0	0	0	0	0	72	17	30	0	0	0	0	30	0	0
17	13	30	0	0	0	0	12	0	0	73	17	30	0	0	0	0	20	0	0
18	14	30	0	0	0	0	15	0	0	74	20	20	0	0	0	0	20	0	0
19	20	30	0	0	0	0	25	0	0	75	17	30	0	0	0	0	20	0	0
20	10	25	0	0	0	0	25	0	0	76	28	30	0	0	0	0	25	0	0
21	20	40	0	0	0	0	22	0	0	77	17	30	0	0	0	0	26	0	0
22	13	30	0	0	0	0	18	0	0	78	18	30	0	0	0	0	20	0	0
23	13	30	0	0	0	0	25	0	0	79	20	25	0	0	0	0	25	0	0

B—Bubble. 0—No gas.

Adonite, erythrol, saccharose, dextrine, lactose, raffinose, inulin, maltose and glycerine produced no gas with any of the cultures.

TABLE 2. — *Gas Production of the 112 Strains of Bacterium Pullorum in Carbohydrate Broth* — Concluded.

Culture Number.	Dextrose.	Mannite.	Galactose.	Levulose.	Arabinose.	Salicin.	Mannose.	Xylose.	Dulcite.	Culture Number.	Dextrose.	Mannite.	Galactose.	Levulose.	Arabinose.	Salicin.	Mannose.	Xylose.	Dulcite.
24 .	17	20	0	0	0	0	5	0	0	80 .	27	40	0	0	0	0	20	0	0
25 .	15	30	10	0	0	0	35	0	0	81 .	20	30	0	0	0	0	25	0	0
26 .	28	30	0	0	0	0	B	0	0	82 .	13	30	0	0	0	0	20	0	0
27 .	23	30	0	0	0	0	25	0	0	83 .	15	25	0	0	0	0	20	0	0
28 .	18	25	0	0	0	0	15	0	0	84 .	27	25	0	0	0	0	25	0	0
29 .	20	45	0	0	0	0	B	0	0	85 .	20	25	0	0	0	0	20	0	0
30 .	10	20	0	0	0	0	B	0	0	86 .	13	25	0	0	0	0	20	0	0
31 .	20	45	0	0	0	0	32	0	0	87 .	15	30	0	0	0	0	25	0	0
32 .	13	40	0	0	0	0	35	0	0	88 .	25	25	0	0	0	0	20	0	0
33 .	30	35	0	0	0	0	15	0	0	89 .	22	30	0	0	0	0	20	0	0
34 .	27	30	0	0	0	0	35	0	0	90 .	20	20	0	0	0	0	25	0	0
35 .	25	25	0	B	0	0	25	0	0	91 .	23	20	0	0	0	0	30	0	0
36 .	27	30	0	0	0	0	25	0	0	92 .	47	45	5	B	0	0	20	0	0
37 .	25	45	0	0	0	0	35	0	0	93 .	10	35	0	0	0	0	25	0	0
38 .	28	35	0	0	0	0	35	0	0	94 .	10	30	0	0	0	0	20	0	0
39 .	25	40	0	0	0	0	0	0	0	95 .	20	25	0	0	0	0	30	0	0
40 .	25	30	0	0	0	0	30	0	0	96 .	25	30	0	0	0	0	0	0	0
41 .	29	35	0	0	0	0	18	0	0	97 .	10	25	0	0	0	0	0	0	0
42 .	45	45	10	10	0	0	5	0	0	98 .	23	20	0	0	0	0	20	0	0
43 .	8	30	0	0	0	0	15	0	0	99 .	27	50	0	0	0	0	25	0	0
44 .	B	20	0	0	0	0	28	0	0	100 .	17	35	0	0	0	0	15	0	0
45 .	20	30	0	0	0	0	22	0	0	101 .	13	30	0	0	0	0	10	0	0
46 .	48	25	15	0	0	0	35	0	0	102 .	17	25	0	0	0	0	25	0	0
47 .	5	25	0	0	0	0	0	0	0	103 .	40	30	10	B	0	0	30	0	0
48 .	27	30	0	0	0	0	0	0	0	104 .	33	25	0	0	0	0	20	0	0
49 .	10	0	0	0	0	0	0	0	0	105 .	30	20	5	B	0	0	20	0	0
50 .	20	50	0	0	0	0	30	0	0	106 .	28	30	0	0	0	0	25	0	0
51 .	17	30	0	0	0	0	B	0	0	107 .	25	25	0	0	0	0	25	0	0
52 .	30	25	0	0	0	0	B	0	0	108 .	17	30	0	0	0	0	30	0	0
53 .	12	25	0	0	0	0	25	0	0	109 .	20	25	0	0	0	0	20	0	0
54 .	32	30	10	0	0	0	B	0	0	110 .	28	20	0	0	0	0	20	0	0
55 .	17	20	0	0	0	0	40	0	0	111 .	10	25	0	0	0	0	25	0	0
56 .	22	30	0	0	0	0	25	0	0	112 .	22	25	0	0	0	0	25	0	0

B=Bubble. 0=No gas.

Adonite, erythrol, saccharose, dextrine, lactose, raffinose, inulin, maltose and glycerine produced no gas with any of the cultures.

TABLE 3.—*Summary of Biochemical Data as Regards Fermentation of the 112 Strains of Bacterium Pullorum.*

[Acid[1] and gas[2] production.]

Culture	Dextrose		Mannite		Galactose		Levulose		Arabinose		Salicin		Mannose		Xylose		Adonite		Erythrol		Saccharose		Dulcite		Dextrine		Lactose		Raffinose		Inulin		Maltose		Glycerine	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
1	+	+	+	+	+	−	+	−	+	−	O	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	O	−	−	−	−	−	−	−	O	−
2	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3	+	+	+	+	+	−	+	B	+	+	−	+	−	+	+	+	−	−	−	−	−	−	−	+	O	−	−	−	−	−	−	−	−	−	O	−
4	+	+	+	+	+	−	+	−	+	−	−	+	−	−	+	−	O	−	−	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−	−	−
5	+	+	+	+	+	−	+	B	+	−	−	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	+	−	+	−	O	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7	+	+	+	+	+	−	+	−	+	−	O	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	O	−
8	+	+	+	+	+	−	+	−	+	−	−	−	−	+	+	−	O	−	−	−	−	−	O	−	−	−	O	−	−	−	−	−	−	−	−	−
9	+	+	+	+	+	−	+	−	+	−	−	−	−	+	+	−	O	−	−	−	−	−	O	−	−	−	O	−	−	−	−	−	−	−	O	−
10	+	+	+	+	+	−	+	−	+	−	−	−	−	+	+	−	O	−	−	−	−	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−
11	+	+	+	+	+	−	+	−	+	−	−	−	−	B	+	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	O	−
12	+	+	+	+	+	−	+	−	+	−	−	−	−	B	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	O	−
13	+	+	+	+	+	−	+	B	+	−	−	−	−	B	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
14	+	+	+	+	+	−	+	−	+	−	O	−	−	B	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
15	+	+	+	+	+	−	+	−	+	−	O	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
16	+	+	+	+	+	−	+	−	+	−	+	−	−	−	+	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
17	+	+	+	+	+	−	+	−	+	−	−	−	−	+	+	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
18	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
19	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	O	−
20	+	+	+	+	+	−	+	−	+	−	O	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	O	−	−	−	−	−	−	−	O	−
21	+	+	+	+	+	−	+	−	+	−	O	−	−	+	+	−	O	−	−	−	O	−	O	−	−	−	−	−	−	−	−	−	−	−	O	−
22	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	O	−	−	−	O	−	O	−	−	−	O	−	−	−	−	−	−	−	O	−
23	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	−	−	−	−	O	−	−	−	−	−	O	−	−	−	−	−	−	−	O	−
24	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	O	−	−	−	−	−	−	−	O	−
25	+	+	+	+	+	+	+	−	+	−	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	O	−	−	−	O	−
26	+	+	+	+	+	−	+	−	+	−	−	−	−	B	+	−	−	−	−	−	−	−	−	−	−	−	−	−	O	−	−	−	−	−	−	−
27	+	+	+	+	+	−	+	−	+	−	O	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
28	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
29	+	+	+	+	+	−	+	−	+	−	+	−	−	B	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
30	+	+	+	+	+	−	+	−	+	−	+	−	−	B	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
31	+	+	+	+	+	−	+	−	+	−	−	−	−	+	+	−	−	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−
32	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
33	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
34	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	O	−	−	−	O	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−
35	+	+	+	+	+	−	+	B	+	−	+	−	−	+	+	−	O	−	−	−	O	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−
36	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
37	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
38	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	O	−
39	+	+	+	+	+	−	+	−	+	−	+	−	−	−	+	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
40	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	O	−	−	−	O	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−
41	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	O	−
42	+	+	+	+	+	+	+	+	+	−	+	−	−	+	+	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	O	−
43	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	O	−	−	−	O	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−
44	+	B	+	+	+	−	+	−	+	−	+	−	−	+	+	−	+	−	−	−	O	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−
45	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
46	+	+	+	+	+	+	+	−	+	−	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
47	+	+	+	+	+	−	+	−	+	−	+	−	−	−	+	−	O	−	−	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
48	+	+	+	+	+	−	+	−	+	−	+	−	−	−	+	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
49	+	+	+	+	+	−	+	−	+	−	+	−	−	−	+	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
50	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
51	+	+	+	+	+	−	+	−	+	−	+	−	−	B	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
52	+	+	+	+	+	−	+	−	+	−	+	−	−	B	+	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
53	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	O	−	−	−	−	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−
54	+	+	+	+	+	+	+	−	+	−	+	−	−	B	+	−	O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	O	−
55	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
56	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−

[1] + = acid production.
— = alkali production.
O = neutral.

[2] + = gas produced.
— = no gas produced.
B = bubble (not enough to measure).

TABLE 3. — *Summary of Biochemical Data as Regards Fermentation of the 112 Strains of Bacterium Pullorum* — Concluded.

[Acid[1] and gas[2] production.]

Culture	Dextrose		Mannite		Galactose		Levulose		Arabinose		Salicin		Mannose		Xylose		Adonite		Erythrol		Saccharose		Dulcite		Dextrine		Lactose		Raffinose		Inulin		Maltose		Glycerine	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
57	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	O	—	—	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—
58	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—
59	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	O	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—
60	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—
61	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	O	—	—	—
62	+	+	+	+	+	+	+	B	+	—	+	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	O	—	—	—
63	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
64	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
65	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
66	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
67	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
68	+	+	+	+	+	—	+	—	+	—	+	—	+		+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
69	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—
70	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	O	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—
71	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—
72	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—
73	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—
74	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—
75	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
76	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
77	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
78	+	+	+	+	+	—	+	—	+	—	O	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
79	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—
80	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—
81	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
82	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	O	—	—	—	—	—	—	—	O	—	—	—	—	—	—	—
83	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	O	—	—	—	O	—	—	—	O	—	—	—	—	—	—	—
84	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
85	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—
86	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
87	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
88	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
89	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
90	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
91	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
92	+	+	+	+	+	+	+	B	+	—	+	—	+	+	+	—	O	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
93	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—
94	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
95	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
96	+	+	+	+	+	—	+	—	+	—	+	—	+	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
97	+	+	+	+	+	—	+	—	+	—	+	—	+	—	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
98	+	+	+	+	+	—	+	—	+	—	O	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
99	+	+	+	+	+	—	+	—	+	—	O	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
100	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
101	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
102	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
103	+	+	+	+	+	+	+	B	+	—	+	—	+	+	+	—	+	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
104	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
105	+	+	+	+	+	+	+	B	+	—	+	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
106	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
107	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
108	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	+	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
109	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
110	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
111	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
112	+	+	+	+	+	—	+	—	+	—	+	—	+	+	+	—	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[1] + = acid production.
— = alkali production.
O = neutral.

[2] + = gas produced.
— = no gas produced.
B = bubble (not enough to measure).

A comparison of the tables which have to do with *Bact. pullorum* with those which have to do with *Bact. sanguinarium* shows that *Bact. pullorum* is maltose-dextrine-dulcite negative, while *Bact. sanguinarium* is maltose-dextrine-dulcite positive. All freshly isolated strains of *Bact. pullorum* (139 strains) have produced gas, while the five strains of *Bact. sanguinarium* have never produced gas. The 112 strains of *Bact. pullorum* studied have been maltose-dextrine-dulcite negative. This agrees very well with the work of Hadley. Thus far we have isolated but one organism from chicks, showing typical symptoms of white diarrhœa, which did not produce gas in dextrose. This particular strain was maltose-dextrine-dulcite negative, and therefore would correspond to *Bact. pullorum* B or the anaerogenic pullorum form. During the current year, 1920–21, several anaerogenic forms have been isolated from adult hens, and they were maltose-dextrine-dulcite negative, which in a way helps to substantiate Hadley's claim that the *Bact. pullorum* infecting adult hens is maltose-dextrine-dulcite negative, but anaerogenic. The number of cases thus studied is meager, and future studies with more cases ought to give sufficient data to establish this point. Since Hadley has been able to isolate both aerogenic and anaerogenic forms of *Bact. pullorum* from the eggs of fowls experiencing infections with the maltose-dextrine-dulcite anaerogenic strains, and since the maltose-dextrine-dulcite negative strains isolated by him from chicks have been aerogenic, while all the maltose-dextrine-dulcite negative strains isolated from infections in adult birds have been anaerogenic, the duality of the *Bact. pullorum* type appears to be justified. The work presented in this paper substantiates Hadley's results. Besides, the gas production is of great value as a differential characteristic. Therefore it is essential in making a differential bacterial diagnosis for *Bact. pullorum* to note its special morphological characteristics; to ascertain its fermentation activities in maltose, dextrine and dulcite, and its aerogenicity. Doubtful cultures of *Bact. pullorum* should be submitted to the above biochemical tests before a differential diagnosis is justified. As a routine in this department, all doubtful cultures are tested for aerogenicity in dextrose, and for acidity in maltose; methyl red being used as an indicator for the increased acid production. The data at hand indicate that there are maltose-dextrine-dulcite negative strains which do not produce gas in dextrose, and these, whether found only in adult birds or not, should be classed as the *Bact. pullorum* B, different from the one so generally isolated from chicks, which is maltose-dextrine-dulcite negative, but produces gas in dextrose.

The fowl typhoid (*Bact. sanguinarium*) is characterized, aside from its specific morphology, as an anaerogenic non-motile bacillus. It does not form indol, nor reduce nitrates. It forms H_2S in lead acetate media. It is a maltose-dextrine-dulcite positive organism.

Distribution of Fowl Typhoid in Massachusetts.

During the seasons of 1919–20 and 1920–21, observations were made on all specimens sent to the laboratory for diagnosis, especially to note the presence of *Bact. sanguinarium*. During that time more than 600 different specimens were examined, and this anaerogenic, non-motile bacillus which was maltose-dextrine-dulcite positive was isolated but six times, — three times in the season of 1919–20 and three times in the season of 1920–21. These cases exhibited all the post-mortem findings peculiar to this disease. Especially noticeable were the enlarged spleen and the marked leukemic condition. There were, however, several maltose-dextrine-dulcite negative forms isolated which were anaerogenic, these classifying as *Bact. pullorum* B. During this same period 289 chicks, sent here with a history of bacillary white diarrhœa, were examined, and the true *Bact. pullorum* was isolated from all but one. This one strain was anaerogenic, and persistently gave a faint acid reaction in maltose when methyl red was used as an indicator. From this it would appear that in this one chick we were dealing with an organism which came close to the *Bact. sanguinarium* type. From these findings the writer is led to believe that the fowl typhoid infection in Massachusetts is infrequent, and that the *Bact. pullorum* B type is far from common. In our work of the last few years we have never isolated from eggs a *Bact. pullorum* form which was anaerogenic. All cultures have been aerogenic and have produced little or no acid in maltose, dextrine or dulcite.

Although this represents but two years' observations, there appears to be sufficient evidence to indicate that fowl typhoid is not widely distributed in Massachusetts; that it is not transmitted by the egg; and that *Bact. pullorum* of the B type is found frequently in adult stock.

Does either Bact. Pullorum or Bact. Sanguinarium play Any Part in the so-called "Paralysis" so widely distributed in Massachusetts?

During the course of the studies concerning the diagnosis of *Bact. pullorum*, there were brought to the laboratory many birds suffering with the so-called "paralysis," which even now is assuming a vast economic importance in the poultry industry in Massachusetts. The weakness of the legs and the listlessness of these birds were not essentially different from conditions produced in rabbits when inoculated with pure cultures of *Bact. pullorum*. With this in mind, all specimens exhibiting the paralytic symptoms were examined bacteriologically, with special reference to *Bact. pullorum* and *Bact. sanguinarium*. There were 83 paralytic specimens examined, and from 5 of them only was isolated *Bact. pullorum* of the aerogenic type. None of the 83 specimens exhibited the marked enlarged spleen and leukemic conditions found in fowl typhoid, as known to us in this laboratory. The anaerogenic maltose-dextrine-dulcite positive organism of fowl typhoid was not isolated from any of the 83 specimens. Cultural examinations were made of liver tissue, spleen, intestinal mucosa,

ovarian tissues, and lumbar region of the spinal cord. In this so-called "paralysis" all birds during life showed a rather bright red comb, the paleness being evident only a short time before death. There was never found at autopsy a marked leukemia. In fowl typhoid this leukemic condition is highly prominent, and for this reason Moore has called this paratyphoid type of infection "infectious leukemia." Hadley has observed a similar epidemic in fowls showing pronounced leukemic symptoms associated with *Bact. pullorum*. The writer has never observed this condition in relation to *Bact. pullorum* infections in adult birds.

From these observations on the 83 paralytic birds, with only 5 showing the presence of the *Bact. pullorum* infection, — these five probably having carried the infection since chickhood, — the evidence does not indicate that the paralytic disease so widely distributed at certain periods of the year in Massachusetts is due to the presence of either the pullorum or sanguinarium type.

Influence of Infection upon the Hatching Quality of Eggs and upon the Viability of Young Stock.

In 1917 and 1918 several sets of experiments were carried out under the best known conditions for poultry husbandry. Eggs from 60 hens known to have reacted positively to the agglutination test were set in an electrobator. When tested at the end of the first seven days of incubation, 30 were found to be infertile and 2 were found dead in the shell. Of the 28 left, 10 were hatched; 3 chicks died at the end of the first day and *Bact. pullorum* (aerogenic type) was isolated from the unabsorbed yolk. All eggs containing fully developed chicks were examined especially for *Bact. pullorum*, with the following results. The egg number in each case represents the number of the hen laying the egg.

TABLE 4. — *Results of Tests for Bacterium Pullorum in Dead Chicks from Eggs laid by Positively Reacting Birds.*

Egg Number.	Bact. pullorum.	Egg Number.	Bact. pullorum.
8001	+	7925	—
8384	+	7998	—
8388	—	8430	+
8002	—	8430	—
8002	—	8565	+
8430	+	8388	+
7925	—	7998	+
8565	—	8430	—
8001	+	8384	—

+=present. —=not present.

From this table it will be seen that with the methods used it was not possible to detect *Bact. pullorum* in all the dead chicks, although adult hens were all positively reacting to the agglutination test. From 8, *Bact. pullorum* was isolated without difficulty; from the other 10, the cultures were negative.

After three months, following out three sets of incubation, the author was able to obtain from the three sets of eggs set, 60 in each lot, all from positively reacting hens, 7 livable chicks on the first set, 9 on the second set, and 9 on the third set, and these chicks were all given the numbers of the parent stock from which they came: 7811, 7895, 7925, 7997, 7998, 8001, 8002, 8020, 8082, 8084, 8094, 8139, 8171, 8180, 8202, 8204, 8294, 8384, 8388, 8389, 8430, 8431, 8544, 8565, 8810. These 25 birds, all reared from positively agglutinating hens, were yarded together and blood taken at various times to determine whether their blood would show any signs of agglutinative powers.

When the chicks had grown to a weight of at least 400 grams, they were put together in the yard on Aug. 10, 1917. The following table shows the weight of each bird at that time: —

TABLE 5. — *Weight of Chicks on Aug. 10, 1917.*

Chick Number.	Weight (Grams).	Chick Number.	Weight (Grams.)
7811	870	8180	680
7895	1,200	8204	450
7925	1,240	8202	580
7997	860	8294	780
7998	1,249	8384	620
8001	1,160	8388	530
8002	1,130	8389	540
8020	680	8430	540
8082	950	8431	380
8084	1,490	8544	510
8094	730	8565	530
8139	1,050	8810	670
8171	780		

Agglutination tests were run on these birds, the first being on July 17, 1917. The following table shows the reactions for this and subsequent tests: —

TABLE 6. — *Records of Agglutination Tests on Chicks hatched from Eggs laid by Positively Reacting Hens.* [1]

CHICK NUMBER.	JULY 17 AND 18, 1917.					JULY 21, 1917.					AUG. 3, 1917.					AUG. 26, 1917.					NOV. 7, 1917.				
	DILUTION OF SERUM.					DILUTION OF SERUM.					DILUTION OF SERUM.					DILUTION OF SERUM.					DILUTION OF SERUM.				
	1–100.	1–200.	1–500.	1–1000.	1–2000.	1–100.	1–200.	1–500.	1–1000.	1–2000.	1–100.	1–200.	1–500.	1–1000.	1–2000.	1–100.	1–200.	1–500.	1–1000.	1–2000.	1–100.	1–200.	1–500.	1–1000.	1–2000.
7811	?	?	?	?	?	C	?	?	?	?	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
7895	?	?	?	?	?	0	0	0	0	0	C	C	C	0	0	?	?	?	?	?					
7925	0	0	0	0	0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
7997	C	C	0	0	0	0	0	0	0	0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
7998	0	0	0	0	0	0	0	0	0	0	C	C	0	0	0	?	?	?	?	?	?	?	?	?	?
8001	0	0	0	0	0	?	?	?	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8002	0	0	0	0	0	C	0	0	0	0	C	C	0	0	0	?	?	?	?	?	0	0	0	0	0
8020	0	0	0	0	0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
8082	C	?	?	?	?	0	0	0	0	0	C	C	0	0	0	C	C	0	0	0					
8084	?	?	?	?	?	0	0	0	0	0	C	C	C	C	C	C	C	C	C	C	C	C	0	0	0
8094	0	0	0	0	0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
8139	?	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8171	?	?	?	?	?	?	?	?	?	?	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
8180	C	C	C	C	C	C	C	C	C	C	C	0	0	0	0	C	C	C	0	0	C	C	C	C	C
8202	0	0	0	0	0	0	0	0	0	0						C	C	C	C	C	C	C	C	C	C
8204	?	?	?	0	0	C	C	C	C	?	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
8294						0	0	0	0	0	C	C	C	C	C	C	C	0	0	0	C	C	C	C	C
8384	C	C	C	C	C	0	0	0	0	0	C	0	0	0	0	C	C	C	0	0	C	C	C	C	C
8388	?	?	?	?	?	C	C	C	C	C	?	?	?	?	?	C	C	0	0	0	C	C	0	0	0
8430	?	?	?	0	0	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
8565	?	?	?	?	?	C	C	C	C	C	C	C	C	C	0	C	C	C	C	C	C	C	C	C	C
8810	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	?	?	?					

[1] The symbols indicating various degrees of agglutination have been taken from Hadley, Journal of Immunology, Vol. 2, p. 463, 1917, as follows: C=complete agglutination; ?=doubtful agglutination; 0=no agglutination.

These experiments indicate that in chicks hatched from eggs laid by positively reacting hens, at least six months' time should elapse before the normal agglutination power of such sera would be sufficiently definite to furnish indication of past or present infection. The birds reared from hens 8001, 8139 and 8810 never showed any agglutinative power to their blood sera. The length of time a serum maintains its agglutination power has not as yet been determined.

The Present Status of the Specificity of the Agglutination Test as a Means of Control of Bacterium Pullorum Infection in Young Chicks.

During the last few years the agglutination test has become a popular means of recognition in the domestic fowl of those individuals which have contracted *Bact. pullorum* infections in chickhood; and consequently, as adult productive fowls, may have become, through infections in their ovaries, carriers of infection to the offspring. Several writers have demonstrated that there are certain factors which have influenced the test and which suggest the need of modification of the method in the direction of securing a higher degree of specificity. Hadley suggests that we stand in need of a means of diagnosis which shall distinguish between a latent (presumably ovarian) and a past infection. The data presented up to date indicate that not all adult hens with *Bact. pullorum* have infections localized in the ovaries; and also that not all infection has its origin in an attack of bacillary white diarrhœa experienced in the chick stage. This point, as Hadley suggests, is of less significance in its bearing upon the validity of the results of agglutination tests for *Bact. pullorum* infection than is the question of the specificity of the test. This author as well as others has demonstrated the interagglutinability of *Bact. pullorum*, fowl typhoid and other antigens in both *Bact. pullorum* and fowl typhoid serum. Fowls which have been experimentally immunized against different types of fowl typhoid possess serum which agglutinates *Bact. pullorum* antigens quite as well as it agglutinates its homologous antigen. According to these data the agglutination test for the recognition of *Bact. pullorum* infection appears to lose some of its claim to specificity; and to this extent, without carefully going over the results as obtained in field and laboratory co-operating, it may be open to criticism.

If all operations both in field and laboratory are considered, however, the reader will be convinced that the test yields valuable results. From our work, already reported, during the seasons of 1919–20 and 1920–21, there were only six cases where the anaerogenic type of organism was isolated and the post-mortem examinations revealed the enlarged spleen associated with leukemic conditions. This indicates that, at least so far as this laboratory has been called upon to make examinations, fowl typhoid infections are infrequent. That all infections are localized in the ovary is yet to be proven. It can be said, however, that the ovarian infections are not rare, and when they are present they persist. During the course of the examination of hundreds of eggs for *Bact. pullorum* infection, only the true aerogenic form of *Bact. pullorum* was isolated. Strains of these cultures, even after four years, maintained this aerogenic property and were maltose-dextrine-dulcite negative. Therefore these studies indicate that fowl typhoid is not transmitted to the egg. In all of our work in the bacteriological examination of young chicks, in all cases showing large unabsorbed yolks, we have been able to isolate only the aerogenic type of organism, and this in hundreds of cases. This shows an apparent lack of

susceptibility of young stock to the *Bact. sanguinarium* type of infection, and appears to substantiate the work of Dr. Hadley, who states that he has examined large numbers of cultures derived from young stock, but has not encountered among them the *Bact. sanguinarium* type.

In this laboratory hundreds of agglutination tests have been made to demonstrate the interagglutinability of *Bact. pullorum* with *Bact. sanguinarium*, *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B. The results obtained here agree with those from other laboratories: *i.e.*, that the agglutinative tests are sufficiently definite for grouping the fowl typhoid and pullorum types together, both demonstrating the same intimate relation to typhoid bacilli. In every test made, the *Bact. pullorum* immune serum agglutinates typhoid antigen better than typhoid serum agglutinates pullorum antigen. *Bact. sanguinarium* immune serum agglutinates *Bact. pullorum* much better than it does typhoid. There has never been demonstrated any indication of an affinity of interagglutinability between *B. avisepticus* (fowl cholera) and the pullorum and sanguinarium types. While it is true that by our present methods it is difficult to differentiate sanguinarium and pullorum by agglutination, this does not mean that application of the test will not yield valuable results. Already, from the work of three years, the typical maltose-dextrine-dulcite positive anaerogenic fowl typhoid organism has been isolated six times, and in this study more than 600 specimens were examined. This indicates that fowl typhoid is not widespread, at least in Massachusetts.

From the preceding biochemical data the establishment of *Bact. pullorum* and *Bact. sanguinarium* as separate types is justifiable. Therefore if it can be proven that breeding birds showing a positive agglutination reaction may lay eggs, from which are hatched chicks developing white diarrhœa symptoms, and at death the internal organs yield cultures which demonstrate morphologically an organism which is slender, non-motile, gram-negative, gelatine non-liquefying, and is aerogenic, demonstrating no acidity in maltose, dextrine and dulcite, the agglutination test would not be invalid as an economic measure in the identification of this infection. With this in mind, an experiment was carried out to this end.

Twenty breeding flocks were selected, all showing positively reacting birds, and the following spring all the dead chicks from these places were examined bacteriologically, with special reference to identifying the small gram-negative, maltose-dextrine-dulcite negative organism which was aerogenic. The following table shows the details of the tests: —

TABLE 7. — *Results on Identification of Cultures isolated from Dead Chicks which had been hatched from Eggs laid by Positively Reacting Breeding Birds.*

[Materials for study obtained from 20 different parts of Massachusetts.]

FLOCK NUMBER.	BREEDING BIRDS.		Cultures isolated from Dead Chicks.	(FERMENTATION) ACID IN —			Gas Production (Aerogenicity) in Dextrose.	Agglutinability by Pullorum Serum.	Identification.
	Number in Flock.	Number with Positive Agglutination Test.		Maltose.	Dextrine.	Dulcite.			
1	51	16	2 Y 3 H 3 Y	— — —	— — —	— — —	+ + +	+C (1-400) +C (1-400) +C (1-400)	*Bact. pullorum* A
2	219	26	1 L 2 Y 3 Y 4 H	— — — —	— — — —	— — — —	+ + + +	+C (1-400) +C (1-400) +C (1-400) +C (1-400)	*Bact. pullorum* A
3	216	45	29 Y	—	—	—	+	+C (1-400)	*Bact. pullorum* A
4	51	20	22 L	—	—	—	+	+C (1-400)	*Bact. pullorum* A
5	36	3	24 Y 25 Y	— —	— —	— —	+ +	+C (1-400) +C (1-400)	*Bact. pullorum* A
6	1,194	244	29 Y	—	—	—	+	+C (1-400)	*Bact. pullorum* A
7	784	14	31 Y 32 Y 33 Y	— — —	— — —	— — —	+ + +	+C (1-200) +C (1-200) +C (1-200)	*Bact. pullorum* A
8	250	51	39 L 40 L	— —	— —	— —	+ +	+C (1-200) +C (1-200)	*Bact. pullorum* A
9	89	13	45 H	—	—	—	+	+C (1-200)	*Bact. pullorum* A
10	393	29	52 L 53 L 54 L	— — —	— — —	— — —	+ + +	+C (1-200) +C (1-200) +C (1-200)	*Bact. pullorum* A
11	138	21	60 L	—	—	—	+	+C (1-200)	*Bact. pullorum* A
12	76	6	61 Y	—	—	—	+	+C (1-200)	*Bact. pullorum* A
13	882	129	1 L 2 Y 3 Y 4 H	— — — —	— — — —	— — — —	+ + + +	+C (1-200) +C (1-200) +C (1-200) +C (1-200)	*Bact. pullorum* A
14	116	33	2 Y 3 H 3 Y	— — —	— — —	— — —	+ + +	+C (1-200) +C (1-200) +C (1-200)	*Bact. pullorum* A
15	264	71	1 Y	—	—	—	—	+C(1-200)	*Bact. pullorum* ?
16	110	46	2 Y	—	—	—	+	+C (1-200)	*Bact. pullorum* A
17	239	33	1 L	—	—	—	+	+C (1-200)	*Bact. pullorum* A
18	66	10	1 Y	—	—	—	+	+C (1-200)	*Bact. pullorum* A
19	38	11	2 Y 3 H 3 Y	— — —	— — —	— — —	+ + +	+C (1-200) +C (1-200) +C (1-200)	*Bact. pullorum* A
20	407	103	1 L 2 Y 3 Y 4 H	— — — —	— — — —	— — — —	+ + + +	+C (1-200) +C (1-200) +C (1-200) +C (1-200)	*Bact. pullorum* A

Y = unabsorbed yolk; H = heart blood; L = liver.

The results presented in this table need no comment. It can readily be seen that, with the exception of one culture obtained from flock No. 15, all cultures obtained from dead chicks which had been hatched from positive-reacting birds were maltose-dextrine-dulcite negative, and produced gas in dextrose. This is significant in that these flocks were widely distributed, and the only exception to this rule was the one noted above. This culture was maltose-dextrine-dulcite negative and was anaerogenic. At any rate, it gave none of the reactions for *Bact. sanguinarium*. On this experiment were 5,619 breeding hens and 924 were positive reactors, giving a positive agglutination up to dilutions of 1,000 and over. It is reasonable to believe that these results would be substantiated by a repetition of the experiment. While there are, as already noted, certain factors which have influenced the test and which may suggest need of modifications, — such as the validity of the agglutination tests, based on interagglutinability of *Bact. pullorum*, *Bact. sanguinarium* and other antigens in both *Bact. pullorum* and *Bact. sanguinarium* serum, — yet the fact remains that in the twenty flocks mentioned the agglutination test definitely located infection in 924 birds in a total number of 5,619. The differential characteristics of the cultures isolated from dead chicks which had been hatched from the eggs laid by these positive-reacting birds proved to be typical *Bact. pullorum*, conforming morphologically and biochemically to the standard set as a result of fermentative, serological and morphological studies completed.

After all is said about chances of error with the test, data are constantly being accumulated which indicate that the agglutination when carefully controlled through epidemiological work is at present the best method we have of locating *Bact. pullorum* infection and furnishing poultrymen a starting point for its elimination.

Summary.

From the foregoing data the following conclusions appear justified concerning the diagnosis of *Bact. pullorum* infection in the domestic fowl: —

1. From the fermentation studies conducted over a period of three years, it was found that *Bact. pullorum* is maltose-dextrine-dulcite negative and aerogenic, while all cultures of *Bact. sanguinarium* studied have been maltose-dextrine-dulcite positive and anaerogenic. These characteristics are constant. Whenever there has been question as to cultural and morphological differentiations, these investigations have shown that the biochemical tests have aided in making a final decision. Variations in morphology of the pullorum strains are frequent; therefore doubtful cultures should be submitted to the maltose-dextrine-dulcite test and checked by gas production in dextrose. Experience has shown that this procedure should be followed as a routine in all laboratories having to do with the pullorum problem.

2. From the examination of 600 avian specimens for the anaerogenic, non-motile, maltose-dextrine-dulcite positive form which produced en-

larged spleens associated with marked leukemic conditions, it was of some significance that the true sanguinarium culture was identified only six times. Chick examinations conducted during this same period, representing several hundred examinations, all yielded typical pullorum cultures. There was but one exception, and this culture was probably an atypical pullorum form which had become anaerogenic. In the examination of the adult avian specimens, the maltose-dextrine-dulcite negative forms isolated from several dead hens indicate that Hadley is correct in his contention that *Bact. pullorum* may assume a dual rôle: *Bact. pullorum* A being maltose-dextrine-dulcite negative and aerogenic, infecting young chicks; and *Bact. pullorum* B being maltose-dextrine-dulcite negative and anaerogenic, infecting adult hens. Cultures from eggs have always been aerogenic. If knowledge of *Bact. sanguinarium* is based upon the anaerogenicity of cultures, the absence of this property in cultures isolated from adult hens, chicks and eggs sent from all parts of the State would appear to indicate that fowl typhoid is not widely distributed in Massachusetts.

3. From pathological and bacteriological examination of 83 birds suffering with the so-called "paralysis," the evidence at hand does not indicate that the disease, so widely distributed at certain periods of the year, is due to the presence of the pullorum or sanguinarium type of organism.

4. The agglutination test has become a popular means of recognition in the domestic fowl of those individuals which have contracted infections in chickhood, and consequently, as adult productive fowls, may have become, through infections in their ovaries, carriers of infection to the offspring. During this investigation hundreds of agglutination tests have been made, demonstrating that there is an interagglutinability of *Bact. pullorum* with *Bact. sanguinarium*, *B. typhosus*, *B. paratyphosus* A and *B. paratyphosus* B antigens, with a consequent tendency to make the test lose in terms of specificity. The fact remains, however, as a result of experiments in this department, that in twenty flocks studied, representing 5,619 breeding birds, the test located infection in 924. Furthermore, the differential characteristics of the cultures isolated from dead chicks which had been hatched from eggs laid by these positively reacting birds proved them to be typical *Bact. pullorum*, conforming morphologically and biochemically to the standard set for this organism. Therefore, from these data, the conclusion seems justified that the agglutination test, when carefully controlled through epidemiological work, is at present the best method we have for locating *Bact. pullorum* infection and furnishing to poultrymen a starting point for its elimination.

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AGRICULTURAL EXPERIMENT STATION

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THE INHERITANCE OF FERTILITY AND HATCHABILITY IN POULTRY

By F. A. HAYS and RUBY SANBORN

Determination of fact as to inheritance of characters is essential to successful poultry breeding. This work is peculiarly within the province of the Agricultural Experiment Station, for records must be made on large numbers of individual birds, the work must extend over a period of years, a wearisome amount of data must be preserved. The data recorded in this bulletin are the result of eleven years' work. Individual records were made on 886 birds. Resulting data are now analyzed statistically in the light of all that genetic science has to offer. It is through work such as this that a basis of sound fact, in poultry breeding work, will ultimately replace one based largely on opinion and tradition.

Requests for Bulletins should be addressed to the
AGRICULTURAL EXPERIMENT STATION
AMHERST, MASS.

Publication of this Document approved by the Commission on Administration and Finance

THE INHERITANCE OF FERTILITY AND HATCHABILITY IN POULTRY.

By F. A. Hays and Ruby Sanborn.

Introduction.

The importance of a thorough understanding of the mode of inheritance of factors affecting fertility of hens' eggs needs no stressing. Neither does the value of a complete understanding of the way hatching power of eggs is inherited require emphasis, for the proper functioning of the factors for high fertility and high hatchability is of fundamental and vital importance to every poultry breeder.

The purpose of this report is to consider only the question of the inheritance of fertility[1] and hatchability[2] from as many angles as our data will permit. The inheritance of these two characteristics is discussed first from the standpoint of the dams and then from the standpoint of the sires. The fact should be recognized at the outset that numerous variable environmental factors such as weather conditions, health of birds, exposure of eggs, variation within the same and different incubators, etc., are in constant operation. The combined action of these constantly varying environmental factors may largely obscure the inherent capacity of the bird to produce fertile eggs that are largely hatchable. A further lack of knowledge of the fundamental factors concerned in breeding for high fertility and high hatchability, as pointed out by Dunn ('23), makes proper matings impossible.

Data Available.[3]

The data used in this bulletin have been collected each hatching season from 1913 to 1923. All records kept represent the pullet year or cockerel year unless otherwise stated. All records were made by pedigreed Rhode Island Red birds. The attention of the reader is called to the fact that stud matings have been used almost exclusively and this will account for a lower degree of fertility than might be obtained from pen matings. Uniform methods of incubation have been used and care has been taken to maintain a definite system of management throughout the eleven-year period. Only females whose daughters were trap-nested are included in this report.

PART I.

The Female's Rôle in the Inheritance of Fertility and Hatchability.

Fortunately a measure of individual fertility and hatchability is possible in the female. The accuracy of such a measure depends very largely upon the number of eggs laid by the pullets in question during the hatching season. Some pullets will lay fifty eggs during a two months' incubation season, while others may lay as few as five or ten eggs. Fertility and hatchability records on the first type would certainly be much more significant than those on the second type. The major portion of the records here reported upon were made between the hatching dates of March 25 and May 15 of the respective years. In some cases chicks were hatched beyond the above dates, but not as a rule. Since the flock was being bred for egg production, considerable care was exercised to use pullet breeders that would lay a goodly number of eggs during the hatching season.

Section 1. Correlation between Fertility and Hatchability.

A hen to be able to produce a large number of chicks must lay highly fertile eggs. Furthermore, her eggs must hatch well. In ordinary usage, good hatching hens are those from which almost all eggs laid give rise to vigorous chicks. Fertility and hatchability are bound together in the sense that there can be no hatch-

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[1] *The term fertility as used here refers to the percentage of eggs that are fertile; the test being made on the fifth day of incubation.*

[2] *The term hatchability as used here refers to the percentage of fertile eggs hatched.*

[3] *The data used in this report were collected by Dr. H. D. Goodale until 1921; for the year 1922, by Professor William Sanctuary and the junior author.*

ability without fertility; but there may be one hundred per cent fertility and zero hatchability, or there may be only five per cent fertility and one hundred per cent hatchability.

The above facts show that the coefficient of correlation between fertility and hatchability could neither be zero nor negative. Pearl ('09) found a correlation of −.127±.071 between the percentage of infertile eggs and the percentage of fertile eggs hatched from pullets. Such a factor, in view of the large probable error, indicates no sensible correlation between the degree of fertility and the percentage of fertile eggs hatched.

In table 1 presented below, the percentage of fertile eggs from 758 pullets is correlated with the percentage of fertile eggs hatched. These percentages represent each pullet's average fertility record and her average hatching record for the season. The records were obtained in eleven breeding seasons. The table includes all pullets used as breeders during the period covered, except those showing zero fertility. The zero-fertility class had to be omitted because zero fertility always means zero hatchability, and if the fifty-three pullets that laid no fertile eggs were included, a spurious correlation would arise and not the true correlation coefficient.

Table 1.— *Correlation Between Fertility and Hatchability.*

Pullets' Fertility, Per Cent.	Pullets' Hatchability, Per Cent.																				f.
	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–100	
1–4																					
5–9	4																			2	6
10–14	2						1						1							1	5
15–19	2					1					1		1							1	6
20–24	5					1	2				1	1				1	1	1		1	14
25–29	1		1			2					1		2			2	1			2	12
30–34	3		1			1				1						1				3	10
35–39	1					1			1		1	1	5	1		1				2	14
40–44	3		1	1				1			2				4		1			1	14
45–49	3					1			2				1		2	3			2		14
50–54	3				1			1			1	2	2	1	1	4	1		1	5	23
55–59	1	1			1			1	1	1			1	1	1	2			1	1	13
60–64		1		1	2		2					1	3	2	2	6	1		2	1	24
65–69	6	1		1	2	2	2	1	1	2	2	1	7	1	3	4	3			1	40
70–74	5	1	1			3	1	2		1	2	3	4	3	2	3	6	2		2	41
75–79	4	1	3	1	1		2	1	1	3	1	4	3	1	3	5	1	3	2	2	42
80–84	1	2	2		4		2	1	2		7	2	6	6	3	4	4	4		3	53
85–89	5	1		3	3	1	3	7	4	7	3	5	7	9	9	4	2	2	1	1	77
90–94	10		3	1	2	3	4	4	3	2	3	3	11	8	5	8	9	8	4		91
95–100	28	5	4	10	5	7	10	9	9	7	13	12	20	22	15	22	17	18	20	6	259
f.	87	13	16	18	21	23	29	28	24	24	38	35	74	55	50	70	47	38	33	35	758

Constants calculated from Table 1.

Mean fertility	.688272±.005466
Fertility standard deviation	.2231±.003865
Mean hatchability	.637875±.007119
Hatchability standard deviation	.2906±.005034
Coefficient of correlation	.0672±.024390

Table 1 gives a positive correlation coefficient of .0672±.02439 which must be interpreted in the light of a probable error of more than one-third as signifying

almost complete independence between degree of fertility and hatchability.

From the genetic standpoint, the results in table 1 are significant. The table shows that a flock of pullets may carry the factors that are conducive to high fertility and yet lack the ability to be good hatchers. Stated simply, these results mean that the degree of fertility in a hen's eggs is an entity independent from the hatchability of her eggs.

The mean fertility shown in table 1 is .6883, while the mean hatchability is .6379. Of the total eggs laid by these pullets during the hatching season, 68.83 per cent were fertile, and 63.79 per cent of these fertile eggs hatched. Two possible avenues are open for increasing the number of chicks per pullet. *First*, Increase the percentage of total eggs that are fertile. *Second*, Increase the percentage of fertile eggs that hatch. Selection for high fertility and high hatchability is possible only where hens are used as breeders. Hens have been used to only a very minor extent in this flock. Hence there has not been much progress in fertility and only moderate progress in hatchability, as will be shown in section 17 of this bulletin. The general deduction must be made, therefore, from the study of table 1, that fertility and hatchability are independent of each other. The stability of each characteristic may next be considered.

Section 2. The Constancy of Fertility in Hens.

In order to test the constancy of fertility in hens, the records of 253 female breeders that were used first as pullets and again as yearlings have been placed in table 2. In practically all cases a different male was mated to these females the second year. If there is a sensible correlation in fertility between the pullet-year record and the yearling record from the same hens, the natural assumption must be that degree of fertility is more or less constant in the female, regardless of the male to which she is mated.

TABLE 2. — *Correlation Between First and Second-Year Fertility.*

Pullets' Fertility, Per Cent.	Yearling Hens' Fertility, Per Cent.																				f.
	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–100	
0–4	3													1						5	9
5–9									1										1		2
10–14							1			1										1	3
15–19		1			2																3
20–24																1		1	2	1	5
25–29	1													1						1	3
30–34														1							1
35–39				1				1				1						1		2	6
40–44	1																			2	3
45–49	1										1			1				1	1	2	7
50–54	1		1															3		2	7
55–59														1						2	3
60–64																	2		3	1	6
65–69	1			1		1								1	1		1	2	3	4	15
70–74	2							1			1			1				1	3	3	12
75–79																1	1	2	2	3	9
80–84	2			1	2										3	1	1	2	2	6	20
85–89	1							1	1		1	2	1		2	1	2	1	2	5	20
90–94			1		2				1				2	1	1		3	7	5	19	42
95–100	2		1				1			1		1		3			7	3	8	50	77
f.	15	1	3	3	6	1	2	3	3	2	3	4	3	11	7	4	17	24	32	109	253

Constants calculated from Table 2.

Pullets' mean fertility	.7589±.011288
Pullets' standard deviation	.2662±.007982
Yearling hens' mean fertility	.7825±.012111
Yearling hens' standard deviation	.2856±.008564
Coefficient of correlation	.2733±.039238

The mean fertility of the birds used in table 2 was slightly greater for the yearling than for the pullet-year. The difference, .0236±.016579, is not great enough to be significant. The range of variability measured by the standard deviation is slightly wider as yearlings than as pullets, but the closeness of agreement in the two years signifies a degree of fixedness. From the breeding standpoint, the chief deduction that may be made from a study of table 2 is that the percentage of fertility for a pullet is a good guide as to her probable fertility as a yearling.

A positive coefficient of correlation, .2733±.039238, between the first and second year fertility supports the view that fertility is a trait that is fairly constant for the individual hen. Lamson and Card ('20) have pointed out this fact in Leghorns. Pearl ('09) found a negative correlation of .1112±.092 between infertility the first year and the second year in Barred Plymouth Rocks. Our data, however, indicate that a bird with good fertility as a pullet will probably show good fertility as a yearling.

Section 3. The Constancy of Hatching Power in Hens.

The group of 253 birds studied in table 2 are correlated for hatchability in table 3 to discover if there is a relationship between the percentage of fertile eggs hatched as pullets and as yearlings. In other words, does hatchability approach any degree of constancy in the same individual in two successive years? Does a good hatching record as a pullet mean a good hatching record as a yearling?

TABLE 3. — *Correlation Between First and Second-Year Hatchability.*

Pullets' Hatchability, Per Cent.	Yearling Hens' Hatchability, Per Cent.																				f.
	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–100	
0–4	10				2		1					2	1	1	2				1		20
5–9	2				1							1				1					5
10–14	3						1				1										5
15–19	3				1		1		1			1									7
20–24	3																				3
25–29	2	2			1							1			1		1				8
30–34								1				1			1			1	1		5
35–39	3			1			2	1					1								8
40–44		2	1		1		1	1					2	2							10
45–49	1					3	1		1		2					1					9
50–54	5		2		2	1		1			2		1	2	1		1	1		1	20
55–59	1		2	1		1	3		2	3	1		2					1			17
60–64	1				1				3	3	2	1		1	2	1	2	1		2	20
65–69	2		1	1								1	3	3	2	2	1	2	1		19
70–74	2		1	1					1	1		1	2	1	1	5	1	1	3	1	22
75–79	4					1	1	1	1	1	3		3	1		3	2	2	1		24
80–84									1		2	2		2			1				8
85–89	1	1					1			1	1		3		2	4	1	2		1	18
90–94	1									1	2		1			1	3	4		1	14
95–100	2										1			4			2	1		1	11
f.	46	5	7	4	9	6	12	5	10	10	17	11	19	17	12	18	15	16	7	7	253

Constants calculated from Table 3.

Pullets' mean hatchability	.5678 ± .011313
Pullets' standard deviation	.2668 ± .008333
Yearling hens' mean hatchability	.4791 ± .012963
Yearling hens' standard deviation	.3057 ± .009166
Coefficient of correlation	.4346 ± .034409

The mean hatchability for pullets is .5678±.011313. The mean hatchability for the same birds as yearlings is .4791±.012963. There is a difference of .0887± .0172 in favor of using pullet breeders. This difference is significant in the light of its probable error. Stewart and Atwood's ('09) records with White Leghorns do not agree with these results. They found both the mean fertility and mean hatchability to be higher in hens than in pullets. Their records are scarcely comparable with ours because they did not compare the same birds. Furthermore, in a yearling or two-year-old flock, most of the poor hatchers will have been discarded if they were tested as pullets. Pearl ('09) obtained a slightly higher mean fertility in the pullet year and an insignificant difference in hatchability between pullets and yearlings, using the same flock of Barred Plymouth Rocks.

The range of variability measured by the standard deviation is significantly greater in the yearling hens. This difference may possibly be ascribed to variability in physical condition in the older birds. Hatchability, however, seems to be a trait that behaves with a good deal of constancy in hens. This fact makes the individual hatching record valuable, at least in making use of a hen for several years to increase flock numbers. The ability of the hen to transmit this hatching power to her daughters will be considered in section 5.

The coefficient of correlation calculated from table 3 is .4346±.034409. Hatchability is therefore more constant than fertility, for the coefficient for fertility in the same flock was only .2733. In breeding for high hatchability there is ample justification for discarding the poor hatchers the first year and retaining the good hatchers to perpetuate the flock.

Section 4. Correlation in Fertility between Mothers and Daughters.

In order to discover if there is any relationship between mothers and daughters in degree of fertility, the average fertility of pullet breeders has been correlated with each of their daughters that were used for breeding as pullets. In case only one daughter was used, there was but one insertion in the table. If a pullet dam had more than one daughter used as a breeder she is paired with each of these daughters and an insertion made in the table.

TABLE 4. — *Correlation in Fertility Between Mother and Daughter.*

Dams' Fertility, Per Cent.	Daughters' Fertility, Per Cent. 0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-100	f.
0-4																					
5-9																					
10-14																					
15-19									1									1			2
20-24																					
25-29	1																				1
30-34												1					2		1	1	5
35-39						1								1		1			1	1	5
40-44				1						1	1			1	2			2	1	4	13
45-49								1			2						2			3	8
50-54										1				1	1			2	1	2	8
55-59	1																1		3	4	9
60-64	2				1					1			1		1		2	1	1	7	17
65-69	3		2		2	1					2		1	2	1	1	2	3	3	8	31
70-74	2				1		1	1	1		1			2	1	5	2	3	4	13	37
75-79								1			1		1	4	2	2	3	2	3	5	24
80-84	5				3	1	1				2		1	1	4	2	1	2	7	17	47
85-89	5	1		1			2	2	3	1	1	2	2	1	2	4	3	7	10	17	64
90-94	12	1	1	1	1	3	1	1	3	4	5		3	7	6	4	5	10	11	45	124
95-100	23	4	2	4	6	5	5	7	6	6	8	9	15	20	21	23	31	44	45	132	416
f.	54	6	5	7	14	11	10	13	14	14	23	12	24	40	41	42	54	77	91	259	811

Constants calculated from Table 4.

Dams' mean fertility	.8765±.003503
Dams' standard deviation	.1479±.002477
Daughters' mean fertility	.7378±.006831
Daughters' standard deviation	.2884±.004830
Coefficient of correlation	.0147±.023679

The standard deviation in dams in fertility is .1479, while the standard deviation of their daughters is twice as great or .2884. There is a positive correlation coefficient in fertility of .0147±.023679 between the dams and the 811 daughters that were used as breeders. Since this coefficient is less than its probable error, it can have no significance. This table must therefore indicate that a pullet with low fertility is as likely to give daughters high in fertility as is a breeding pullet that shows high fertility herself. These observations are essentially in agreement with Pearl ('09), for he found a negative correlation of .035±.072 in infertility between mother and daughter. The conclusion seems justified, therefore, that the fertility of the dam's eggs is no indication as to the probable fertility of her daughter's eggs. In section 2, the fertility record of a pullet was shown to be a guide as to her second-year fertility. Since the dam's fertility record is not a dependable index of her ability to breed true for fertility, the only satisfactory test is the progeny test, for fertility seems to depend upon many as yet unrecognized factors, or else is not an inherited characteristic.

Section 5. Correlation in Hatchability between Mothers and Daughters.

The identical group of dams and daughters used in table 4 has again been correlated in table 5, using percentage of fertile eggs hatched.

TABLE 5. — *Correlation in Hatchability Between Mother and Daughter.*

Dams' Hatchability, Per Cent.	Daughters' Hatchability, Per Cent.																				f.
	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–100	
0–4																					
5–9							1														1
10–14													1								1
15–19	1																				1
20–24	1										1										2
25–29	3											2									5
30–34	1	1		1			1			1		2	1				1				9
35–39	4					2	1						5	3	1		2	1			19
40–44	6			2	2				3	3	1	1		1			1	1		3	24
45–49	7	3	2	1	2	1		3			1	2	3		2						27
50–54	7	2	1		1	1	1		3	1	1	1	3	1	1	7	1	1	1	2	36
55–59	10	1	3	1	4	4	3	2	4	1	2	3	9	3	5	5	2	1	3	1	67
60–64	13				3	3	4	4	3	6	3	2	8	4	7	9	4	3	3	5	84
65–69	18		1	2	4	2		2	4	2	5	4	11	6	4	7	5	2	4	3	86
70–74	8	2	1	2	2	2	4	3	1	1	9	3	6	2	3	8	5	4	3	1	70
75–79	28		4	4	3		4	3	2	4	6	2	7	12	5	8	6	9	1	7	115
80–84	11			2		1	6	3	2	2	3	4	8	5	6	6	4	10	5	4	82
85–89	11	3	3	1	1	3	2	2		1	1	4	3	8	6	9	6	6	4	5	79
90–94	6	2	1	1		2	1	3	1	2	3	2	7	6	7	7	5		6	5	67
95–100	2					2		2			2	2	3	2	3	4	6	2	3	3	36
f.	137	14	16	17	22	23	28	27	23	24	38	34	75	53	50	70	48	40	33	39	811

Constants calculated from Table 5.

Dams' mean hatchability	.7064± .003891
Dams' standard deviation	.1643± .002752
Daughters' mean hatchability	.5091± .007340
Daughters' standard deviation	.3099± .005190
Coefficient of correlation	.1960± .022805

Table 5 undoubtedly shows that hatching power is transmitted from mother to daughter, yet while the dam's mean hatchability is .7064, her daughter's mean was only .5091. The standard deviation of dams was .1643 and their daughters' standard deviation was .3099. Thus the range of variation in daughters as measured by the magnitude of their standard deviation is almost double that of their dams. Such would be the case if a dominant factor is present for high hatchability. This relative variability is in exact agreement with the same observation on fertility as pointed out in section 4.

There is a positive correlation coefficient of .1960±.022805 between dams and daughters in hatchability. During the progress of the experiment, the pullet breeders used on successive years came from pullet mothers that showed a good hatching percentage. In other words, the pullets that were used as breeders in any one year came from pullet dams that had laid eggs of good hatching power. According to Pearson ('03) rigid selection in parents may reduce the correlation between parent and offspring for the character in question. Since we have no fertility and hatchability records for the flock as a whole, it is impossible to mathematically measure the effect of such selection on our flock.

Pearl ('09) reports a correlation coefficient of only .031±.072 between mothers and daughters in hatchability, but only 87 individuals were studied. Dunn ('23) states that he was unable to separate high and low hatching lines by two generations of selection. He did find, however, that families tend to become different in hatching power and to retain this difference.

Table 5 clearly indicates that hatching power is transmitted from mother to daughter, even though rigid control of the many environmental factors that modify the hatching power is very difficult. These varying conditions often obscure the true hatching ability of the pullet as an individual. The use of breeding females of high hatching power is the first step toward improving the flock in this particular characteristic. We have shown in section 3 that the hatching power of a pullet is sensibly correlated with her later hatching power. Follow this by using breeding hens that transmit high hatchability to all of their daughters. The male's part in heredity of hatchability will next be considered.

PART II.

The Male's Rôle in Inheritance of Fertility and Hatchability.

Section 6. The Constancy of Fertility in Males.

In studying the question of the inheritance of fertility and hatchability, much importance should be attached to the male side of the flock, for the male is more than half the flock from a genetic standpoint because each male furnishes half the inheritance to the progeny of several hens.

The measure of the male's fertilizing ability is the mean degree of fertility from his different matings. The accuracy of such a measure will of course depend upon whether or not high fertility is governed in inheritance by dominant or recessive factors, or whether it is independent of Mendelian factors. If high fertility depends upon recessive factors, we should expect less variation in the daughters from a hen that carries these factors pure, so that she herself is genetically highly fertile, than would be the case if high fertility is dependent on dominant factors and these were not in homozygous condition. The fact that manifestation of fertility in the eggs is probably dependent on both male and female makes the classification of either males or females with regard to this characteristic a hazardous undertaking. A careful analysis of the results from mating specific males to a number of females in successive years with conditions kept uniform would help much to explain this confusing problem.

The problem of the constancy of a male's ability to transmit a certain degree of fertility to his daughters may be elucidated by correlating the fertility of his daughters sired during his first breeding year with that of his daughters sired during the second breeding year, using pullet records in all cases. In other words, if males transmit a certain degree of fertility to their daughters in successive years, a positive correlation will exist. Such a tabulation is made from data available in table 6. Unfortunately, records on only 51 pairs of daughters are obtainable for study. The number is small because few males are used as breeders after their cockerel year.

TABLE 6. — *Correlation in Fertility between Males' First and Second-Year Daughters.*

Fertility of Males' First Year Daughters, Per Cent.	Fertility of Males' Second Year Daughters, Per Cent.																				f.
	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–100	
0–4	2											1					1		1		5
5–9											1									1	2
10–14																					
15–19																			1		1
20–24																	1		1		2
25–29																		1			1
30–34																					
35–39																					
40–44															1					1	2
45–49																				1	1
50–54																					
55–59																				1	1
60–64			1																		1
65–69															1	1					2
70–74																	1			2	3
75–79								1		1			1							2	5
80–84											1					1				4	6
85–89																				2	2
90–94											1							1	1	1	4
95–100					1			1			1			1	1				3	5	13
f.	2	0	1	0	1	0	0	2	0	1	4	1	1	1	3	2	3	2	7	20	51

Constants calculated from Table 6.

First-year daughters' mean fertility	.6651±.031064
First-year daughters' standard deviation	.3289±.021966
Second-year daughters' mean fertility	.7700±.025001
Second-year daughters' standard deviation	.2647±.017678
Coefficient of correlation	.2151±.090076

In table 6 the mean fertility of the first-year daughters was .6651 while the mean for the second-year daughters was .77. There is a difference of .1049±.0399, which, judged by the magnitude of its probable error, is of doubtful significance. There is also no sensible difference in the standard deviation of first-and second-year daughters. A sensible degree of correlation between first-and second-year daughters is questionable because $r = .2151 \pm .090076$. The probable error is almost half as great as the coefficient itself. The only logical interpretation that can be placed on the limited data in table 6 is that mean fertility in the daughters of the same group of males in successive years is strikingly constant, and in the second place that a positive correlation coefficient of questionable magnitude exists between first-and second-year daughters in fertility. More data of a similar nature are required to clear up this question.

Section 7. The Constancy of Hatchability in Males.

The male's ability to transmit fertility is still questionable, as has been pointed out in section 6. In the present section the subject of the constancy of hatchability in the male, as measured through his daughters, will be considered. The same difficulties are encountered in studying this question that have already been men-

tioned for fertility. Possibly environmental factors are of less importance in hatchability than in fertility. Pearl ('09) believes that hatching quality is more of an innate constitutional character than is fertility. If hatching quality is dependent upon Mendelian factors in inheritance, the degree of correlation between hatchability of the eggs of first-year daughters and the eggs of second-year daughters would vary with the number of factors concerned, and with the degree of homozygosity in the males for these factors. Should there be a sensible positive correlation, it would indicate that the male as well as the female transmits hatching power to the offspring.

In table 7, the group of 51 pairs of daughters studied in section 6 is tabulated for hatchability.

TABLE 7.— *Correlation in Hatchability between Males' First and Second-Year Daughters.*

Hatchability of Males' First Year Daughters, Per Cent.	Hatchability of Males' Second Year Daughters, Per Cent.																				f.
	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-100	
0-4	6		1	2	1			1				2	2		3			1	1		20
5-9																					
10-14		1																			1
15-19							1														1
20-24	1																				1
25-29	1														1		1				3
30-34	1						1				1		1		1						5
35-39									1												1
40-44									1		1										2
45-49	1																	1			2
50-54	1														1		1	1			4
55-59	2																		1		3
60-64												1				1					2
65-69																1	1				2
70-74																					
75-79															1					1	2
80-84																					
85-89					1										1						2
90-94																					
95-100																					
f.	13	1	1	2	2	0	2	1	2	0	2	3	3	0	8	2	3	3	2	1	51

Constants calculated from Table 7.

First-year daughters' mean hatchability	.2965± .025445
First-year daughters' standard deviation	.2694± .017992
Second-year daughters' mean hatchability	.4484± .031130
Second-year daughters' standard deviation	.3296± .022013
Coefficient of correlation	.2996± .085972

Referring to table 7, the mean hatchability of first-year daughters is.2965, while the second-year daughters of the same male have a mean of .4484. The difference is .1519±.0336, which is a significant difference. The second-year daughters appear to be superior to the first-year daughters in hatching power. To draw any conclusion, however, on such meager data would be more than hazardous. The standard deviation does not differ significantly in the two groups of daughters.

A sensible positive correlation of .2996±.085972 appears between first-year pullet daughters and second-year pullet daughters in hatchability. Table 7 thus furnishes a very small amount of evidence that hatching power is transmitted through the male, and that it is a more constant character than would be possible were it independent of heredity.

Section 8. Relation between the Fertility of the Sire's Dam and His Phenotypical Fertilizing Ability.

As there is no direct measure of a sire's phenotypical fertilizing power, it is necessary to resort to the indirect, which is the average fertility of his mates. The degree of fertility in the sire's dam may be something of a guide to his inheritance. The pertinent question at this point is: Is the degree of fertility of a cockerel's mother a guide to his ability to fertilize the eggs of his mates? If such be the case, there should be a sensible positive correlation between sire's dam's fertility and his mates' fertility. In table 8 the dams of cockerels used throughout the eleven-year period have been tabulated with the mates of these cockerels. The record of any particular dam was used against each of the mates of her son. The total number of mates was 647.

TABLE 8. — *Correlation in Fertility Between Sires' Dams and Sires' Mates.*

Sires' Dams' Fertility, Per Cent.	Sires' Mates' Fertility, Per Cent.																				f.
	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-100	
0-4																					
5-9																		1	2	1	4
10-14																					
15-19																					
20-24				1											1	1		3	3	2	11
25-29																					
30-34																					
35-39												1	1		1			1		9	13
40-44																					
45-49																					
50-54																					
55-59															1	1	1	2	1	12	18
60-64							1	2		1	1	1	2	1	3		7	5	4	15	43
65-69						1			1	2		1	1	7	4	4	4	2	7	15	49
70-74						1	1		1					2	2	2	3	4	14	21	51
75-79					1					1				2		1		2	2	6	15
80-84			2		2		3	3	2	1	5		5	5	10	6	9	11	23	48	135
85-89									1						1	1		2	3	18	26
90-94						1		2	1	1		4	2	1	3	2	5	10	15	46	93
95-100				1			1	2	3	3	1	1	6	5	4	9	14	14	26	99	189
f.			2	2	3	3	6	9	9	9	7	8	17	23	30	27	43	57	100	292	647

Constants calculated from Table 8.

Sires' dams' mean fertility	.8157±.004492
Sires' dams' standard deviation	.1694±.003176
Sires' mates' mean fertility	.8531±.004587
Sires' mates' standard deviation	.1730±.003244
Coefficient of correlation	−.1890±.025363

The mean fertility of the sires' mates is .0374±.00642 greater than the mean of the sires' dams. This is a small but significant difference and indicates that more attention was given to fertility from the female standpoint than from the male standpoint. The standard deviation is almost identical for both groups of females. A negative coefficient of correlation of .1890±.025363 appears rather difficult to explain. It certainly does indicate that the degree of fertility shown by sire's mother is not an index to the degree of fertility that such a sire will exhibit in his mates — his phenotypical fertilizing ability. This negative correlation may be due to selection of females to be used as breeders with more regard to high fertility in ancestry than is practised in selecting male breeders; or possibly males from the very fertile ancestry were mated to pullets that were lacking in fertility but otherwise desirable.

Section 9. Relation between the Hatchability of the Sire's Dam and His Phenotypical Hatching Ability.

The question of hatchability may be considered by the same methods used in section 8 in studying fertility. The identical group of birds is again tabulated for hatchability in table 9.

TABLE 9. — *Correlation in Hatchability between Sires' Dams and Sires' Mates.*

Sires' Dams' Hatchability, Per Cent.	Sires' Mates' Hatchability, Per Cent.																				f.
	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-100	
0-4													1	1					1	1	4
5-9																					
10-14																					
15-19					1							1			2	1					5
20-24																					
25-29									1		1		1	2	1	2		2			10
30-34		1			1		1		4	3	4	2	4	3	1	1		1		1	27
35-39										1	1		1				1		1		5
40-44									1	4	1	1	2	1		2	2				14
45-49				1	1	1	3	3	2	3	2	4	2	4	3	2	1	3			35
50-54						2	1		4	1	3	4	4	4	4	2	2	1	2		34
55-59						1	3	3	1	1	5	2	7	5		3	3	3	2	2	41
60-64				1		1		2	3	4	6	3	8	4	5	10	5	2	3	2	59
65-69				1		2	2	1	2	2	2	1	2	2	7	6	3			2	35
70-74	1	1	1		1	2	4	6	4	3	4	7	6	7	8	9	7	6	2	8	87
75-79				1	2	1	2	1	1	3	4	2	10	9	7	11	3	4	2	3	66
80-84		2	1		2		1	1	1	1	8	7	5	6	6	6	7	10	9	1	74
85-89				1	1	2	1	3		3	3	3	5	7	7	6	2	3	1	1	49
90-94				1			1		1				3	2	6	8	4	5	5	1	37
95-100				1		2	2		2	1	2	1	6	10	4	8	8	9	6	3	65
f.	1	4	2	7	9	14	21	20	27	30	46	38	67	67	61	77	48	49	34	25	647

Constants calculated from Table 9.

Sires' dams' mean hatchability	.6977±.005115
Sires' dams' standard deviation	.1929±.003617
Sires' mates' mean hatchability	.6488±.005229
Sires' mates' standard deviation	.1972±.003698
Coefficient of correlation	.1579±.025856

The average hatching ability of the sires' dams is .6977±.005115, while that of the sires' mates is .6488±.005229. There is a difference of .0489±.007314, which means that the males used as breeders came from dams of higher hatching power than was inherent in the pullets to which they were mated. The almost identical standard deviation for the two groups points to a similar variability in hatching power for the two.

The coefficient of correlation between the sires' mothers and their mates is .1579 ±.025856, a small but sensible correlation. Possibly this can be interpreted as meaning that males tend to show a phenotypical hatching power comparable with that of their dams. In selecting cockerels for breeders, hatching power of their dams is something of a guide to their ability to contribute hatching power to the eggs they fertilize. There is considerable probability that the male does influence the hatching power of his mates' eggs.

Section 10. Relation of Sire's Average to his Daughters' Individual Fertility.

In considering the fertilizing and hatching power of males, it is necessary to use some measure of their phenotypical character. This fact has been pointed out by Pearl ('09) and, as he states, the average fertility and hatching power of hens mated to a male may be used as his index. In table 10 the average fertility of each sire from his different mates is tabulated against the fertility of each of his daughters. This average figure for each sire is thus inserted a number of times to correspond with the number of his daughters that were used as breeders.

TABLE 10. — *Correlation in Fertility Between Sires' Mates and Sires' Daughters.*

Sires' Mates' Fertility, Per Cent.	Sires' Daughters' Fertility, Per Cent.																				f.
	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–100	
0–4																					
5–9																					
10–14																					
15–19																					
20–24																					
25–29																					
30–34																					
35–39																					
40–44																					
45–49																		1			1
50–54																					
55–59											1	1		2	1		2		2	4	13
60–64																			1		1
65–69								1							1		2	4	6	3	17
70–74								1	1		1		1	1			1	1	1	11	19
75–79					1		1	1		1			1		2	4	1	5	2	14	33
80–84				1							1	1	2	3	4	3	5	4	11	11	46
85–89						1				2	1	1			5	2	8	4	8	22	54
90–94						1		1	3	1	1		5		4	3	3	10	12	34	78
95–100				1	1	1		2	2	1	1	2	4	13	8	5	13	17	21	58	150
f.				2	2	3	1	6	6	5	6	5	13	19	25	17	35	46	64	157	412

Constants calculated from Table 10.

Sires' fertility mean	.8761 ± .003522
Sires' fertility standard deviation	.1060 ± .002491
Sires' daughters' mean fertility	.8416 ± .005599
Sires' daughters' standard deviation	.1685 ± .003959
Coefficient of correlation	.0244 ± .033211

A difference, amounting to .0345±.006614, will be observed between the sires' mean fertility and their daughters' mean fertility. This significant difference is easily explained if the same factors are operating to affect fertility of males and females. A wider range of variability in the daughters as compared with their sires, measured by the standard deviation, seems to indicate that there is little or no constancy in fertility between father and daughter.

No sensible correlation in fertility exists between sire and daughters as table 10 shows. In the face of this fact, there is no evidence that factors for fertility are transmitted from sire to daughter. In other words, fertility does not seem to be an inherited trait that is transmitted from parent to offspring, as has already been shown in both tables 4 and 10.

Section 11. Relation of Sire's Average to Daughters' Individual Hatchability.

The same group of birds used in table 10 is correlated in table 11 to study the relationship between sire and daughters in hatching power.

TABLE 11.—*Correlation in Hatchability between Sires' Mates and Sires' Daughters.*

Sires' Mates' Hatchability, Per Cent.	Sires' Daughters' Hatchability, Per Cent.																				f.
	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–100	
0–4																					
5–9																					
10–14																					
15–19																					
20–24																					
25–29									1		2										3
30–34																					
35–39																					
40–44								1		1		1				2					5
45–49			1							2	3	1				2	1	2			12
50–54								4	4	1	2	5	4	8	2	4	1	2	2	2	41
55–59							1	1	1	1		6	10	1	5	3	3	4	3	1	40
60–64				2	3	1	3	2	1	4	5	1	6	6	8	6	4	5	2	3	62
65–69				2		2	1	3	2	2	2	3	5	2	5	9	4	3	1	2	48
70–74					1	2	3	1	2	2	5	5	10	10	8	10	3	8	8	4	82
75–79						1	1	2	1	2			2	1	2	5	4	2	3		26
80–84	1						1				6	1	7	10	4	11	8	4	4	3	60
85–89														1	2	2	3	2	4	1	15
90–94										1			2		5	3	2	2	2	1	18
95–100																					
f.	1		1	4	4	6	10	14	12	16	25	23	46	39	41	57	33	34	29	17	412

Constants calculated from Table 11.

Sires' hatchability mean	.6824± .004084
Sires' hatchability standard deviation	.1229± .002888
Sires' daughters' mean hatchability	.6753± .006217
Sires' daughters' standard deviation	.1868± .004396
Coefficient of correlation	.2268± .031523

The mean hatchability of the sires is almost identical with that of the daughters. This is in striking contrast to the mean of dams and daughters given in table 5 where the figures are .7064±.003891 and .5091±.003740, respectively. Such evidence might be interpreted as showing that a closer relationship exists between sires and daughters than between dams and daughters in hatching power. Such a relationship is probably due entirely to the somewhat dissimilar methods for measuring hatching power in sire and dam. The range of variability is greater in daughters than in sires evidently because of the variable nature of the males mated to these daughters.

The coefficient of correlation between sires and daughters is .2268±.031523. Comparing this factor with the factor calculated from table 5 where mothers and daughters are concerned, the two are found to be of almost identical magnitude when their probable errors are considered. Table 11 furnishes convincing evidence of the heritability of hatching power. In this instance, hatching power of sires is carried on in their daughters. Table 11 further points to the necessity of using tested males in developing a flock carrying uniformly high hatching power.

Section 12. Relation of Sire's Dam to his Daughters' Fertility.

In section 8 the relation between sire's dam and his phenotypical fertilizing ability has been considered. A negative relationship was found to exist in that case. The present section is an attempt to discover if the sire transmits to his daughters a degree of fertility similar to that of his dam, so that when these daughters are mated with other males their probable fertility may be forecasted. In table 12, 748 pullet fertility records are tabulated with the fertility records of their sire's mother as a pullet.

TABLE 12.—*Correlation in Fertility between Sires' Dams and Sires' Daughters.*

Sires' Dams' Fertility, Per Cent.	Sires' Daughters' Fertility, Per Cent. 0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–100	f.
0–4																					
5–9															1		1	1	1		4
10–14																					
15–19																					
20–24	1										1		1	1			1	2	1	3	11
25–29																					
30–34																					
35–39													1			2			1	3	7
40–44																					
45–49																					
50–54																					
55–59	1										2				1	1	1	2	4	5	17
60–64	2	1			4	1	1	1	1		1		2	5	4	4	2	1	3	17	50
65–69	1			1	2	3		2		2	2			1	4	4	6	5	6	28	67
70–74	5				1	2		4	2	2	2	2	1	7	4	2	7	3	7	21	72
75–79		1					1				1					1	1	3	1	9	18
80–84	15	2	1	2	1			3	1	1	3	2	4	6	6	9	5	9	27	52	149
85–89	1				1				1	1		1	1	1	1	3	3	4	4	7	29
90–94	14	1	1			1	3		2	3	6	3	4	5	7	5	10	16	14	29	124
95–100	12		1	3	5	1	3	4	6	5	4	4	9	7	12	8	13	27	19	57	200
f.	52	5	3	6	14	8	8	14	13	14	22	12	23	33	40	39	50	73	88	231	748

Constants calculated from Table 12.

Sires' Dams' Mean Fertility	.8183 ± .003909
Sires' Dams' Standard Deviation	.1585 ± .002764
Sires' Daughters' Mean Fertility	.7364 ± .007108
Sires' Daughters' Standard Deviation	.2882 ± .005026
Coefficient of Correlation	−.0501 ± .024599

The mean fertility of the dams of the males used in this study is .0819±.008112 greater than the mean for the daughters of these males. The males used, therefore, came from dams of high fertility but the daughters of these males failed to measure up to such a standard. The standard deviation of the daughters is almost twice as great as for the sires' dams, showing that the daughters are a highly variable lot. The coefficient of correlation is negative but insignificant because of the magnitude of its probable error. The conclusion seems justified, therefore, that the degree of fertility of a sire's dam is no index to the degree of fertility that his daughters will exhibit.

Section 13. Relation of Sire's Dam to his Daughters' Hatchability.

If the hatching power of a sire's dam is something of an index to his probable inheritance of factors affecting hatchability, such relationship will appear when the hatchability records of the daughters are tabulated with the records from the sires' dams. Table 13 is thus made up of the same birds used in table 12.

TABLE 13.—*Correlation in Hatchability between Sires' Dams and Sires' Daughters.*

Sires' Dams' Hatchability, Per Cent.	Sires' Daughters' Hatchability, Per Cent.																				f.
	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–100	
0–4																					
5–9																					
10–14																					
15–19				1			1				1		2			1				1	7
20–24																					
25–29	2							1	1	1		1	1	1		1		2			11
30–34	3	3	1		2	2	2	2	1		2	1	4	1	1	3	1		1	1	31
35–39		1					1	1		1			1								5
40–44	6	1	1		2		1	1	2	2	1	2	2	3		3	2				29
45–49	16	2	4	2	2	1		1	1	2	2	4			1	1		3		1	43
50–54	2			1	2	1	3		4		2	1	3	1	1	1	2	2	2	2	30
55–59	6			1	2		1	2	1	1	3	1	1	8	2	3	2	2	3	3	42
60–64	12			1	1	3	1	1		3	3	3	10	3	6	11	6	5		3	72
65–69	1						1						1		2	1	4				10
70–74	13	2	1	1	4	3	5	6	7	2	4	8	10	12	7	11	4	12	8	2	122
75–79	12		1	3	2	3	1	3		1	4	3	6	6	7	9	8	2	4	3	78
80–84	18	2	2	2		2	5	5	3	5	3	5	14	8	9	7	7	3	6	9	115
85–89	7		1		2	1	1				3		4	4	2	1	1			2	29
90–94	2					3	1	3	2	1	1	1	2	3	2	4	4	4	1	4	38
95–100	26	1	1	4		2	4	1	1	4	3	5	5	2	5	9	3	1	7	2	86
f.	126	12	12	16	19	21	28	27	23	23	32	35	66	52	45	66	44	36	32	33	748

Constants calculated from Table 13.

Sires' Dams' Mean Hatchability	.7019±.004664
Sires' Dams' Standard Deviation	.1891±.003298
Sires' Daughters' Mean Hatchability	.5096±.007588
Sires' Daughters' Standard Deviation	.3077±.005366
Coefficient of Correlation	.0588±.024576

The mean hatching power of the hens whose sons were used for breeding was .7019. The daughters of this group of males averaged only .5096 of fertile eggs hatched. This difference in the means amounts to .1923±.008906 and is a much more striking difference than was observed between the same group of females in fertility. The standard deviation of the two groups agrees with that found for fertility in table 12. Again the daughters of the males show almost double the range in variability of their sires' dams.

The coefficient of correlation is here positive, but of no significance since it is a little more than twice its probable error. The lack of correlation between sire's dam and sire's daughters in hatchability can scarcely be interpreted to show that hatchability is not governed by factors transmitted from sire to daughter. The hatching power of a cockerel's dam is only the phenotypical manifestation of her ability and may be affected by her mate as well as by numerous environmental factors. She furnishes, moreover, but a part of the heritage of her son. If several factors governing hatchability are transmitted equally by males and females and if both parents have an influence on the hatching power of eggs laid and fertilized, respectively, this apparent independence of hatching power in inheritance will be explained.

If fertility be governed by genes transmitted in Mendelian fashion and without sex-linkage, this fact should be brought out by correlating the sire's record with his son's record. The only measure is the fertility record of the eggs laid by females mated to such males. If it were possible to compare males by a system of mating to the same group of females, the variable factors could be reduced to the male side alone. Such a system seems impossible to attain because of numerous factors too well understood to require mention.

Section 14. Relation of Sire and Son in Fertility.

TABLE 14.—*Correlation in Fertility between Sires and Sons.*

Sires' Fertility, Per Cent.	Sons' Fertility, Per Cent.																				f.
	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–100	
0–4																					
5–9																					
10–14																					
15–19																					
20–24																					
25–29																					
30–34																					
35–39														1		2					3
40–44																		1			1
45–49					1															2	3
50–54																					
55–59																	1			1	2
60–64																				1	1
65–69										1				1	1					3	6
70–74								1								1	4	1	3	4	14
75–79																		1	2	4	7
80–84						1					1		1		1		3	3	3	6	19
85–89								1							2	1	2	2	2	5	15
90–94								1	1				1	2	2	1	2	3	3	9	25
95–100								1	3	2		1	2	3	3	3	6	6	12	32	74
f.					1	1		4	4	3	1	1	4	7	9	8	18	17	25	67	170

Constants calculated from Table 14.

Sires' Mean Fertility	.8682 ± .007041
Sires' Standard Deviation	.1361 ± .004979
Sons' Mean Fertility	.8441 ± .008660
Sons' Standard Deviation	.1674 ± .006124
Coefficient of Correlation	.0685 ± .051486

In table 14 each pullet mate of a sire is paired with a pullet mate of his son. The number of pairs concerned is 170 and the number of sires included is about the same as the number of sons included. The mean fertility of the sires and their sons is not significantly different, and the range in variability of sires and sons, as measured by the standard deviation, is about the same. The coefficient of correlation is very small and its probable error renders it negligible. The only conclusion that may be drawn from this small amount of data is that either the fertility record of a male's mates is not a reliable index to his inherent fertilizing ability, or else degree of fertility is not transmitted from sire to son.

In the next section the relation of hatchability of sire and son will be considered for the same birds that were used in studying fertility.

Section 15. Relation of Sire and Son in Hatchability.

TABLE 15. — *Correlation in Hatchability Between Sires and Sons.*

Sires' Hatchability, Per Cent.	Sons' Hatchability, Per Cent.																				f.
	0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–100	
0–4																					
5–9																					
10–14																					
15–19											1										1
20–24																1					1
25–29							1		1							1	1			1	5
30–34												1	1								2
35–39					1		1	1			1		1	1			1				7
40–44					1					3	1			1	2	1		1			10
45–49							2				1	2	1	2	1	1					10
50–54								1			1	1	2	1	1			1	1		9
55–59							1	1	1		1	1	4	4	1					2	16
60–64										1	3		1	1	3	2	1		1	1	14
65–69					1								1	1	2	4	1	2	1		13
70–74									2	1	1		1	1	1	1		1		1	10
75–79				1						1		1	3	3		4	1	2	1	1	18
80–84						1		1		1	1	2	3		1		1	1		1	13
85–89									1	2	2		3	1	3	1		1			14
90–94				1				1			1			2	2	3	1	1			12
95–100				2		1		1			1		1	1	2	2	1	1	1	1	15
f.				4	3	2	5	6	5	9	15	8	22	19	19	21	8	11	5	8	170

Constants calculated from Table 15.

Sires' Mean Hatchability	.6738 ± .010274
Sires' Standard Deviation	.1986 ± .007265
Sons' Mean Hatchability	.6418 ± .009720
Sons' Standard Deviation	.1879 ± .006873
Coefficient of Correlation	.0755 ± .051440

Reference to table 15 shows that the mean degree of hatchability is almost the same in sires and sons. The two groups are also closely similar in standard deviation. There is no sensible correlation between father and son in hatchability. The degree of correlation here is practically the same as that observed for fertility in table 14. If we are using the correct measure for a male's hatchability, there is no evidence in these data to show that hatching power is transmitted from sire to son.

Section 16. Mendelian Interpretation of the Inheritance of Fertility and Hatchability.

Before entering upon a discussion of the possibilities of Mendelian inheritance of factors governing fertility and hatchability, it would seem desirable to present the mean records in the flock from year to year. These means are given below in table 16 along with the number of birds tested each year.

TABLE 16. — *Mean Fertility and Hatchability Records from the Massachusetts Agricultural Experiment Station Flock.*

YEAR.	Average Fertility.	Average Hatchability.	Number of Birds.
1913	.7562±.016855	.5910±.016578	73
1914	.8300±.015294	.5793±.016514	67
1915	.8308±.012692	.5613±.013015	118
1916	.8834±.010973	.6469±.015942	62
1917	.9158±.009776	.6217±.014709	78
1918	.8821±.009917	.6502±.013599	89
1919	.8882±.014611	.6941±.014602	56
1920	.8647±.014243	.6861±.017473	51
1921	.9107±.012241	.7483±.014129	59
1922	.8746±.010910	.7449±.011125	89
1923	.7749±.011944	.7051±.011399	144

The fertility mean has fluctuated appreciably from year to year and has not increased during the past six years. The low fertility of 1923 can be attributed to no other cause than adverse weather conditions throughout the winter and spring months. The majority of the males seem to have suffered from more or less frosting of combs and wattles during the winter of 1922–23. The basis of selecting breeding males for 1923 was not voluntarily changed from that of previous years. The general deduction must therefore be made, as Pearl ('09) has done, that fertility is dependent largely upon environmental factors and that it is not an inherent characteristic that is transmitted in inheritance.

Table 16 indicates an increase of .1141±.0206 in mean hatchability from 1913 to 1923. This increase is mathematically significant. There has been a gradual upward trend in mean hatching power since 1915. This increase has accompanied the use of breeding pullets and breeding cockerels from mothers showing good hatching power. The .04 drop in hatchability in 1923 is within the range of probability and need not be considered.

RELATION OF MALE TO THE HATCHING POWER OF HIS MATES' EGGS.

Unmistakable evidence is available to show that the male contributes to the hatching power of his mates' eggs. For want of any more suitable term we have used "male's phenotypical hatching power" to express the male's part. In table 9 a positive correlation coefficient of .1579±.025856 was observed between the sire's dam, and his phenotypical hatching power. A sensible correlation could not exist unless the male contributes to the hatching power of his mates' eggs.

The most conclusive evidence that the male influences the hatching power of his mates' eggs lies in the fact that the same hen shows different hatching power when mated to different males in successive years or even in the same year. Such data should be placed beside data showing the degree of constancy of hens in hatchability when mated to the same male on successive years. No data are available on the last-named question from our flock, although table 3 brings out a degree of correlation between first and second year hatchability in hens, amounting to .4346±.034409. The correlation should be much greater if the male did not play a part. In section 5 a sensible correlation between mothers and daughters was discovered. Reference to the constants calculated from table 5 shows that the hatching power of a hen is an uncertain guide to the probable hatching power of her daughters. The relative magnitude of the standard deviation of dams and daughters indicates that the phenotypical hatching power of a hen is an uncertain index of her true genetic constitution. This fact would seem to indicate that the male obscures the true genotype of the hen.

Data from the flock of the Massachusetts Agricultural Experiment Station on the constancy of hatching power in males is very limited. In table 17 a comparison is made between the first-year hatching power and second-year hatching power of

15 males. The figure used for each male represents the mean for all of his mates. These males were used on the following years: — 2 in 1913 and 1914, 4 in 1914 and 1915, 2 in 1915 and 1916, 2 in 1916 and 1917, 1 in 1917 and 1918, 2 in 1919 and 1920, 2 in 1922 and 1923.

TABLE 17. — *Mean Hatchability of Males.*

Male No.	First Year.	Second Year.
A323	57.00	55.80
A324	59.19	57.93
68	38.67	52.17
228	59.50	67.75
619	59.00	49.75
A271	70.71	67.40
A274	50.23	63.50
3617	53.93	64.40
5470	62.00	70.75
5581	59.29	65.00
8528	71.83	72.62
B2776	67.00	75.00
B2828	64.13	85.50
C901	76.20	65.00
C938	70.57	74.44

Mean first year, .6128±.016043; Mean second year, .6580±.015825; Difference in means, .0452±.0225.

Although the data are meager in table 17, we can give it no other interpretation than as indicating that the male does partly control the hatching power of his mate through dominant factors.

The mean hatchability for the fifteen males during the first year is .6128± .016043, for the second year .6580±.015825. There is a difference of .0452±.0225. This difference is just double its probable error and can therefore be of no consequence. The point we wish to emphasize in table 17 is the striking constancy in phenotypical hatching power of the same male, even when mated to different hens on two successive years. Such a degree of constancy was not found to exist in hens, as table 3 shows. The mean pullet-year hatching power of the hens was .5678±.011313. The mean second-year hatching record of the same hens was .4791±.012963. The standard deviation is nearly three times as great for the hens as for the males. The difference in the mean hatching power for the same hens on two successive years is .0887±.0172, which is significant. The genetic interpretation given below will serve to elucidate several apparent complications.

Genetic Factors Concerned [1]

One dominant gene seems to be concerned in the production of high hatchability. We use the symbol H to designate this gene. There is no sex linkage and all results obtained are to be expected in a simple mono-hybrid ratio. With this hypothesis, three possible genotypes of males and females exist, namely, HH, Hh, and hh individuals. The genotype is obscured in most cases for both males and females. Such being the case, only the breeding test can be used as a guide for matings.

Hatching records on 886 females studied in this report show that these birds divide themselves into three general classes or phenotypes: — (1) Those showing hatchability of 85 per cent or above, we call high. (2) Those with a hatchability of 55 to 84 per cent, we call medium. (3) Those below 55 per cent, we call low. Since factor H has a cumulative effect, the range for the medium class is twice as great as for the high class. The minimum for the low class has not yet been determined. Below are summarized the males' phenotypical and genotypical classes:

[1] A detailed report on the genetics of hatchability will appear in another publication.

Males' Phenotypical Character.

HH males on HH hens give all high hatchability.
HH males on Hh hens give all medium hatchability.
HH males on hh hens give all medium hatchability.
Hh males on HH hens give all high hatchability.
Hh males on Hh hens give all medium hatchability.
Hh males on hh hens give all low hatchability.
hh males on HH hens give all medium hatchability.
hh males on Hh hens give all low hatchability.
hh males on hh hens give all low hatchability.

Males' Genotypical Character.

HH males on HH hens give all HH daughters.
HH males on Hh hens give 50% HH and 50% Hh daughters.
HH males on hh hens give all Hh daughters.
Hh males on HH hens give 50% HH and 50% Hh daughters.
Hh males on Hh hens give 25% HH, 50% Hh, and 25% hh daughters.
Hh males on hh hens give 50% Hh and 50% hh daughters.
hh males on HH hens give all Hh daughters.
hh males on Hh hens give 50% Hh and 50% hh daughters.
hh males on hh hens give all hh daughters.

Both parents must carry the H factor in order to be phenotypically good hatchers. Hens cannot rank in the first class unless they carry the gene H in homozygous condition and are mated to H-bearing males. These observations indicate a cumulative value for the factor H and show why the male by failure to contribute at least one-half H-bearing sperm ranks a genotypically high hen as a medium hatcher. Furthermore, both HH and Hh males probably give about the same hatching record from HH hens. The progeny test alone can give a clue to the genetic composition of males if pullets of unknown formulæ are used as breeders.

Selection for high and low hatchability did not give results in two generations according to Dunn ('23). The probable explanation is that he used in his low line genotypically high (HH) hens that gave medium hatching records because they were mated to hh males. If such were the case, no appreciable separation could take place in but two generations. There may also have been a lack of HH or Hh males in his high line. Selection for high hatchability with the female as a guide and using cockerels from hens that hatched well has been a slow but progressive process in our flock, as already shown in table 17. In table 9, the mean hatchability of the dams of the males used for breeders is about 70 per cent. This would indicate that, on the average, the breeding males came from Hh hens. Thus, only in the later years of the period could any considerable percentage of males have been of the formula Hh. A study of earlier records shows that practically all the males must have been of hh composition, because they came from medium or low-hatching dams.

SUMMARY.

1. No correlation was found between fertility and hatchability in 758 pullets.
2. Fertility in the hen behaves as an individual characteristic with a fair degree of constancy from year to year.
3. Fertility does not appear to be transmitted from mother to daughter.
4. Hatching power is more constant from year to year in the same hen than is fertility.
5. Hatching power gives evidence of being transmitted from mother to daughter.
6. Fertility in the male behaves as an individual characteristic and probably with some constancy in the same individual from year to year.
7. The fertility record of a hen is no index to the fertilizing ability of her sons.
8. Fertility does not appear to be transmitted from sire to daughter.
9. Hatchability is more constant from year to year in the same male than is fertility.
10. Fertility does not appear to be transmitted from sire to son.
11. The hatching power of a male cannot be judged by his dam's hatching record.

12. Hatching power gives evidence of being transmitted from sire to daughter.

13. Insufficient data are available on the transmission of hatching power from sire to son.

14. Fertility is evidently not an inherited characteristic.

15. Hatchability is evidently an inherited trait. High hatchability is dependent in inheritance upon one dominant gene. Both male and female parent govern the hatching record, thus obscuring the true genetic composition of either parent.

16. Genetically pure hens for high hatchability may be discovered through their own hatching record. Genetically pure males for high hatchability can be distinguished from males heterozygous for the factor only by the progeny test combined with mating tests. Both the mating and the progeny test should be used for choosing males to improve the flock in hatchability.

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TECHNICAL BULLETIN No. 7 MARCH, 1926

BROODINESS IN RELATION TO FECUNDITY IN THE DOMESTIC FOWL

By F. A. HAYS and RUBY SANBORN

This bulletin is the seventh in the series of bulletins reporting the investigations of the Massachusetts Agricultural Experiment Station on heredity in the Rhode Island Red breed of poultry; and the second giving report of the study on broodiness in the same breed. In addition there have been published at various times scientific papers presenting the results of certain more or less minor phases of this study.

Expressed in terms of change in the character of the breeding flock, the data show that the percentage of broody birds has decreased from 90 in the foundation flock of 1912 to 27 in 1923, the last year reported in this publication. Associated with this decrease in broodiness, the average annual egg production has increased from 114 to 200 eggs. The data show, however, that decrease in broodiness is but one of many factors which have contributed to increased production.

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AGRICULTURAL EXPERIMENT STATION
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BROODINESS IN RELATION TO FECUNDITY IN THE DOMESTIC FOWL

By F. A. HAYS and RUBY SANBORN

NATURE OF CHARACTER BEING STUDIED

Broodiness is the tendency of female birds to incubate or attempt to incubate eggs. The broody hen stays on the nest, clucks, ruffles feathers when disturbed, etc. It is a recurring cyclical trait in birds and should be considered as a normal phase of their reproductive process. It has no homologue in mammals since they reproduce viviparously (developed young). In reptiles, which are closely related to birds, we have oviparous reproduction, but the eggs are hatched without the attention of the mother.

All breeds of domestic chickens exhibit broodiness to some extent. The Asiatic or meat breeds are all intensely broody; the American breeds all exhibit the trait to a considerable extent; and the Mediterranean breeds, although said to be non-broody, always give some broody females.

There thus appear to be widely different degrees of broodiness. There have been birds in the Massachusetts Station flock that first showed broodiness in November of their pullet year and continued to exhibit its cyclical recurrence to the extent of ten or twelve times during the first laying year. Contrasted with this is hen C 960—non-broody during pullet year, twice broody the second year, and non-broody the third year. Also hen C 1347—non-broody as a pullet, broody once for 23 days her second year, and non-broody her third year. Hen C 4765 was broody once as a pullet for 17 days and non-broody her second year. On the other hand, we now have two hens (B [illegible] and B 8797) that have completed four annual records without going broody. In general, three measures of broodiness may be used: namely, (1) the number of broody periods per year, (2) mean length of each broody period, and (3) total days of non-productiveness associated with broody periods. In all cases the length of a broody period has been taken as the period between last egg previous to going broody and first egg following "recovery."

Effect of Method of Handling

With the domestic fowl efforts are made to check the manifestation of broodiness so that the hen may begin laying again. Modern practice is to coop such hens in slat-bottom coops, making nesting almost impossible. After four to six days of such confinement, the bird may ordinarily be returned to the flock without resuming nesting. Such hens show wide diversity in length of time before resuming laying.

Trapnesting and regular removal of all eggs from the nests seem to "discourage" the onset of broodiness. Punnett reports two cases of hens from a broody-free race that were themselves non-broody for two years, later actually incubating and hatching eggs. This particular phase of the problem needs further elucidation.

Broodiness thus appears to be a normal phase of the reproduction of domestic chickens. Its occurrence seems to depend upon environmental and physiological stimuli as will be pointed out later.

WORK ALREADY DONE.

By Other Investigators.

Bateson (1902) and Hurst (1905) both present data on crosses between broody and non-broody races, indicating that broodiness is a dominant character. No further information was obtained at that time.

Punnett and Bailey (1920) report some results using Black Langshans, Brown Leghorns and Gold-pencilled Hamburgs. Results:

Langshan ♀ x Leghorn ♂ gave all F_1* pullets broody. Of the F_2* generation 16 pullets were retained, 8 of which went broody as pullets. Punnett states that if the Langshans were of composition AABB and Leghorns aabb, F_2 should give 9 broody to 7 non-broody, a close approximation to actual ratio. The reciprocal cross, Leghorn ♀ x Langshan ♂ gave all broody in F_1, but in F_2 there were but 19 broody to 47 non-broody. Most of these birds were retained but one year. A few that were kept the second year added more broodiness so that the ratio is not 9 to 7, probably because of delayed appearance of broodiness.

In the Hamburg-Langshan cross, the F_1 hens were either non-broody or showed very little broodiness. Of 38 F_2 pullets, 4 were broody, 34 non-broody. These results suggest a third factor, N, which inhibits. F_1 birds would be NnAaCc, but factor N did not inhibit in all cases. The F_2 ratio gave 4 broody to 34 non-broody. Punnett states that his results are far from conclusive as to the true nature of the broody trait.

Pearl (1914) found much less broodiness in Barred Plymouth Rocks than exists in Reds. His method of measuring the intensity of broodiness was by the length of non-productive period. Other known factors, such as winter pause and molt make such a measure subject to error.

Work Done by the Massachusetts Station.

Goodale began the study of this trait in 1912. From that time up to 1921, when he severed his connections with the Station, very satisfactory progress was made in eliminating the tendency from the egg-laying strain of Rhode Island Reds.†

Recent Work at the Massachusetts Agricultural Experiment Station.

In the fall of 1922 the writer took up this project using the same general plan with some modifications. The non-broody strain has been carried along with the intense broody strain and not as a part of the general flock in so far as the matings are concerned. The non-broody birds are now being carried along through the second and third laying years to definitely test their behavior with regard to broodiness. Similarly, the breeding males are being carried over and tested for genetic composition. In a paper entitled, "Inbreeding the Rhode Island Red Fowl with Special Reference to Winter Egg Production," the broody trait has been shown to confirm Goodale's AC theory which suggests that broodiness is due to the presence of two dominant, autosomal, complementary genes, A and C. Both must be present to produce broodiness, but either may be carried alone by non-broody birds.

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* F_1 and F_2 refer to generations one and two.

† See Mass. Agri. Expt. Sta. Bulls. 199 and 211.

Progress to Date.

General Progress from Year to Year.

Table I. Mean Degree of Broodiness by Years.

Year Hatched	Birds broody, per cent	Average number of broody periods per broody hen	Total number of birds available*	Annual Production
1912	89.60	4.4	125	114
1913	91.03	5.4	78	124
1914	85.95	4.3	121	103
1915	89.25	4.3	428	122
1916	86.31	3.5	431	134
1917	48.84	2.7	432	166
1918	61.40	2.9	215	169
1919	No annual records			
1920	46.03	2.9	126	200
1921	44.56	2.7	285	200
1922	28.91	1.9	399	200
1923	27.35	1.9	340	189

* This column includes all Rhode Island Reds except intense broodies and inbreds.

It will be observed that the percentage of broody birds has been reduced from 90 in 1912 to 27 in 1923. Great significance should also be attached to the fact that the mean annual egg yield has increased from 114 to 200 in the same period. In the 1912 flock each broody hen lost 75 days in broodiness her first year, while in the 1922 flock each broody hen lost but 29 days. The assumption seems justified, therefore, that progress in eliminating broodiness has been two-fold: namely, reduction in the percentage of broody birds, and reduction in the mean degree of broodiness.

The average number of days spent in broodiness for the 112 broody birds in the 1912 flock is 74.8. For the 71 broody birds in the 1913 flock the figure is 78.8 days. In the 1922 flock, made up of all birds except those bred for intense broodiness, there were 112 birds that were broody, with a mean of 28.71 days spent in broodiness. In the 1922 flock there were 33 birds bred for intense broodiness. These birds averaged 42.94 days broody for the pullet year.

Specific Results.

The non-broody strain has been strengthened during the past two years by the retention of non-broody hens up to five years old. Such hens have been used as breeders each season so that their genetic character for broodiness may be confirmed by the progeny test. Aged breeding males have also been retained for similar purposes.

An intense broody strain has been carried on from year to year. Females selected to perpetuate this strain have been selected with a view of combining the maximum number of broody periods with desirable traits from the standpoint of annual fecundity. This intense broody strain will eventually differ from the non-broody strain only in possessing the broody trait. It is possible

in this manner to measure directly the effect of broodiness on fecundity. This intense broody strain differs from the foundation birds more in the distribution of broody periods throughout the laying year than in the number of broody periods.

Complete records of broodiness are also maintained on every female of the experimental flock to augment data collected in the broody experiment.

End to Be Attained

A flock of poultry breeding *true* for broodiness and non-broodiness.

Scope of This Report.

In this bulletin consideration is given to the actual relationship between pullet-year egg production and the broody trait as manifested during the first laying year. Coefficients of correlation have been calculated as follows:

Between broodiness and rate.
December rate—Sections 1, 2, 3, 4, 5, 16, 17.
Winter rate—Sections 6, 7, 8, 9, 10, 18, 19.
Annual rate—Sections 11, 12, 13, 14, 15, 20, 21.

Between times broody and length of broody periods.
Section 22.

Between winter rate and annual rate.
Section 23.

Between winter rate and annual egg yield.
Section 27.

Between annual rate and annual egg yield.
Section 28.

Between broodiness and egg yield.
Winter production—Sections 24, 25, 26.
Annual production—Sections 29, 30, 31, 32, 33, 34, 35.

Coefficient of Correlation.

The coefficient of correlation furnishes a concrete measure of the tendency of two characteristics to move together, to move in opposite directions, or to behave independently. In this particular study the characteristics studied both belong to the same individual fowl. Either a significant positive or negative correlation coefficient is useful to the breeder as a guide, and the magnitude of the coefficient shows him the relative amount of dependence between the traits or characters considered. The value of a coefficient of correlation from the biological standpoint depends upon its absolute magnitude and upon its relation to its probable error. A coefficient at least three times as great as its probable error is generally considered significant, even though its absolute magnitude is small. The deductions reported in this bulletin are based on the above conception. King (1923), however, states that the correlation coefficient should be more than six times its probable error. He further states that a correlation coefficient of less than .30 indicates a lack of marked correlation, that over .50 shows decided correlation. Furthermore, the correlation coefficient with its regression coefficients may be used for purposes of prediction. The value of a knowledge of the degree of correla-

tion lies mainly in its use for selecting a group of breeders and not in the selection of individual breeders.

The true coefficient of correlation may only be calculated for a race pure with regard to the characteristics being studied, as Harris (1915) points out. False correlations result when two or more genetically different races are concerned in any calculation. Broody birds have been shown to be genetically different (Hays, 1924) from non-broody birds. In studying the relation of broodiness to fecundity, it has been deemed advisable to make three general groupings: namely, (1) total population of broody and non-broody combined, (2) only birds that went broody during the pullet year, and (3) broody or non-broody without regard to the degree of broodiness. The first series of calculations was made for two purposes: first, to confirm that broody and non-broody races are genetically different; second, to furnish evidence on the intensity characteristics in relation to the broody trait even in a mixed population of broodies and non-broodies. The third series of calculations was made by Yule's formula for presence and absence of a character, as given by Davenport (1907). All other calculations were made by the ordinary method for calculating the correlation coefficient for fluctuating variables.

The regression coefficient is readily calculated after the correlation coefficient is determined. It is useful to the breeder for selection purposes. If a group of hens, each five times broody, were selected, the regression coefficient might be used to estimate its probable average egg production. If the degree of correlation between days broody and annual production is known, it is a simple matter to calculate the probable annual egg record of hens broody for 25 days or for any other period of days. Thus the regression coefficient merely represents the amount of change in one character with respect to a unit change in another. For example, the regression coefficient of days broody on annual production is —.1171, and the regression of annual production on days broody is —.3295. What should be the average annual egg yield of hens broody for thirty days?

42.87 average days broody of all hens
30.00

—12.87 days broody below the average
—12.87 x—.3295 = 4.2407 + 164.885 (average production of all)=

169.1257, probable record of hens broody for 30 days

The correlation ratio is comparable to the correlation coefficient and has a similar use. The former is made use of where the correlation coefficient would be false. As a measure of association in mixed races the correlation ratio is reasonably accurate, but it is of less value than the correlation coefficient for prediction purposes. Since a constant is calculated for each of the two variables in correlation ratio, a difference in magnitude of these two constants sometimes occurs, probably due to genetic impurity. Correlation ratio has not been used extensively in these studies because the correlation coefficient has been calculated on the three classes of hens with respect to broodiness: namely, broody and non-broody, different degrees of broody, and broody or non-broody, so that regressions closely approach linearity.

Character of Birds Used.

Beginning in the spring of 1916 the plan of breeding Rhode Island Reds for high egg production was somewhat modified. On that year matings were planned to consider early sexual maturity, no winter pause, intensity, persistency, and especially non-broodiness. Particular attention was given to the elimination of the broody tendency by using females non-broody during the pullet year and males from non-broody mothers for breeding purposes. The original foundation stock was all standard-bred Rhode Island Red. No new blood has been introduced into the flock since the plan of mating for the five characteristics above referred to was inaugurated in 1916. Inbreeding has not been practiced to any considerable extent, but the line of ancestry has been markedly reduced so that the present flock traces to but a small number of the best foundation birds.

Records Kept

Records used in the study of broodiness include complete pedigree of all birds used; complete trapnest records of every female as long as retained; date hatched; date of first egg; age at first egg; weight at first egg; nesting records; date of appearance of broodiness; date of placing into broody coop; date of return to laying house; hatching record of females used as breeders; complete family record of the progeny from each mating; and daily, winter and annual records on all surviving females.

Intensity.

Intensity and rate are terms used interchangeably in this report. They refer to the number of eggs laid in a specific interval of time on a percentage basis of the maximum possible number of eggs in the time considered. *December Rate*, as used here, is a figure obtained by dividing the number of eggs laid by 31 if the hen began laying on or before December first. For birds that laid their first egg later than December first, the rate was calculated by dividing the number of December eggs by the number of days *from first egg* to the end of December. As a short-time measure of intensity this may be considered more accurate than the actual number of eggs laid during December, for obvious reasons. *Winter Rate* is calculated by dividing the total number of eggs from first egg to March first by the number of days from first egg to March first, less all pauses of four or more days in duration from November first to March first. *Annual Rate* is calculated by dividing the total eggs from first egg to 364 days thereafter, for all birds that showed no 30-day pause after March first, by the number of days from first to last egg. When a bird stopped laying for thirty or more days after March first, her laying year is assumed to terminate at the beginning of this pause, and her annual rate is calculated by dividing the number of eggs laid by the number of laying days before the pause.

Broodiness.

Broodiness has already been defined as the tendency of the female fowl to incubate or attempt to incubate eggs. The intensity of broodiness may be

measured by the number of broody periods and by the mean length of broody periods. Both Pearl (1914) and Goodale (1920) have measured the length of each broody period by the cessation of egg production associated therewith. Goodale (loc. cit.), however, stresses the fact that winter pause and fall molt may prolong the non-productive period for a considerable time interval beyond the normal broody period.

In the present studies, the observation has been made that there is a remarkable degree of uniformity in length of broody periods in the same individual. In the occasional bird that goes broody during the fall or winter of her pullet year, the winter pause may greatly lengthen the period of non-production. In such cases we have allowed four days for the bird to begin laying after removal from the broody coop to the laying house. Such birds are removed from the broody coop only when they no longer show signs of broodiness. In such cases any pause up to March first, of greater duration than four days following removal of hen from broody coop to laying house, is not considered a broody pause.

Very frequently the laying year terminates with a broody period and no more eggs are laid for two or three months. In such cases the length of the last broody period is calculated in the same manner as outlined above for the winter season. This long period of non-production is without question due largely to the onset of complete molt and not to broodiness. The fact that non-broody birds exhibit this long period of non-production during molt is very convincing evidence on the point in question.

Relation of Broodiness to Fecundity.

In studying the relation of broodiness to fecundity, it has been necessary to study the degree of correlation between broodiness and rate of laying, times broody and mean length of broody periods, winter rate and annual rate, winter rate and annual egg yield, annual rate and annual egg yield, and broodiness and annual egg yield.

Unpublished data at this Station indicate that rate of laying or intensity is the most important single characteristic affecting egg yield. For this reason, the relation between broodiness and rate is of extreme importance. Either a positive or negative correlation between broodiness and rate would be far more significant genetically than would the absolute correlation between broodiness and egg production; for egg production has already been shown by Goodale and Sanborn (1922) to depend upon at least five characteristics and one of these characteristics is rate. In the present study of the relation of broodiness to fecundity these facts are fully considered.

1. *Correlation Between Times Broody and December Rate—Pullet Year.*

In this study pullets are included that were hatched on the following years: 1916, 1917, 1918, 1920, 1921, 1922 and 1923. The flock hatched in 1919 is not included because no annual records are available for that year on account of a disease epidemic. All Rhode Island Red pullets with normal records are included. In addition to the major portion of each flock that was bred for egg production, there are included a small number of inbred birds, a small number bred for intense broodiness, a small number bred for color, and a small number used in studying the inheritance of hatchability. Inasmuch as this report is a study of the relationship between broodiness and fecundity, there is no conceivable reason why a rather heterogeneous flock should not

be as valuable for study as one of marked uniformity for all characteristics.

Some short-time record of production is often made use of by commercial poultrymen in predicting the laying ability of a pullet for the year. Winter pause is likely to appear in many birds during December and is very pronounced in earlier birds. Other birds beginning their laying year early and continuing to lay regularly through December, as well as those starting their laying year in December, will as a rule have high December rate. Possibly November records would be freer from the winter pause, but such records would be less valuable than December records for predicting either winter or annual egg records, as Harris and Goodale (1922) have shown. It therefore seems advisable to use December rate in studying the relation of broodiness to rate.

A total of 1945 birds consisting of both broody and non-broody are included in the study. The range in times broody is from 0 to 12, divided into 13 classes. The range in December rate is from 1 to 100, divided into five-unit classes. Constants calculated from this study follow:

Number of birds	1945
Mean times broody	1.41
Times broody standard deviation	±1.98
Mean December rate	59.60
December rate standard deviation	±20.40
Coefficient of correlation	+.0639 ± .0152

The constants given above impress the reader with the marked variability in the birds studied, both with regard to times broody the first year and December rate of production. The apparently abnormal standard deviation in times broody is due to the large percentage of non-broody birds (51.23 per cent). In other words, an impure population is concerned.

The magnitude of the standard deviation in December rate signifies very marked variation in rate of laying for December. Even such a short-time measure of fecundity is subject to excessive variability.

The coefficient of correlation between times broody and December rate, although more than three times as great as its probable error, is of questionable magnitude and is a false correlation as Section 2 shows.

2. Correlation Between Times Broody and December Rate for Broody Birds Alone—Pullet Year.

In order to measure the relation of degree of broodiness, as indicated by the number of periods, to December intensity, only birds actually going broody have been used in the calculation of the correlation coefficient. Of the group of 1945 individuals studied in section 1, 949 birds actually went broody during the pullet year. This number has been used to study the relation of degree of broodiness to December rate. Constants arrived at follow:

Number of birds	949
Mean times broody	2.89
Times broody standard deviation	±1.95
Mean December rate	61.24
December rate standard deviation	±20.11
Coefficient of correlation	+.0145 ± .0219
Regression broodiness on rate	+.0014
Regression rate on broodiness	+.1498

The very large standard deviation in times broody suggests a most pronounced variability in number of broody periods. The actual range is from 1 to 12. Since the modal class is broody but once, there can be but little further progress in reducing the mean number of broody periods within the broody race.

The mean December rate is slightly higher than that for both broodies and non-broodies combined, in section 1. The standard deviation in rate is of the same magnitude as that in section 1.

The coefficient of correlation between degree of broodiness and December rate is actually less than its probable error, and since it is of very small magnitude, the interpretation seems justified that December rate is independent of degree of broodiness, and that the correlation in section 1 is false.

3. Correlation between the Presence of Broodiness and December Rate above the Mean of Broodies and Non-broodies Combined—Pullet Year.

The actual correlation between the presence of broodiness and high rate is of much importance to the breeder. Such a constant was calculated for the 1915 broody and non-broody birds by the method of Yule (loc. cit.).

December Rate	Broody	Non-Broody
Number above population mean	632	595
Number below population mean	317	401
Totals	949	996

Coefficient of correlation $+.1466 \pm .0450$

Although the degree of correlation between the presence of broodiness and high December rate is not large, there can be no justification for any other deduction than that the presence of broodiness is partially linked with high December intensity. The elimination of the broody trait should result in something of a reduction in December rate for the flock as a whole.

A further consideration of this relationship in a flock high in broodiness and in a flock low in broodiness seems advisable. The 1916 flock showed 86.31 per cent broody and is unimproved, at least for broodiness. The 1923 flock showed 27.35 per cent broody and may be classified as an improved flock.

4. Correlation Between the Presence of Broodiness and December Rate above the Mean of Broodies and Non-broodies Combined—Pullet Year (Unimproved Flock 1916).

In the total of 253 birds the following results were obtained:—

December Rate	Broody	Non-Broody
Number above population mean	138	14
Number below population mean	85	16
Totals	223	30

Coefficient of correlation $+.2996 \pm .0386$

The above constant suggests that in the 1916 flock there was a rather distinct tendency for broody birds to lay at a higher rate during December than non-broody birds. The constant given in section 3 for the entire period reported upon is $+.1466 \pm .0150$. A comparison of the two constants assigns them a similar value in comparison with their probable error, as each is about eight times its probable error. There is the possibility that December rate is higher in the early flock because they were slow to reach sexual maturity, so that winter pause was less pronounced in December than in later flocks.

5. Correlation Between the Presence of Broodiness and December Rate above the Mean of Broodies and Non-Broodies Combined—Pullet Year (Improved Flock 1923).

A total of 404 birds is studied in the 1923 flock, distributed as below:—

December Rate	Broody	Non-Broody
Number above population mean	78	157
Number below population mean	51	118
Totals	129	275

Coefficient of correlation $+.0695 \pm .0334$

The degree of correlation amounts to insignificance compared with its probable error. It indicates no dependence between the presence of broodiness and December rate above the mean. It is conceivable that early maturity may affect December rate, and winter pause is more pronounced in the flocks since the age at maturity has been reduced.

6. Correlation Between Times Broody and Winter Rate—Pullet Year.

This study included 2221 pullets hatched the same seven years as those studied for December rate. Winter rate is calculated on the period from first egg to March first, as already explained. Unpublished data at this Station indicate a rather intimate correlation between winter rate and annual production. Winter rate was calculated on a greater number of pullets than were studied for December rate, because the latter could only be calculated on individuals laying one or more eggs in December. The same classes were used in tabulating times broody and winter rate as were used in studying December rate. Constants calculated are as follows:—

Number of birds	2221
Mean times broody	1.43
Times broody standard deviation . . .	1.99
Mean winter rate	66.45
Winter rate standard deviation	±9.37
Coefficient of correlation	+.0706 ± .0142

The above constants show the mean winter rate to be greater than the mean December rate previously calculated. The above winter rate really signifies that, on the average, the birds laid 66.45 per cent of the maximum possible number of eggs when they were laying, since all pauses of four or more days have been deducted in calculating winter rate. The standard deviation in winter rate is only ± 9.37 compared with a figure of ± 20.10 for December rate. The winter pause and the fact that many of the birds actually lay their first egg during December account for the wider variability in December rate.

The coefficient of correlation between times broody and winter rate is almost identical with that between times broody and December rate. This is a constant of small magnitude, and is a false correlation because the population is made up of both broody and non-broody birds.

7. Correlation Between Times Broody and Winter Rate for Broody Birds Alone—Pullet Year.

In order to ascertain any possible relationship between winter rate and degree of broodiness, the correlation between times broody and winter rate has been calculated for broody birds alone. The constants obtained are as follows:—

Number of birds	1098
Mean times broody	2.89
Times broody standard deviation	±1.93
Mean winter rate	67.57
Winter rate standard deviation	±9.63
Coefficient of correlation	—.0314 ± .0203
Regression broodiness on rate	—.0063
Regression rate on broodiness	—.1564

The mean winter rate in those birds that actually went broody during their pullet year is 67.57 compared with 66.45 for broodies and non-broodies combined. Such a difference is of no significance.

The coefficient of correlation is negative. Its small magnitude, together with the size of its probable error, leads to the assumption that there is absolute independence between winter rate and degree of broodiness as measured by times broody.

8. Correlation Between the Presence of Broodiness and Winter Rate above the Mean of Broodies and Non-Broodies Combined—Pullet Year.

The absolute correlation between the presence of broodiness and high rate is of importance to the breeder. Such a constant will indicate whether or not the broody trait carries with it higher winter intensity than does the non-broody trait. The coefficient of correlation is calculated below according to Yule.

Winter Rate	Broody	Non-Broody
Number above population mean	674	558
Number below population mean	422	565
Totals	1096	1123

Coefficient of correlation — .2358 ± .0135

The magnitude of the above constant points to a linkage between broodiness and high winter intensity. Herein lies a probable explanation why the heavier breeds, all of which carry the broody trait, are in general superior winter layers to the non-broody lighter breeds. In the history of the flock under consideration, the highest average winter records, 67.65 and 74.5 eggs, were made by the 1920 and 1921 flocks with a percentage of broodiness amounting to 16.03 and 14.56 respectively of birds included. The 1923 flock, for example, showed 27.35 per cent broody and a mean winter egg record of but 51.04. Probably broody birds carrying early sexual maturity and no winter pause are superior as winter layers to non-broody birds possessing the same two traits, because of some linkage between broodiness and high intensity. Further consideration is given to this important question in sections 9 and 10.

9. Correlation Between the Presence of Broodiness and Winter Rate above the Mean of Broodies and Non-broodies Combined—Pullet Year (Unimproved Flock 1916).

Winter rate and broody records are complete for 332 birds in the 1916 flock. These have been correlated below:

Winter Rate	Broody	Non-Broody
Number above population mean	174	15
Number below population mean	115	28
Totals	289	43

Coefficient of correlation +.4770 ± .0286

This is a rather pronounced correlation and shows winter intensity was associated with broodiness in an early flock.

10. Correlation Between the Presence of Broodiness and Winter Rate above the Mean of Broodies and Non-Broodies Combined—Pullet Year (Improved Flock 1923).

Winter rate and broody records for 430 birds hatched in 1923 are tabulated below:

Winter Rate	Broody	Non Broody
Number above population mean	87	141
Number below population mean	51	151
Totals	138	292

Coefficient of correlation + .2925 ± .0297

A significant coefficient of correlation between broodiness and high winter rate suggests that there is linkage between broodiness and high winter intensity. Further evidence has already been presented in sections 8 and 9. Herein lies the probable superiority of broody breeds over non-broody breeds in winter intensity.

11. Correlation Between Times Broody and Annual Rate—Pullet Year.

The method used in calculating annual rate does not allow for winter pause or for time lost while broody. It is simply a figure intended to measure the actual rate of laying between the time of laying the first pullet egg, and time of laying the last egg before the complete molt. Winter pause birds and broody birds are actually penalized in calculating annual rate. If there is absolute independence between broodiness and winter pause, the only normal handicap that the broody bird carries over the non-broody is the production loss during broody periods. Inasmuch as the magnitude of the annual rate depends most largely upon yearly egg production, this method of measuring rate should be most significant in breeding for fecundity. It is believed that this is a true measure of actual rate of laying during the year. Constants calculated from the 2245 individuals studied follow:—

Number of birds	2245
Mean times broody	1.44
Times broody standard deviation	± 1.98
Mean annual rate	56.48
Annual rate standard deviation	± 9.85
Coefficient of correlation	—.2620 ± .0133

The above constants show that the 2245 birds actually laid on 56.48 per cent of the possible days between their first egg and the time they ended their year with the complete molt. The standard deviation agrees closely with that for the winter rate. A mean rate of such a magnitude immediately suggests high annual production.

The coefficient of correlation between times broody and annual rate is negative; and its magnitude, together with its small probable error, suggests that broodiness and low rate tend to move together.

By the use of the regression coefficient we find that those birds with a mean rate of 60.48 will be less broody than the mean of all birds studied (1.44 — .21 = 1.23). The fact is very evident, therefore, that broodiness tends to lower annual rate of laying. The coefficient as determined, however, does not represent the true correlation, since the flock of 2245 birds is made up of both broody and non-broody races.

12. Correlation Between Times Broody and Annual Rate for Broody Birds Alone—Pullet Year.

A pure race in so far as the broody trait is concerned is to be found in the birds actually going broody during their first laying year. The total number of birds in this class for the seven years is 1122. By tabulating the annual rate of each individual against her number of broody periods a measure of the degree of correlation between degree of broodiness and annual rate is obtained. Constants calculated on this group follow:—

Number of birds	1122
Mean times broody	2.89
Times broody standard deviation	±1.91
Mean annual rate	54.93
Annual rate standard deviation	±9.24
Coefficient of correlation	—.3232 ± .0180
Regression broodiness on rate	—.0669
Regression rate on broodiness	—1.5610

The mean annual rate for the broody birds is slightly lower than was found for the total population in section 11 (56.48). No significant change is observable in standard deviation.

The coefficient of correlation is slightly larger than that obtained for the total number of birds, and represents a rather intimate negative correlation between times broody and annual rate. Degree of broodiness as measured by number of periods is therefore very inimical to high annual rate.

13. Correlation Between the Presence of Broodiness and Annual Rate above the Mean of Broodies and Non-broodies Combined—Pullet Year (Flocks 1916-1923).

The true relation or correlation between the presence of broodiness and annual rate above the mean is of interest and value to poultrymen. Such a determination has been made for the 2245 birds being studied, by Yule's method.

Annual Rate	Broody	Non-Broody
Number above population mean	513	675
Number below population mean	609	448
Totals	1122	1123

Coefficient of correlation —.2828 ± .0131

The above constant does not differ significantly from that representing the whole population. In this particular case the mingling of a broody and a non-broody race in the same correlation table did not result in skew correlation. The constant —.2828 ± .0131 is known to represent a true value for the flock in question, and emphasizes the importance of breeding for non-broodiness to secure maximum annual records.

The next two sections are devoted to the correlation between the presence of broodiness and annual rate above the mean of broodies and non-broodies combined, using the high broody flock of 1916 and the low broody flock of 1923. Such a study shows the relative importance of broodiness in determining annual rate in a flock of low and high fecundity.

14. Correlation Between the Presence of Broodiness and Annual Rate above the Mean of Broodies and Non-broodies Combined—Pullet Year (Unimproved Flock 1916).

Annual Rate	Broody	Non-Broody
Number above population mean	165	31
Number below population mean	159	18
Totals	324	49

Coefficient of correlation −.2480 ± .0328

This constant agrees well with that for the whole eight-year period. It is significant and illustrates the negative relation between broodiness and high annual rate in an unimproved flock.

15. Correlation Between the Presence of Broodiness and Annual Rate above the Mean of Broodies and Non-broodies Combined—Pullet Year (Improved Flock 1923).

Annual Rate	Broody	Non-Broody
Number above population mean	60	164
Number below population mean	76	129
Totals	136	293

Coefficient of correlation −.2338 ± .0308

This coefficient does not differ significantly from the coefficient obtained on the 1916 flock in section 14 or from the constant on all flocks in section 13. Evidently the relation of broodiness to annual rate has not changed with the improvement in fecundity.

In the previous sections, the relation between *times broody* and rate or intensity of production has been considered. A considerable body of evidence has been presented to indicate first, that hens with the broody trait do tend to lay more intensely during the winter season than non-broody hens; second, that broodiness is a considerable handicap to annual production in that it lowers the annual rate. The next consideration is the relation of *total days broody* during the pullet year to December rate, winter rate and annual rate.

16. Correlation Between Total Days Broody and December Rate—Pullet Year.

In this study the same group of 1945 birds both broody and non-broody that were studied in relation of times broody to December rate (section 1) is considered. It is important to know which is the more important from the standpoint of rate, the number of broody periods or the actual number of days spent in broodiness calculated so as to avoid winter pause and fall molt. Constants calculated on this group of birds follow:—

Number of birds	1945
Mean total days broody	23.20
Total days broody standard deviation . . .	±27.07
Mean December rate	59.60
December rate standard deviation . . .	±20.40
Coefficient of correlation	+.0529 ± .0153

The standard deviation in total days broody exceeds the mean total days broody because of the large percentage of non-broody birds in the group studied.

The coefficient of correlation agrees rather closely with the figure given in section 1 where times broody and December rate are considered. Evidently broodiness may be measured either by periods or by total days. The degree of correlation is slight, and it is really a false correlation because based upon a mixed population—broody and non-broody.

17. Correlation Between Total Days Broody and December Rate for Broody Birds Alone—Pullet Year.

The relation between degree of broodiness, as measured by total days of non-production associated with broodiness during the pullet year, and December rate may be determined by using only the birds that went broody the first year. Such a determination was made for the same group of 949 birds that was considered in section 2. The following are the constants:—

Number of birds	949
Mean total days broody	42.84
Total days broody standard deviation . . .	±27.38
Mean December rate	61.24
December rate standard deviation . . .	±20.11
Coefficient of correlation	—.0002 ± .0219
Regression broodiness on rate	—.0003
Regression rate on broodiness	—.0001

That degree of broodiness and December rate are entirely independent is shown by the above coefficient of correlation which is practically zero. This is rather conclusive evidence that December intensity bears no relation to the presence or absence of the broody trait.

18. Correlation Between Total Days Broody and Winter Rate—Pullet Year.

A total of 2221 birds studied in section 6 are included in this study to discover the degree of dependence or independence between total days broody and winter rate. The constants calculated follow:—

Number of birds	2221
Mean total days broody	23.50
Total days broody standard deviation	27.01
Mean winter rate	66.15
Winter rate standard deviation	±9.37
Coefficient of correlation	−.0178 ± .0142

The coefficient of correlation is practically the same figure as was obtained between times broody and winter rate. This is also a false correlation because broodies and non-broodies each represent a genetic type. Since winter production and annual production are so intimately correlated (Hervey 1923; Hays, Sanborn and James 1924), high winter record is of very great importance in breeding for fecundity.

Blakeman's test for linearity of regression has been applied in this study with the following results:

Correlation ratio for days broody	±.1131
Correlation ratio for winter rate	±.1695
(Cor. Ratio)^2 — (Cor. Coeff.)^2 =	.0075 ± .0024
(Cor. Ratio)^2 — (Cor. Coeff.)^2 =	.0233 ± .0013

The difference between the correlation ratio for winter rate squared and the correlation coefficient squared is .0233 ± .0013, a difference more than five times as great as its probable error. This fact indicates that the coefficient of correlation is false, as might be anticipated from the fact that two genetic races are concerned.

19. Correlation Between Total Days Broody and Winter Rate for Broody Birds Alone—Pullet Year.

Winter rate records are available on 1098 birds that were broody the first year. In this study days broody is tabulated against winter rate to further discover the correlation between degree of broodiness and winter rate. Constants are as follows:—

Number of birds	1098
Mean total days broody	12.85
Total days broody standard deviation	±27.14
Mean winter rate	67.57
Winter rate standard deviation	±9.63
Coefficient of correlation	.0241 ± .0203
Regression broodiness on rate	.0679
Regression rate on broodiness	−.0085

The coefficient of correlation as shown above signifies independence between degree of broodiness and winter rate. The intensity of the broody trait is therefore of no concern in affecting winter intensity.

20. Correlation Between Total Days Broody and Annual Rate—Pullet Year.

The total days broody for each bird are tabulated against her annual rate. The lowest rate class is 16-20; the highest rate class is 86-90. The lowest broody class is 0-9; the highest broody class is 150-159 days. This study on the 2245 birds used in section 11 will show if broodiness is an advantage or disadvantage from the standpoint of annual rate. Are broodiness and high intensity linked together? Constants calculated are:—

Number of birds	2245
Mean total days broody	23.68
Total days broody standard deviation	±26.98
Mean annual rate	56.48
Annual rate standard deviation	±9.85
Coefficient of correlation	—.2720 ± .0132

The coefficient of correlation between days broody and annual rate is negative and of such magnitude as to be of considerable significance, were it not for the fact that the two races of birds give a false correlation.

21. Correlation Between Total Days Broody and Annual Rate for Broody Birds Alone—Pullet Year.

The same group of birds considered in section 12 is used in this study. The coefficient of correlation is here used to measure the degree of association between degree of broodiness and annual intensity. Constants obtained are the following:—

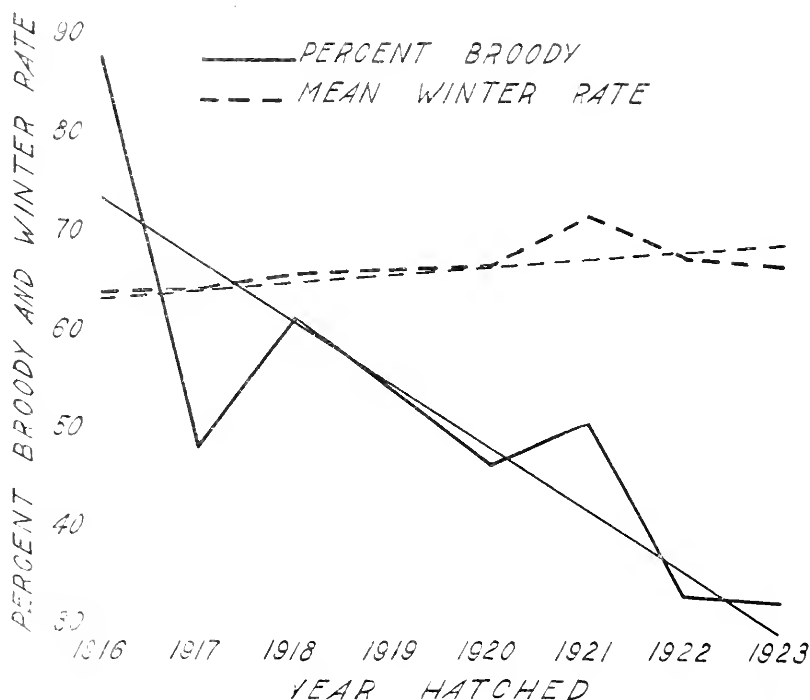

CHART 1. Relation of percentage of birds broody to mean winter rate by years.

Number of birds	1122	
Mean total days broody	12.87	
Total days broody standard deviation	26.84	
Mean annual rate	51.93	
Annual rate standard deviation	9.21	
Coefficient of correlation	.3622	.0175
Regression broodiness on rate	1.0526	
Regression rate on broodiness	.1236	

A rather marked degree of negative correlation exists between days broody and annual rate. The degree of broodiness influences annual rate because of the loss of time while broody. This constant agrees substantially with the constant for times broody and annual rate (–.3232 ± .0180).

Relation of Broodiness to Winter Rate and Annual Rate.

In charts 1 and 2 the mean percentage of birds broody on the different years is illustrated graphically by a solid line. The mean winter rate and

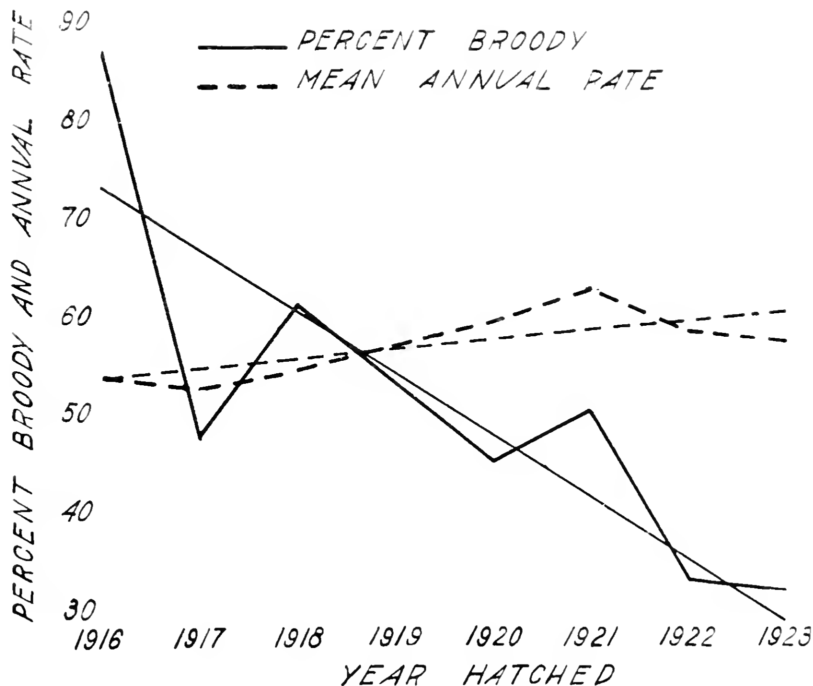

CHART 2. Relation of percentage of birds broody to mean annual rate by years.

mean annual rate are represented by broken lines. The groups of birds used in making the two charts are not identical because winter rate records are available on a considerable number of individuals that did not survive to complete annual records. However, the two groups are so nearly identical that the mean percentage of broody birds closely agrees in the two charts.

The increase in mean winter rate from 1916 to 1923 is 4.06, while the increase for annual rate in the same period is 6.00. This fact indicates that annual rate has increased more rapidly than winter rate as the percentage of broody birds has been reduced from year to year. The greater degree of parallelism in the two graphs on chart 1 suggests that a change in percentage of broody birds is usually accompanied by a change in winter rate. Chart 2 shows a lesser relationship between percentage broody and annual rate.

In general the two charts furnish evidence that both mean winter rate and mean annual rate may be increased while the percentage of broody birds is being reduced. The lowering of the percentage of broody birds to at least 30 per cent, as has been accomplished in the flock studied, appears to be advantageous from the standpoint of annual production.

The next section is devoted to a study of the relation between the number of broody periods and the mean length of broody periods. It seems desirable to ascertain if the average length of broody period is affected by the number of periods. Does the frequency of onset of broodiness tend to shorten or lengthen the period? The coefficient of correlation is again made use of and the number of broody periods is tabulated against the mean length of period, using 1135 birds that were broody in the pullet year.

22. Correlation Between Times Broody and Mean Length of Broody Periods—Pullet Year.

Any attempt to decrease the intensity of broodiness must be accomplished either by reducing the number of periods or by reducing the length of these periods. The coefficient of correlation is here calculated to discover a possible relationship between number and length of broody periods. Constants calculated are as follows:

Number of birds	1135
Mean times broody	2.89
Times broody standard deviation	±3.67
Mean length of periods	15.10
Length of periods standard deviation	±3.78
Coefficient of correlation	—.2338 ± .0189
Regression times broody on length	—.4620
Regression length on times broody	—.1183

The total number* of birds showing one or more broody periods is slightly greater than the number in sections 12, 21, 30 and 32, broody records being available on a few birds on which annual rate records are lacking. The stand-

*The total number of birds going broody was 1135. Of this group, 1017 individuals were first broody after March first so that the actual length of the period of nonproduction attributable to broodiness could be definitely recorded. There were 118 birds broody before March first. The mean length of broody period for the 1017 birds is 15.95 days, while that for the group of 1135 birds is 15.10 days. This slight difference in mean length of period is not significant and may be attributed to our inability to separate broody pause from winter pause in those 118 birds going broody before March first. The method of allowing a bird but four days to begin laying after her return to the laying house following broodiness during the winter season is faulty in that it actually assigns a shorter broody period during winter than the mean of summer broody periods.

ard deviation in times broody is greater than the mean because 951 birds (83 per cent) fell into classes 1-4, leaving only 17 per cent in classes 5-13. The modal class is 2.

The mean length of broody periods is 15.40 days with a standard deviation of ± 3.78. There is, therefore, much greater uniformity in length of period than is observed for number of periods. Evidently the number of periods offers a more fertile field for improvement than is offered by the length of period.

The negative coefficient of correlation indicates that an increase in number of broody periods is accompanied by a decrease in their average length. A reduction in number of periods would therefore be accompanied by an increase in their length. That this relationship is far from absolute is shown by the magnitude of the correlation coefficient. Certainly the time lost in non-production has been very significantly reduced by decreasing the number of broody periods, as table 1 shows.

23. Correlation Between Winter Rate and Annual Rate—Pullet Year.

The records for 2242 individuals both broody and non-broody are available for study. This relationship is important because both rates bear a rather intimate relation to egg production. The fact has previously been pointed out that broody birds tend to be more intense winter layers than are non-broody birds, but that the former are likely to carry a lower annual rate. An intimate correlation between winter rate and annual rate would suggest that rate of laying for the year may be predicted from the winter rate. Constants calculated are as follows:—

Number of birds	2242
Mean winter rate	66.11
Winter rate standard deviation	±9.38
Mean annual rate	56.16
Annual rate standard deviation	±9.85
Coefficient of correlation	+.4900 ± .0108

The above constants indicate a slightly greater relative standard deviation in annual rate than exists for winter rate. Such a condition might be surmised from the fact that broodiness and complete molt may both affect annual rate but for the most part are not concerned in winter rate.

A rather intimate correlation exists between winter and annual rate. Evidently those birds above the average in winter rate would be expected to be above the average in annual rate. The practice of selecting for high winter rate is without doubt sound from the standpoint of securing high annual rate.

24. Correlation Between the Presence of Broodiness and Winter Production above the Mean of Broodies and Non-broodies Combined—Pullet Year (Flocks 1916-1923).

The absolute correlation between the presence of broodiness and winter production above the mean of all birds is of much concern to poultrymen striving for high winter records. Such information will show whether or not broody birds tend to lay more eggs before March first than do broody-free birds. In section 8 some evidence is presented to indicate that broody birds do actually lay at a slightly higher rate than non-broodies when they are laying; but late sexual maturity, winter pause and the occasional winter broody period may

possibly be more pronounced in the broody population. The actual correlation between the presence of broodiness and winter production above the mean is shown by the following table:—

Winter Production	Broody	Non-Broody
Number above population mean	561	561
Number below population mean	534	563
Totals	1095	1124

Coefficient of correlation $- .0264 \pm .0143$

The above coefficient is so small as to be of no significance and it is less than three times the magnitude of its probable error. The deduction must be made from this study that broodiness and winter egg production are entirely independent even though broody birds do lay at a slightly higher rate in winter when they are laying.

25. Correlation Between the Presence of Broodiness and Winter Production above the Mean of Broodies and Non-broodies combined—Pullet Year (Unimproved Flock 1916).

Winter Production	Broody	Non-Broody
Number above population mean	149	14
Number below population mean	140	29
Totals	289	43

Coefficient of correlation $+ .3759 \pm .0318$

The above constant shows that broodiness bears a rather intimate correlation to high winter production in the 1916 flock. Such an assumption is based on the conclusion that the individuals laying more eggs in winter than the average of the flock (46.87 eggs) are high producers. Even though a small percentage of the 1916 flock reduced this winter record by being broody before March first, broody birds appeared to carry intensity to a sufficient extent to enable them to lay more eggs for the period than did the non-broody birds. It is rather striking that the total population (section 24) should not exhibit a constant similar to that for the 1916 flock. No doubt changes in early maturity and winter pause have operated to modify winter production to a greater extent than any possible lowering of intensity by the elimination of broodiness has been responsible for.

26. Correlation Between the Presence of Broodiness and Winter Production above the Mean of Broodies and Non-broodies Combined Pullet Year (Improved Flock 1923).

Winter Production	Broody	Non-Broody
Number above population mean	77	140
Number below population mean	61	152
Totals	138	292

Coefficient of correlation + .1563 ± .0317

This constant is of questionable magnitude and signifies that winter production of the 1923 flock above the mean of 53.62 eggs is but little dependent upon the presence of the broody trait. The fact should be recalled, however, that the maximum winter production (74.5 eggs) was made by the 1921 flock with 44.56 per cent of the birds broody during the pullet year.

The later sections of this report are devoted to a consideration of the correlation between rate and egg yield and broodiness and egg yield.

27. Correlation Between Winter Rate and Annual Egg Production—Pullet Year.

In commercial poultry breeding for fecundity, a short-time measure of probable annual production is of vast importance. If the winter rate could be used as a basis for selecting breeding females as efficiently as the yearly record, it would be of vast economic importance. By making use of the coefficient of correlation, a measure of the probable worth of the winter rate in selecting for large yearly records is obtained. The constants arrived at in this study are given below:—

Number of birds	2242
Mean winter rate	66.11
Winter rate standard deviation	±9.38
Mean annual production	174.37
Annual production standard deviation	± 44.59
Coefficient of correlation	+.4561 ± .0113

The mean annual egg production of the 2242 birds used in section 27 was 174.37, with a standard deviation of 44.59, or a coefficient of variation of about 25 per cent. The class range in egg production is from 21 to 300 with class intervals of 10. This wide range in production is due to the heterogeneity of the flock and to the number of characteristics that affect production.

The magnitude of the correlation coefficient, together with the small probable error, suggests that winter rate is rather intimately correlated with annual egg production.

28. Correlation Between Annual Rate and Annual Egg Yield.

Annual rate as calculated for this flock is a rather concise measure of in-

tensity for the entire pullet laying year. It should furnish a reasonably true measure of the bird's ability to lay throughout the year. Since the relation of broodiness to annual rate has already been considered, it seems advisable to correlate annual rate with annual yield. The calculations gave the following constants:

Number of birds	2289
Mean annual rate	56.38
Annual rate standard deviation	±9.86
Mean annual egg yield	172.21
Annual egg yield standard deviation	±46.61
Coefficient of correlation	+.6717 ± .0077

A very sensible positive correlation was found between annual rate and annual egg yield. Annual rate is thus a very dependable measure of a bird's ability to lay during her pullet year.

29. *Correlation Between Times Broody and Annual Production—Pullet Year.*

The records of 2215 birds broody and non-broody are tabulated and the coefficient of correlation calculated between times broody and annual production. Constants arrived at follow:—

Number of birds	2215
Mean times broody	1.41
Times broody standard deviation	±1.98
Mean annual production	173.06
Annual production standard deviation	±46.40
Coefficient of correlation	—.2126 ± .0136

This constant is false because the table is made up of two genetically distinct races, namely, broody and non-broody.

TABLE 2.—Relation of Broodiness to Egg Record.

Times Broody	Number of Birds	Egg Record
0	1121	181.31
1	312	178.32
2	259	156.62
3	220	156.50
4	149	158.65
5	72	162.58
6	47	153.59
7	28	140.14
8	17	155.50
9	9	147.72
10	5	145.50
11	2	160.50
12	1	155.50
13	1	145.50

Reference to table 2 above shows that the 1121 non-broody birds averaged 181.31 eggs per year. Close to this group in production is the class of 312 birds with but one broody period, averaging 178.32 eggs. A somewhat gradual but not regular decline begins with the group broody twice. No further

decline is observed until the group with six broody periods is reached, after which the mean egg yield falls significantly. The probable error increases so rapidly due to small numbers when the class with eight broody periods is reached that very little significance can be attached to the mean in this and later classes. On the whole, this table suggests in a general way that increased broodiness does lower the annual record.

30. Correlation Between Times Broody and Annual Egg Yield for Broody Birds Alone Pullet Year.

A true measure of the correlation between times broody and annual production can only be found within the broody population as previously stated. In the eight-year period 1122 broody birds are concerned. This group has been tabulated and constants calculated.

Number of birds	1122
Mean times broody	2.89
Times broody standard deviation	±1.94
Mean annual production	161.89
Annual production standard deviation . . .	±45.03
Coefficient of correlation	.1791 ± .0195
Regression broodiness on production . . .	.0076
Regression production on broodiness . . .	4.2167

A negative coefficient of correlation of .1791 ± .0195 indicates that the correlation between times broody and annual production may not be considered intimate. Such a constant leads to the assumption that broodiness as measured by periods has played some part in limiting annual production for the eight-year period studied.

31. Correlation Between Total Days Broody and Annual Production Pullet Year.

Broodiness may next be measured in total days for the year. This method of measuring has been made use of between total days broody, and annual egg production. The birds used in this tabulation are 2245 in number, both broody and non-broody. Constants calculated follow:

Number of birds	2245
Mean total days broody	23.68
Total days broody standard deviation	±26.98
Mean annual production	173.06
Annual production standard deviation . . .	±46.40
Coefficient of correlation	.2200 ± .0135

An interesting and important fact is brought out by the above constants in that the correlation between total days broody and annual egg record is negative and of almost the same magnitude as that obtained between times broody and annual egg record (section 29). The deduction that broodiness lowers annual record again seems warranted, but the coefficient obtained in this table is false because of the presence of the genetically different broodies and non-broodies.

32. Correlation Between Total Days Broody and Annual Egg Yield for Broody Birds Alone—Pullet Year.

A tabulation made of the 1122 broody birds gives the true correlation between degree of broodiness and pullet-year production. Constants obtained are the following:—

Number of birds	1122
Mean days broody	42.87
Days broody standard deviation	±26.84
Mean annual production	164.89
Annual production standard deviation . .	±45.03
Coefficient of correlation	—.1964 ± .0194
Regression days broody on production . . .	—.1171
Regression production on days broody . . .	—.3295

The degree of correlation between total days broody and annual egg record is not at all intimate. It is in very close agreement with the constant for times broody and annual egg yield, in section 30. On the whole, broodiness has been shown to be negatively correlated with annual production to a rather moderate degree over the eight-year period covered in this report.

33. Correlation Between the Presence of Broodiness and Annual Production above the Mean of Broodies and Non-broodies Combined—Pullet Year (Flocks 1916-1923).

The true correlation between the presence of broodiness and high annual egg production is determined below.

Annual Production	Broody	Non-Broody
Number above population mean	514	665
Number below population mean	608	458
Totals	1122	1123

Coefficient of correlation —.2640 ± .0132

The above constant is statistically significant and is of sufficient magnitude to warrant the assumption that broodiness is negatively correlated with high annual production. The fact that annual egg record depends upon a vast array of genetic and non-genetic factors should not be overlooked. During the eight-year period being considered there has been constant progress in eliminating broodiness, yet the mean annual egg records of the flocks have been stable since 1920. Very likely broodiness has played a greater part in affecting production in some years than on others. The two following sections show that the correlation between broodiness and annual record has not been intimate either in 1916 or 1923.

34. Correlation Between the Presence of Broodiness and Annual Production above the Mean of Broodies and Non-broodies Combined—Pullet Year (Unimproved Flock 1916).

Annual Production	Broody	Non Broody
Number above population mean	165	27
Number below population mean	159	22
Totals	324	49

Coefficient of correlation .0837 ± .0347

The correlation coefficient is negative and less than three times its probable error. The conclusion seems to be warranted that in the 1916 flock there is no significant correlation between broodiness and annual production.

35. Correlation Between the Presence of Broodiness and Annual Production above the Mean of Broodies and Non-broodies Combined—Pullet Year (Improved Flock 1923).

Annual Production	Broody	Non-Broody
Number above population mean	67	164
Number below population mean	69	129
Totals	136	293

Coefficient of correlation −.1339 ± .0320

This coefficient is about four times its probable error and is probably of some significance. The degree of correlation between broodiness and annual production is slight on the two years studied; a fact that probably indicates more correlation on the intervening years.

General Summary and Deductions

The means for the broodies and non-broodies for the three rates and for winter and annual production for the eight-year period are as follows:

	Broody		Non-broody	
	Number of Birds	Mean	Number of Birds	Mean
December Rate	949	61.24	996	58.05
Winter Rate	1098	67.57	1123	65.36
Annual Rate	1122	54.93	1122	58.04
Winter Production	1094	69.46	1123	58.79
Annual Production	1122	164.89	1124	181.34

1. Birds carrying the broody trait lay at a slightly higher rate when they are laying than do non-broody birds. This characteristic is observable in both December records and in winter records.

2. A much more intimate correlation between the presence of broodiness and high December intensity was observed in the 1916 flock than in the 1923 flock. This difference may probably be attributed to changes in sexual maturity and to the time of onset and termination of winter pause.

3. Intensity for the winter period is rather intimately correlated with the presence of broodiness. Such a relationship is observed in the total population for the eight years, in the 1916 flock and in the 1923 flock.

4. Degree of broodiness is not correlated with either December rate or winter rate.

5. The degree of correlation between broodiness and high annual rate is constant but negative and significant.

6. Degree of broodiness may be measured with equal accuracy by number of broody periods or by total days broody during the pullet year.

7. Winter rate and annual rate are distinctly positively correlated.

8. The duration of the broody period is somewhat lessened as the number of periods increases.

9. Correlation between the presence of broodiness and winter production above the average is negligible when all the birds are considered over the eight-year period. There is a significant positive correlation for the 1916 flock and probably a slight correlation within the 1923 flock.

10. The mean winter egg record of broodies is not significantly greater than that of non-broodies.

11. Annual egg production is significantly negatively correlated with broodiness in the total population studied; to a very minor degree in the 1916 flock; and to a rather moderate degree in the 1923 flock.

12. The elimination of broodiness has had but little significance in breeding for high winter production but a pronounced significance in breeding for high annual records.

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AGRICULTURAL EXPERIMENT STATION

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WINTER CYCLE AND WINTER PAUSE IN RELATION TO WINTER AND ANNUAL EGG PRODUCTION

By F. A. HAYS and RUBY SANBORN

In this bulletin are reported the results of a statistical study of winter cycle and winter pause records taken over a period of nine years, with as many separate flocks. The records show that winter cycle furnishes a significant short-time measure of probable annual egg production in the flock. Winter pause is shown to be a potent cause of lowered annual egg production; and any increase in length of pause is but partially compensated for by later increased activity in egg production.

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AGRICULTURAL EXPERIMENT STATION

AMHERST, MASS.

WINTER CYCLE AND WINTER PAUSE IN RELATION TO WINTER AND ANNUAL EGG PRODUCTION.

By F. A. HAYS and RUBY SANBORN

INTRODUCTION

Winter cycle is represented by a period of continuous egg laying during the winter season. February 28 or 29th is arbitrarily chosen as the closing date of the winter period. A distinct cessation of laying before the end of February may be assumed to mark the end of the winter laying cycle. Just what length of pause should be chosen to mark the end of the winter laying cycle is purely arbitrary. Goodale (1918) suggested that a ten-day cessation of laying is sufficiently small to mark the end of the winter cycle. In the studies reported here, a four-day interval is considered as a winter pause because, in the flock studied, such a pause generally means the omission of one clutch* of eggs. The omission of one clutch of eggs really marks a distinct break in the functioning of the reproductive system and probably represents the termination of a laying cycle. Pauses due to broodiness or to injury or disease are not considered, and only pauses occurring between November 1 and February 28 or 29 are classed as winter pauses or as marking the end of the winter cycle.

Winter cycle may further be defined as total days from date of first egg to a pause of four or more days, the pause being considered only between the dates November first and March first. Winter cycle length can therefore be determined only for the pause class of birds, as Goodale (1918) pointed out. Winter cycle is probably inherited as a recessive, according to Goodale (loc. cit.); but he failed to discover a relationship between number of eggs laid in the winter cycle and duration of winter pause.

Winter pause may be defined as the period when egg production ceases, following the termination of the winter cycle and previous to the initiation of the spring laying cycle. Pearl (1912) and Goodale (1918) make reference to winter pause in relation to fecundity. Pearl (loc. cit.) found that the winter cycle in Barred Plymouth Rocks was characteristically terminated by a cessation of production. Goodale (loc. cit.), on the other hand, observed a cessation of production previous to March first in but a part of a Rhode Island Red flock. Goodale (1922) states that winter pause is due in part to inherited characteristics and in part to environmental conditions. He discovered a rather intimate relation between the time of beginning to lay in the fall and the appearance and duration of the winter pause.

Winter pause is usually associated with the shedding of some feathers, or partial molt, as Hays (1921) suggested. Furthermore, a cessation of production during broodiness or at any other time during the laying year is

* The term "clutch" refers to the number of eggs laid on successive days, which is more or less characteristic of the individual hen.

generally characterized by partial or complete molt. Apparently molting, which precedes the development of a new growth of feathers, is a phenomenon initiated by a cessation of active functioning of the reproductive system. A number of environmental influences as well as inherent fecundity thus appear to be concerned in the different pauses of the pullet laying year. The sum total of the winter pause may or may not be represented by a continuous period of non-production. In other words, there may be several pauses of four or more days with some production intervening. In these studies such pauses have been added together and such individuals placed in the same class with birds whose pause is unbroken.

The duration* of winter pause is recognized to be dependent upon environmental conditions such as hatching date, feeding and housing, weather conditions, and all other influences that may affect the physical condition and state of metabolism of the pullet. Since age at which sexual maturity is attained is modified by such controllable conditions as date of hatching, as Hays and Sanborn (1924) point out, and since age when sexually mature largely governs the time of beginning to lay, a complex relationship must exist between age at first egg and winter pause.

The presence or absence of winter pause depends upon inheritance, as Hays (1924) points out. Pullets that exhibit a winter pause of a week or more before March first are known to carry a dominant factor M, while non-pause pullets are recessive and lack factor M. This factor is transmitted equally by both males and females. In this connection, breeders should bear in mind that genetically non-pause pullets may exhibit a winter pause brought on by disease, abrupt changes of feed, moving to new quarters, and other environmental influences largely within control of the poultryman. In the breeding flock of the Massachusetts Agricultural Experiment Station extreme care has been exercised to keep environmental conditions constant from year to year in order that inherited traits affecting fecundity may be studied.

Character of Birds Used

This study includes all Rhode Island Red females, hatched from 1916 to 1924, on which pullet-year trapnest records are available. The flocks each year are made up of all the daughters from each individual female whose progeny was retained. Culling within the family has not been practiced. The major portion of birds in each flock belong to the fecundity experiment. There are, however, a limited number of birds bred for non-broodiness, some for intense broodiness, some for hatchability, some for color, and a few inbreds that are included. Pullet-year records alone are used in this study. The heterogeneity of the flock can scarcely be considered as a factor affecting the constants presented in this report.

Two possible methods are open for attacking these questions in a large population made up from nine years' breeding for fecundity. The first method is the use of the coefficient of correlation. The second possible mode of attack is through the presentation of actual data by families tracing to both foundation males and females through nine successive years. The possibilities of the two methods may be briefly considered.

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* In a small percentage of the flock the pause began late in February and such birds did not resume laying until some time in March or later. In these cases, duration of pause is calculated when production is actually resumed.

The Coefficient of Correlation.

A general survey of a series of individual egg records may lead to certain general deductions some of which may actually be true and others false. The breeder needs to know just how much stress to lay upon different characteristics associated with the traits concerned in high fecundity. The simple correlation coefficient affords a concise measure of the degree of association between specific traits and high fecundity as well as a measure of the relationship between the presence of particular traits and high fecundity. The correlation coefficient may thus be made use of by the breeder in two ways: first, for prediction purposes; and second, in the selection of breeding stock to obtain the most valuable combination of traits. Stated concisely, the correlation coefficient is the only direct and specific measure for degree of association of characteristics where large numbers of individuals are concerned. Fecundity records may be modified by a vast number of environmental conditions as well as by the five traits pointed out by Goodale and Sanborn (1922) which are shown to be inherited. Hays (1924). Only by the use of large numbers of records made over a period of years under uniform conditions of management and in a flock bred for uniformity can a true value of the relative importance of characteristics concerned with fecundity be approached. The coefficient of correlation thus becomes an invaluable tool in breeding for fecundity.

Presentation of Data by Families.

A study undertaken to consider the winter cycle and winter pause by separate families would necessitate the presentation of page after page of abstract data. Such data should be accompanied by detailed and complete discussions and such general deductions as would seem justified. No definite constants could be determined on numbers so small as the individual family. Possibly all the descendants of particular individuals could be considered as units, but from the genetic standpoint such a consideration should be classed as questionable. A general tabulation of the whole population, giving such information as mean hatching date, mean age at first egg, mean weight at first egg, percentage of birds pausing, mean length of pause, mean winter production, mean annual production, etc., by years could be made. Such a tabulation would again be open to the criticism of not furnishing specific information. Only general deductions could be made and no evidence would be furnished as to relative values. In view of the above facts, this method of handling the data is not considered feasible.

WINTER CYCLE

Winter cycle may be considered in three general categories: namely, (a) in its relation to environmental conditions, (b) in its relation to heritable characteristics concerned in fecundity, and (c) in its absolute relation to egg production.

(a) Relation of Environmental Conditions to Winter Cycle.

Hatching date belongs to the definitely controllable class of conditions in that it may be varied at will of the investigator. Date of first egg depends

both upon environment and inheritance. The time when a group of pullets will begin to lay depends in part upon hatching date, method of feeding and management, and upon weather conditions all of which may be classed as environmental. The dependence of date of first egg upon age when beginning to lay, however, is a relation to a heritable trait, since Hays (1921) has shown age at first egg to be inherited.

1. Correlation Between Hatching Date and Length of Winter Cycle.

Time of hatching is generally believed to hold an important relation to the time of appearance of winter pause. Since the appearance of winter pause marks the termination of the winter cycle, the possibility exists of a relationship between hatching date and length of winter cycle. The table presented below tends to substantiate a relation between date of hatching and length of winter cycle for the *total* population of birds actually manifesting a winter cycle terminated by a pause:

Hatches	Number of Birds	Mean Length of Winter Cycle
1	329	68.33 Days
2	267	62.54 "
3	286	59.56 "
4	281	51.91 "
5	258	47.05 "
6	237	42.00 "
7	225	36.21 "
8	195	38.78 "
Grand Average		52.26 "

The mean length of winter cycle is shown to consistently decrease as the hatching date advances, with but a single exception in the last hatch. There are eight hatches each year at one week intervals from March 25 to May 15. The total difference in age between the first and last hatches is 49 days, while the difference in mean winter cycle length is 30 days. The ability of the later hatched pullets to reach sexual maturity at a slightly earlier age than do early hatched pullets (Hays, Sanborn, and James, 1924) probably accounts for the minor inconsistencies in the above table. The means of the eight different hatches for the nine-year period covered by the table indicate a rather important relationship between date of hatching and length of winter laying cycle, which is determined by the onset of winter pause. In this connection, the reader should bear in mind that only winter pause birds are included in the tabulation because no winter cycle can be ascertained in non-pause birds.

The absolute relation between hatching date and length of winter cycle may be discovered by means of the coefficient of correlation. Available for study are 2078 birds. Class intervals of ten days are used for winter cycle in calculating the following constants:

Number of birds	2078
Mean hatching date (Apr. 17)	4.18
Hatching date standard deviation	$\pm$2.26
Mean length of winter cycle	52.26
Winter cycle standard deviation	$\pm$34.23
Coefficient of correlation	$-.3174 \pm .0133$
Regression of hatching date on winter cycle length .	$-.021$
Regression of winter cycle length on hatching date .	-4.811

A significant negative coefficient of correlation informs that, in general, early-hatched pullets have a longer winter laying cycle than late-hatched pullets of the same flock. The magnitude of the constant does not establish an intimate relationship, however, and for this reason the influence of other forces is evident. An increase in length of laying cycle is important from the breeding standpoint, because it signifies a greater mean winter record, and winter production is intimately correlated with annual production (Hays, Sanborn and James, 1924).

2. Correlation Between Date of First Egg and Length of Winter Cycle.

Date of first egg is very important economically. Its significance biologically depends upon the influence of weather conditions on egg production. Specific data concerning the influence of weather on fecundity are not available, however. There is a considerable body of evidence pointing toward a seasonal periodicity of production which has led a number of workers to consider winter, spring, summer and autumn cycles of laying.

In this experiment 2078 pullets with definite winter cycles are available for study. Fifteen-day class intervals are used in grouping data of first egg, and the range in dates is August 24 to February 20. Below are the constants calculated:

Number of birds	2078
Mean date of first egg (Oct. 29)	5.93
Date of first egg standard deviation	±2.09
Mean length of winter cycle	52.26
Winter cycle standard deviation	±34.23
Coefficient of correlation	—.5307 ± .0106
Regression of date of first egg on winter cycle .	—.032
Regression of winter cycle on date of first egg .	—8.689

The date of first egg fluctuates widely in the population studied. The mean date of first egg for the 2078 birds studied is October 29. In breeding for fecundity this variability in time of beginning to lay may be reduced genetically and also by providing a more uniform environment.

A negative coefficient of correlation of substantial magnitude demonstrates that early laying makes for a long winter cycle. The relation that winter cycle length holds to egg production remains to be considered in sections 4 and 5 of this report.

(b) Relation of Heritable Traits to Winter Cycle.

Age at first egg is a definitely heritable trait (Hays, 1924). It has been shown by a number of workers to be intimately correlated with both winter and annual fecundity. This study shows how age at first egg is related to length of the winter laying cycle. Sexual maturity is the only heritable characteristic reported on in relation to winter cycle.

3. Correlation Between Age at First Egg and Length of Winter Cycle.

The same group of 2078 pullets has been studied to ascertain the correla-

tion between age at first egg and length of the winter laying cycle. Class intervals of ten days have been used for age, and the respective ages of the individuals tabulated against their winter cycle length. The constants determined are as follows:

Number of birds	2078
Mean age at first egg	203.66
Age at first egg standard deviation	±25.92
Mean length of winter cycle	52.26
Winter cycle standard deviation	±34.23
Coefficient of correlation	—.4529 ± .0118
Regression of age on winter cycle	—.343
Regression of winter cycle on age	—.598

Age at first egg varies within moderate limits. Since genetically-early and genetically-late birds are concerned, and because environment probably modifies age and sexual maturity, the standard deviation of age is not excessive.

A significant negative correlation is shown, as might be anticipated from the constants obtained in section 2. Age of sexual maturity may be classified as a characteristic influencing the length of winter cycle as determined by the onset of winter pause. Here is an example of a heritable trait being negatively correlated with duration of winter cycle.

(c) Relation of Winter Cycle to Egg Production.

A knowledge of the relation of winter cycle length to winter fecundity and annual fecundity is of value for prediction purposes. If any short-time measure of fecundity that is reasonably accurate in predicting winter and annual production is discovered, it will be of much economic import. Proper culling enables the poultryman to raise mean flock production by disposing of mediocre layers. If a relatively short season of trapnesting gives a clue to probable production for the year, such information will greatly assist poultrymen. This section considers the correlation between length of winter cycle and winter production and length of winter cycle and annual production. Since the winter cycle length for each bird is tabulated against her egg record, a true measure of degree of correlation is arrived at.

4. Correlation Between Length of Winter Cycle and Winter Egg Record.

Both length of winter cycle and winter egg record are placed in class intervals of ten for the 2078 individual pullets studied. The following constants were determined:

Number of birds	2078
Mean length of winter cycle	52.26
Winter cycle standard deviation	±34.23
Mean winter production	56.99
Winter production standard deviation	±23.40
Coefficient of correlation	+.6538 ±.0085
Regression of winter cycle on production	+.956
Regression of production on winter cycle	+.447

Mean length of winter cycle is 52.26 days while mean winter production is 56.99 eggs. Winter production exceeds the production of the winter laying cycle because most of the pullets resume laying previous to March first following a pause. An arbitrary termination of the winter season at the close of February in all cases does not give a true measure of winter production and no definite calendar date will suffice.

The standard deviations of both winter cycle and winter production are excessive. This fact further establishes the variability as due to inheritance and environment.

The above constant discloses a very intimate positive correlation between length of winter cycle and winter production. Here is concrete evidence establishing an important relation between long winter cycle and high winter egg record.

The importance of optimum hatching date, age at first egg, and date of first egg in relation to the length of the winter laying cycle has been presented in sections 1, 2, and 3. Possibilities of shortening the pause period by breeding methods are to be handled in another publication. Probably the most important consideration is the correlation between length of winter cycle and annual egg production, studied in the next section.

5. Correlation Between Length of Winter Cycle and Annual Production.

Annual egg record depends upon a vast array of environmental forces and upon a series of Mendelian factors. Specific information concerning many of these influences has never been presented. This section attempts to present in concrete form the relation of length of winter cycle to annual production over a period of years. On 1314 pullets the following constants appear:

Number of birds	1314
Mean length of winter cycle	53.52
Winter cycle standard deviation	±34.87
Mean annual production	172.53
Annual production standard deviation . . .	±41.13
Coefficient of correlation	±.4027 ± .0156
Regression of winter cycle on production . . .	+.341
Regression of production on winter cycle . . .	+.475

This group of birds averaged slightly under 173 eggs during their pullet laying year of 365 days beginning with their first egg. The standard deviation in production illustrates a wide range of fluctuation. The actual range in annual egg production was from 35 to 275.

A positive correlation of substantial magnitude exists between winter cycle length and annual egg record. Length of winter cycle, therefore, furnishes a rather dependable short time measure of probable annual production for a population. Winter cycle length is discernable only for pullets exhibiting winter pause. By trapnesting during the first part of the laying year, it is possible to discover the length of the winter cycle and consequently the time of appearance of its complement, the winter pause.

Winter Pause

In the following table are given the number of pause and non-pause birds by years, together with the percentage of birds in the pause class.

Year	Number of Non-Pause Birds	Number of Pause Birds	Total	% With Pause
1916	120	159	279	56.99
1917	153	239	392	60.97
1918	115	248	363	68.32
1919	59	109	168	64.88
1920	48	133	181	73.48
1921	276	175	451	38.80
1922	201	376	577	65.16
1923	109	353	462	76.41
1924	160	340	500	68.00

The average length of pause for those birds pausing is as follows:

1916 flock	29.46 days
1921 flock	19.23 days
1924 flock	32.97 days

6. *Correlation Between Length of Winter Cycle and Length of Winter Pause.*

The range in cycle length was found to be from 5 days to 175 days. The class interval used was 10 days. Since winter cycle and winter pause are complementary to each other, it is important to discover their possible relation to each other. The coefficient of correlation will illustrate any tendency for length of winter cycle and length of winter pause to move in the same or in opposite directions. Such information will make clearer their physiological relationships and possible genetic linkage.

Number of birds	2078
Mean length of winter cycle	52.26
Length of winter cycle standard deviation	±34.23
Mean length of winter pause	31.91
Length of winter pause standard deviation	±21.68
Coefficient of correlation	—.1385 ± .0145
Regression winter cycle on pause	—.219
Regression pause on winter cycle	—.088

The length of winter cycle as measured in these studies is subject to wide fluctuation as indicated by the relative magnitude of the mean and its standard deviation. Such fluctuations are to be anticipated in a population highly variable for the seven pairs of inherited factors concerned in winter production, subjected to uncontrollable variation in environmental influences. The fact should be observed, however, that the mean length of winter cycle is about 63 per cent greater than the mean length of pause.

A small negative correlation coefficient indicates a very slight tendency for long-cycle birds to pause for a shorter period than do short-cycle birds. This

constant is subject to error, however, in that any increase in length of winter cycle reduces the possible pause interval before March first. The coefficient of correlation as calculated is of value in that it gives the actual relationship between length of winter cycle and duration of pauses within the winter season. These data furnish very good evidence that the length of winter pause does not depend upon the length of the winter cycle of laying.

In this report consideration is given to the winter pause from three general standpoints, namely, (a) Environmental factors affecting duration of the pause: (b) Inherited characteristics concerned with fecundity in relation to winter pause, and (c) The absolute relationship of winter pause to egg production.

(a) Environmental Factors Affecting Duration of Pause.

Much concern should be given to the relation of environmental factors affecting the duration of the winter pause since these conditions are more or less under the control of the poultryman. In the group of environmental factors the following have been placed: hatching date and time of beginning to lay in the fall. The time of year when pullets begin to lay is clearly dependent both upon management and inheritance. Management is a factor when the hatching date remains constant because housing, range, and feeding may either retard or accelerate sexual maturity. Just how significant these environmental influences are on time of beginning to lay in comparison with inherited early or late sexual maturity remains to be determined. At any rate, hatching date can be definitely controlled and time of beginning to lay may be considered as partially controllable.

7. Correlation Between Hatching Date and Length of Winter Pause.

A very common observation among poultrymen is that early-hatched pullets are more likely to exhibit winter pause than are late-hatched birds of the same flock. In other words, the belief is prevalent that the earlier the hatching date, the longer the winter pause. Such observations have naturally led to the assumption that the pullet possesses capacity to lay a certain number of eggs in the fall and winter and if this number is laid early there will be a cessation of laying until the spring season. That hatching date is only one of several conditions operating to affect the onset and duration of winter pause has been shown by Hays (1924) and Hays, Sanborn, and James (1924). Age at sexual maturity has been pointed out as an inherited characteristic, and as a characteristic having greater effect than hatching date upon winter egg production. Furthermore, winter pause of seven or more days' duration is an inherited characteristic. The importance of knowing just how intimate a relationship exists between date of hatching and duration of winter pause becomes apparent and may be discovered by means of the coefficient of correlation.

A total of 2134 birds exhibited a pause of four or more days and are included in these calculations. The winter pause class interval is ten days in all cases. Constants obtained from this study follow:

Number of birds	2134
Mean hatching date (Apr. 17)	4.15
Hatching date standard deviation	±2.26
Mean length of winter pause	32.26
Winter pause standard deviation	±21.92
Coefficient of correlation	—.2480 ± .0137
Regression of hatching date on winter pause	—.026
Regression of winter pause on hatching date	2.404

The mean length of the winter pause over the entire nine-year period amounts to 32.26 days for the pause birds, but a striking fluctuation in the duration of the pause is revealed by its standard deviation. Environmental influences may be considered as largely responsible for the fluctuations. Any possible changed environment to shorten the pause would be advantageous economically.

The coefficient of correlation measuring the degree of association between time of hatching and duration of winter pause is negative, of moderate magnitude, and certainly significant. Clearly, a reduction in the range of hatching dates would tend to reduce the length of the period of non-production during the winter season. The mean length of winter pause of the eight different hatches studied follows:

Hatches	No. of Birds	Mean days Paused
1	353	39.41
2	271	37.35
3	293	36.46
4	289	33.84
5	261	28.22
6	245	25.99
7	227	25.85
8	195	24.32
Grand Average		32.26

8. Correlation Between Hatching Date Earlier Than The Mean and the Presence of Winter Pause For Entire Population.

Yule's short method as cited by Davenport (1907) is used in this study. This tabulation includes the total population, 3375 Rhode Island Reds classified as pause and non-pause individuals.

Hatching Date	Pause	Non-Pause
Earlier than Population mean	1206	555
Later than Population mean	928	686
Totals	2134	1241

Coefficient of correlation . . . . +.2326 ± .0110

This positive coefficient of correlation is of sufficient magnitude to establish a definite relationship between early hatching and the appearance of winter pause. This being the case, the assumption must be made that inheritance is not the sole controlling force concerned in the manifestation of winter pause.

9. *Correlation Between Date of First Egg and Length of Winter Pause.*

The date on which a pullet lays her first egg is dependent upon many factors. Among the most important of these are hatching date and age at first egg. Environmental influences such as character of ration, amount of free range, and weather conditions may, to some extent, hasten or retard the date of first egg. Date of first egg is important economically if not biologically.

The relation between date of first egg and duration of winter pause has been determined by means of the coefficient of correlation on the same group of 2134 birds studied in section 7. The birds were again classified as to winter pause into class intervals of ten days. The class interval used for date of first egg was fifteen days. Constants calculated are as follows:

Number of birds	2134
Mean date of beginning to lay (Oct. 29)	5.88
Date of beginning to lay standard deviation	±2.13
Mean length of winter pause	32.26
Winter pause standard deviation	±21.92
Coefficient of correlation	—.3205 ± .0131
Regression of date of first egg on winter pause	—.031
Regression of winter pause on date of first egg	—3.297

The date on which the birds began to lay ranged from August 16 to March 29 making 15 class intervals. Its standard deviation may be expected to be of considerable magnitude in relation to the mean as is shown above.

A negative coefficient of correlation of .3205 ±.0131 between time of beginning to lay and pause duration stresses an important relation between the two. Early laying, on the average, tends to increase the duration of the pause.

10. *Correlation Between Time of Beginning to Lay Earlier Than the Mean and the Presence of Winter Pause for Total Population.*

Time of Beginning to Lay in the Fall	Pause	Non-Pause
Earlier than Population mean	1210	456
Later than Population mean	924	785
Totals	2134	1241

Coefficient of correlation . . . . +.3854 ± .0099

A definite and significant correlation exists between early laying and the presence of winter pause. This fact suggests the importance of breeding for a specific age at first egg, and hatching on some special date to meet conditions of environment.

(b) Inherited Characteristics Concerned With Fecundity In Relation to Winter Pause.

In the category of inherited fecundity traits that may be considered in their relation to winter pause, the following may be grouped: age at first egg, weight at first egg, winter rate or intensity, length of winter cycle, size of winter clutch, annual rate or intensity and annual persistency. A study of the relative degree of correlation between these inherited characteristics and duration of winter pause as well as its presence or absence furnishes constructive information in breeding for high egg yield. Such analyses bring out important relationships as well as pointing out possible cases of genetic linkage.

11. Correlation Between Age at First Egg and Length of Winter Pause.

Age at first egg marks sexual maturity in the pullet. Age at first egg is inherited in Mendelian fashion according to Hays (loc. cit.). The importance of early laying to high winter and annual egg yield has been stressed in our publications as well as in those of other workers. The significance of knowing if there is a correlation between age at first egg and duration of winter pause is therefore very evident, since both are inherited traits and both are concerned in winter and annual egg yield. A study was therefore made on the 2134 pause birds already considered in sections 7 and 9. Age at first egg class intervals of ten days are used and the same class interval used for length of winter pause. The following constants were calculated:

Number of birds	2134
Mean age at first egg	203.26
Age at first egg standard deviation	±26.28
Mean length of winter pause	32.26
Winter pause standard deviation	±21.92
Coefficient of correlation	—.2329 ± .0138
Regression of age at first egg on winter pause . .	—.279
Regression of winter pause on age at first egg . .	—.194

The above coefficient of correlation is almost identical with that between hatching date and winter pause duration given in section 7. The range in hatching date covers 49 days, while the range in age at first egg covers 180 days. The fact therefore becomes evident that a slight change in hatching date would cause a greater change in winter pause duration than would the same change in age at first egg, as brought out by their respective regression coefficients. Herein lies the reason for emphasizing hatching date as of greater significance in relation to winter pause than age at first egg when they exhibit identical coefficients of correlation to winter pause duration.

12. Correlation Between Age at First Egg Below the Mean and the Presence of Winter Pause for the Total Population.

Age at first egg	Pause	Non-Pause
Earlier than Population mean	1337	549
Later than Population mean	797	692
Totals	2134	1241

Coefficient of correlation . . . . +.3578 ± .0101

Attention should be called to the fact that time of beginning to lay and age at first egg each show almost identical correlation coefficients to the presence of winter pause. The interpretation is that age at first egg is the chief determinant of time of beginning to lay when the hatching dates are constant from year to year.

13. Correlation Between Weight at First Egg and Length of Winter Pause.

Available for this study are the records of 2106 birds, classed as pause birds, on which the body weight on the day of laying their first egg was secured. Thus a very small number of the 2134 birds previously considered is omitted from this study. The class interval used for body weight was the half pound and the ten-day class interval was again used for winter pause. The following constants were determined:

Number of birds	2106
Mean weight at first egg	5.55
Weight at first egg standard deviation . . .	±.72
Mean length of winter pause	32.32
Winter pause standard deviation	±22.01
Coefficient of correlation	+.0161 ±.0147
Regression of weight on winter pause . . .	+.0005
Regression of winter pause on weight . . .	+.4908

A range in body weight from 3.25 to 8.25 lbs. occurs in the population studied. The magnitude of the standard deviation in weight indicates, however, that extremely small or extremely large birds are the exception, since the coefficient of variability for body weight is only about 13 per cent.

The coefficient of correlation between body weight and winter pause duration is mathematically insignificant. This furnishes rather concrete evidence that a pullet's body weight when she lays her first egg bears no relation to the length of her winter pause.

14. Correlation Between Body Weight at First Egg Lower Than the Mean and the Presence of Winter Pause for Entire Population.

Weight at First Egg	Pause	Non-Pause
Below Population Mean	1139	655
Above Population Mean	967	544
Totals	2106	1199

Coefficient of correlation . . . . —.0110 ± .0117

The complete independence between weight at first egg and the presence of winter pause is shown by the above correlation coefficient. Evidently body weight is not a factor in either the manifestation of winter pause or its duration.

15. Correlation Between Winter Rate and Length of Winter Pause.

The group of 2134 birds exhibiting winter pause is used in these calculations. Winter rate or intensity was calculated for each individual bird in the following manner:—

The total number of eggs from first egg to March first was divided by the number of days from first egg to March first, less all pauses of four or more days in duration from November first to March first. By this method of calculation the actual net rate of laying is arrived at if the assumption is correct that a cessation of laying for four or more days during winter actually constitutes a winter pause. A four-day cessation of laying may generally be assumed to necessitate the omission of one clutch of eggs for the average bird and such omissions suggest the manifestation of winter pause. The following constants were calculated:

Number of birds	2134
Mean winter rate	65.69
Winter rate standard deviation	±8.74
Mean length of winter pause	32.26
Winter pause standard deviation	±21.92
Coefficient of correlation	—.1023 ± .0144
Regression of rate on winter pause	—.041
Regression of winter pause on rate	—.257

The above mean winter rate expresses the net rate of laying of all birds exhibiting winter pause. This rate of laying is compared in section 16 with that of the total population and that of the non-pause group above. The standard deviation for rate is of moderate magnitude compared with the standard deviation of many other fecundity characteristics.

A small but significant negative correlation suggests a very moderate tendency for high-rate birds to pause for a shorter period than do low-rate birds. Such a relationship is important from the breeding standpoint in that it

hints at some linkage relation between the dominant genes for high winter intensity and the recessive gene for non-pause.

16. Correlation Between Winter Rate Below the Mean and the Presence of Winter Pause for the Total Population.

Winter Rate	Pause	Non-Pause
Below Population Mean	1688	784
Above Population Mean	446	454
Totals	2134	1238

Coefficient of correlation . . . . +.3734 ± .0100

Winter rate as used in all the calculations is the net rate of laying with all pauses of four or more days deducted. The above table shows the relation of net rate of laying to the presence of winter pause. This table displays a moderately intimate relation between low net rate and the presence of winter pause.

17. Correlation Between Size of Winter Clutch and Length of Winter Pause.

Size of clutch represents the number of eggs laid on successive days. In very extreme cases a pullet may lay as many as fifty eggs in succession previous to March first and the same bird may exhibit a few clutches of one. In order to arrive at a constant to represent the clutch size of an individual bird, it has been necessary to calculate mean clutch size during the winter. Such calculations have been made on all pause birds. The range in mean clutch size of individuals was found to be from 1 to 11.9. The class interval used was 1. Only one bird was omitted from this study because its class range fell between 15 and 15.9. Clutch size is really a measure of intensity of laying. Its relation to winter pause duration is of marked significance in breeding for fecundity.

Constants obtained in this correlation study are as follows:

Number of birds	2133
Mean winter clutch size	2.41
Winter clutch standard deviation	±1.11
Mean length of winter pause	32.27
Winter pause standard deviation	±21.92
Coefficient of correlation	—.0674 ± .0145
Regression of winter clutch on winter pause . .	—.003
Regression of winter pause on winter clutch . .	—1.325

On the average, winter clutch size closely approaches 2.5 but the magnitude of its standard deviation indicates considerable variability in clutch size. A small negative correlation was discovered between clutch size and winter pause duration. While this correlation is significant as judged by its prob-

able error, it is of such small magnitude as to indicate practical independence between the characteristics being considered.

18. Correlation Between Winter Clutch Size Below the Mean and the Presence of Winter Pause for Entire Population.

Winter Clutch	Pause	Non-Pause
Below Population Mean	1425	616
Above Population Mean	709	624
Totals	2134	1240

Coefficient of correlation . . . . . . +.3412 ± .0103

A significant positive correlation between small winter clutch and the presence of winter pause appears above. In general, there is a greater tendency for birds that lay in small clutches to pause than for birds laying in larger clutches. The rate of functioning of the reproductive system must therefore bear a relation to winter pause.

19. Correlation Between Annual Rate or Intensity and Length of Winter Pause.

Annual rate represents or approximates the intensity of each individual bird for the pullet laying year. Inasmuch as this constant has been discussed in Technical Bulletin No. 7 of this station, space will not be occupied here by further discussion. Since winter pause represents a period of non-production, there must of necessity exist a negative correlation between annual rate and length of winter pause unless pause birds lay at a higher net rate than non-pause birds. This last point is, in part, discussed in section 16 of this report, where the net winter rate of the total population in relation to the pause and non-pause groups is considered. The important positive relation between annual rate and annual egg record makes the correlation between annual rate and length of winter pause of importance. Included in this study are the 1348 birds exhibiting winter pause and having complete annual records. The following constants were determined:

Number of birds	1348
Mean annual rate	53.79
Annual rate standard deviation	±9.07
Mean length of winter pause	32.29
Winter pause standard deviation	±21.77
Coefficient of correlation	—.4091 ± .0153
Regression of annual rate on winter pause	—.170
Regression of winter pause on annual rate	—.982

The mean annual rate of laying is lower than the mean winter rate of laying, which is 65.69. This difference may be attributed largely to the fact that in calculating annual rate no account is taken of winter pause or of broody pauses. In the winter rate calculations, winter pause days are not included and very few birds become broody before the end of the winter season. The standard deviation in annual rate is relatively small and suggests uniformity in annual rate of laying.

The coefficient of correlation is negative and of such magnitude as to indicate a significant relation between rate and length of pause. In other words, low annual rate and long winter pause tend to move together. In breeding for high annual intensity, winter pause must certainly be reduced in duration.

20. Correlation Between Annual Rate or Intensity Below the Mean and the Presence of Winter Pause for the Total Population.

Annual Rate	Pause	Non-Pause
Below Population Mean	858	209
Above Population Mean	490	593
Totals	1348	802

Coefficient of correlation . . . . +.6619 ± .0081

The substantial magnitude of the above coefficient of correlation points to a pronounced tendency for low annual rate to occur with winter pause. The table above also shows that 80 per cent of the low-rate birds are pause birds while only 45 per cent of the high-rate birds are in the pause group. The conclusion, therefore, seems justified that winter pause operates very significantly to lower the annual rate of laying.

21. Correlation Between Annual Persistency and Length of Winter Pause.

Annual persistency represents the number of days of laying from the first egg to a pause of thirty or more days after March first. If no thirty-day pause occurs between March first and the date 364 days after the first egg, the bird is given a persistency of 365 days on ordinary years and 366 days on leap years. A cessation of laying for a period of thirty days or more during summer is a rather dependable indication of the onset of complete molt, which always signifies the conclusion of the biological laying year.

Persistency as indicated by time of molting has long been recognized as affecting egg yield, and poultry investigators have recommended the use of late molting birds for breeding purposes. Hurst (1921) was the first to offer a definite hypothesis concerning its mode of inheritance. He believes high persistency is transmitted as a single factor recessive. If a rest period in winter enables the bird to lay later in the fall than does the bird without the rest period, then persistency must depend in part upon the previous physiological activity of the reproductive organs, or possibly there is linkage between winter pause and high persistency. The same group of 1348 birds used in the two previous sections is studied below. Persistency range lies between 67 and 366 days with class intervals of 15 days. Following are the constants:

Number of birds	1348
Mean annual persistency	309.03
Annual persistency standard deviation	±54.89
Mean length of winter pause	32.39
Winter pause standard deviation	±21.77
Coefficient of correlation	+.1017 ± .0182
Regression of persistency on winter pause	+.256
Regression of winter pause on persistency	+.040

Mean annual persistency closely approaches ten months, but the range of variability is rather wide as shown by its standard deviation. This variability is no doubt due in part to many environmental influences as well as to differences in the inherited capacities of the birds. Only about five per cent of the population fall below 200 days in persistency so that the range 200 to 366 is a close approximation of the actual range. A study of frequency distribution for persistency does not reveal a bimodal curve as might be expected for a population made up of genetically early and late molting birds. Such information suggests two possibilities, namely, that environmental infiuences completely obscure the genetic phenotypes, or else that high persistency is not inherited in simple Mendelian fashion. The mode of inheritance of persistency is out of the scope of this report.

A small but significant positive correlation coefficient exists between persistency and winter pause duration. Thus there is a very slight tendency for birds with long winter pause to lay later in the fall than do short pause birds. Relatively little significance should be attached to a constant of such small magnitude, however.

22. *Correlation Between Annual Persistency Greater Than the Mean and the Presence of Winter Pause for the Total Population.*

Annual Persistency	Pause	Non-Pause
Above Population Mean	855	423
Below Population Mean	493	378
Totals	1348	801

Coefficient of correlation . . . . +.2156 ± .0139

A moderate degree of correlation is shown between the presence of winter pause and high persistency. There is thus a slight tendency for pause birds to lay later in the fall than do non-pause birds. Possibly the functional ability of the reproductive organs is somewhat extended by a period of nonproduction in winter. The relation does not appear to be pronounced, however.

(c) The Absolute Relationship of Winter Pause to Egg Production.

The duration of winter pause may be considered a factor affecting the number of eggs laid before March first as well as for the entire year. Since winter fecundity alone depends upon the inheritanee of seven pairs of Mendelian factors (Hays, 1924), it is desirable and necessary to know something of the relation of winter pause to winter and annual egg record. Although fecundity is very complex in its mode of inheritance, its manifestation depends in part upon environmental conditions as division (a) of this report shows. The correlation between size of winter clutch and winter egg yield is first considered, then the correlation between winter pause and winter egg record, and finally the correlation between winter pause and annual production is studied.

23. Correlation Between Size of Winter Clutch and Winter Egg Yield.

The relation of winter clutch size to winter pause has already been considered in sections 17 and 18. In this section the relation of winter clutch size and winter egg production are studied. Since size of winter clutch is so often used as a criterion for selection by poultrymen, knowledge of its relation to winter fecundity is important. Records are available on 3376 birds upon which the following constants were ascertained:

Constant	Value
Number of birds	3376
Mean size of winter clutch	2.57
Winter clutch standard deviation	±1.23
Mean winter production	61.08
Winter production standard deviation	±25.79
Coefficient of correlation	+.4725 ± .0090
Regression of winter clutch in production	+.023
Regression of production on winter clutch	+9.884

The fact will be observed that the mean winter production above is greater than the mean length of winter cycle given in section 6. The mean length of winter cycle is less than mean winier production because the end of the winter cycle is determined by a four-day pause before March first while winter egg record does not cease until February 28 or 29. Winter egg record is highly variable on account of the complexity of its inheritance.

The magnitude of the above correlation coefficient emphasizes an important tendency for clutch size and winter production to move together. As a criterion of winter fecundity large clutch size is very important.

24. Correlation Between Length of Winter Pause and Winter Egg Record.

Winter pause represents a definite period of non-production, but the tendency of winter pause and winter production to move in opposite directions can only be measured by means of the coefficient of correlation. The group of 2134 pause birds has been tabulated to give this relationship. The following are the constants obtained:

Number of birds	2134
Mean length of winter pause	32.26
Winter pause standard deviation	± 21.92
Mean winter production	56.87
Winter production standard deviation	± 23.51
Coefficient of correlation	− .2873 ± .0131
Regression of winter pause on production	− .268
Regression of production on winter pause	− .308

Mean winter production is lower in the above group of pause birds than for the total population given in section 23 because non-pause birds tend to have higher winter records than do pause birds. About the same degree of variation in winter records occurs in both cases.

A rather significant negative correlation coefficient shows that in general an increase in length of pause is associated with a decrease in number of winter eggs. A coefficient of much greater magnitude would appear if the time element were the only consideration. There is the possibility that pause birds tend to possess desirable fecundity traits that are lacking in non-pause birds.

25. Correlation Between Winter Production Below the Mean and Presence of Winter Pause for Total Population.

Winter Production	Pause	Non-Pause
Below Population Mean	1273	463
Above Population Mean	861	777
Totals	2134	1240

Coefficient of correlation . . . . +.4255 ± .0095

The above table illustrates a rather pronounced correlation between low winter egg production and the presence of winter pause. Winter pause has, therefore, proven to be a trait inimical to high winter egg record throughout the nine-year period of the experiment here reported.

26. Correlation Between Length of Winter Pause and Annual Production.

There are available for study 1318 pause birds with annual egg records. Tabulations have been made to discover how the length of winter pause affects annual egg production. Following are the constants:

Number of birds	1348
Mean length of winter pause	32.39
Winter pause standard deviation	±21.77
Mean annual production	172.51
Annual production standard deviation	±41.07
Coefficient of correlation	—.2107 ± .0176
Regression of winter pause on production	—.112
Regression of production on winter pause	—.398

The mean annual record of the pause birds throughout the period is about 173 eggs. The range of variation in annual egg yield is wide, as is shown by its standard deviation. Greater homogeneity in heritable factors concerned in fecundity should reduce such variability.

The magnitude of the coefficient of correlation is sufficient to indicate that, in general, an increase in length of winter pause is accompanied by a decrease in annual egg production. The time lost in winter pause is not compensated for by heavier production either before or after the pause in any class of pause birds.

27. *Correlation Between Annual Production Below the Mean and the Presence of Winter Pause for the Total Population.*

Annual Production	Pause	Non-Pause
Below Population Mean	715	301
Above Population Mean	633	501
Totals	1348	802

Coefficient of correlation . . . . +.3056 ± .0132

Low annual production is significantly correlated with the presence of winter pause as shown in the above table. Even though such a short period as a four-day pause is considered, this correlation coefficient is of appreciable magnitude. Winter pause must, therefore, be classed as inimical to highest annual egg yield, for the pause birds averaged but 173 eggs while the non-pause group averaged 189 eggs.

General Discussion and Summary.

The length of the winter laying cycle is unquestionably modified by a series of environmental influences. Some of these influences are within while others are beyond control of the poultry breeder. Winter pause is the complement of the winter laying cycle and is important in that it vitally affects total fecundity.

Two distinct classes of pullets appear in the flock studied, namely, pause and non-pause. A group of pause birds studied beside a group of non-pause birds, both groups hatched on the same date and both groups starting

to lay at the same age, yet the first showing a distinct winter pause, places the difference in the groups as inherent. Such a study within the family gives definite ratios of pause and non-pause pullets as Hays (1924) points out.

The line of demarkation between genetically non-pause birds that exhibit winter pause due to environmental influences and birds carrying the dominant factor (M) for pause cannot be drawn. The present paper is devoted to a consideration of the non-heritable and some heritable factors that may or may not affect winter cycle and winter pause. Genetic factors concerned with the inheritance of winter cycle length and winter pause duration have not been dealt with.

The major teachings of this study may be summarized:

1. In general, early-hatched pullets have a longer laying cycle than late-hatched pullets of the same flock.

2. Date of first egg exhibits a rather intimate negative correlation to length of winter cycle.

3. Age at first egg shows an appreciable negative correlation to length of winter cycle.

4. The winter egg record is intimately positively correlated with length of winter cycle.

5. Annual egg production is significantly correlated with length of winter cycle though less intimately than is winter record.

6. A minor though significant degree of negative correlation appears between length of winter cycle and length of winter pause.

7. Hatching date bears a significant but not intimate negative correlation to length of winter pause in the pause population.

8. Early hatching is positively correlated with the presence of winter pause in the total population of pause and non-pause birds.

9. Time of beginning to lay is significantly negatively correlated with length of winter pause in the pause population.

10. Time of beginning to lay is appreciably positively correlated with early hatching in the total population.

11. Age at first egg shows the identical degree of negative correlation to length of winter pause that it shows to *length* of winter cycle.

12. Early sexual maturity is positively correlated with the presence of winter pause in the total population.

13. Weight at first egg is independent of length of winter pause.

14. Light weight at first egg is not correlated with the presence of winter pause in the total population.

15. The net winter rate of laying holds a very slight negative correlation to length of winter pause in the pause population.

16. Slow rate of winter laying is rather intimately positively correlated with the presence of winter pause in the total population.

17. The average size of winter clutch is but very slightly correlated with length of winter pause in the pause population.

18. Small size of winter clutch is moderately positively correlated with the presence of winter pause in the total population.

19. Annual intensity shows a considerable degree of negative correlation to length of winter pause.

20. A very intimate positive correlation exists between low annual intensity and the presence of winter pause.

21. Annual persistency is but slightly positively correlated with length of winter pause.

22. Birds that pause during winter show a tendency to lay later in the fall than non-pause birds.

23. The mean size of winter clutches is rather intimately positively correlated with winter fecundity. Clutch size is a very good measure of intensity.

24. Length of winter pause is negatively correlated with winter production.

25. Low winter production exhibits a considerable degree of correlation to the presence of winter pause in the total population.

26. Length of winter pause is negatively correlated with annual egg record in the pause population.

27. Annual production below the mean is substantially correlated with the presence of winter pause in the total population.

28. Although winter cycle and winter pause are complements of each other, they are practically independent in duration in the pause group.

29. Winter pause is definitely shown to be a characteristic detrimental to both winter and annual fecundity, and should therefore be eliminated from flocks bred for egg production.

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MASSACHUSETTS

AGRICULTURAL EXPERIMENT STATION

TECHNICAL BULLETIN No. 9 OCTOBER, 1926

ANNUAL PERSISTENCY IN RELATION TO WINTER AND ANNUAL EGG PRODUCTION

By F. A. HAYS and RUBY SANBORN

This bulletin is the third in a series dealing with the five inherited traits in relation to fecundity. Those already published show the relation of broodiness and of winter pause to egg production; while a later publication will consider intensity in relation to fecundity.

The records show that high persistency is a trait much to be desired from the standpoint of production, and that there is no reason why it may not be combined with other desirable traits in the same individual.

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AGRICULTURAL EXPERIMENT STATION

AMHERST, MASS.

ANNUAL PERSISTENCY IN RELATION TO WINTER AND ANNUAL EGG PRODUCTION

By F. A. HAYS and RUBY SANBORN

Annual persistency, as terminated by the onset of complete molt, has been emphasized for more than the past three decades as of marked significance in the selection of breeding females for egg production. The cessation of egg production in summer or fall is generally accompanied by a complete change of plumage and this period of non-production may continue for 30 to 120 days. The exceptional hen may lay a considerable number of eggs while molting, but such individuals are of infrequent occurrence.

Hurst (1925) classifies laying hens into complete and partial-molt classes and states that there is complete cessation of laying in the first class while the second class sheds its feathers gradually and continues to lay for 13 or 14 months after the first pullet egg. According to Hurst, complete early molt depends upon the inheritance of a dominant Mendelian gene.

Goodale and Sanborn (1922) note that cessation of production in the summer or fall at the end of the pullet laying year has a genetic foundation as indicated by the behavior of families in this respect. Data collected on the Massachusetts Agricultural Experiment Station flock of Rhode Island Reds show that the biological laying year may extend to 14 or 15 months as a maximum with 6 or 7 months as the minimum for normal birds. A study of all factors affecting the duration of the pullet laying year in the flock in question has not yet been completed.

A flock bred for egg production should theoretically consist of two general classes of birds with respect to persistency, namely, a high persistent class and a low persistent class. In reality these two classes do not stand out distinctly to form a bimodal curve when all the birds with annual records for the nine-year period are tabulated in persistency classes using 15-day class intervals. (See chart 1.) The probability exists, however, that environmental forces largely obscure these expected classes. A tabulation of the 2179 birds with annual persistency records does give a frequency distribution that is indistinctly bimodal and furnishes the basis for classification of those birds laying for a shorter period than 315 days as low in persistency and those laying for 315 days or longer as high in persistency. Such a classification is largely arbitrary, however, and is used in these studies only as a working basis until the true genetic point of division may be discovered.

Scope of This Report

This study was undertaken for a three-fold purpose, namely, to show (a) the relation between controllable environmental conditions and persistency, (b) the relation between inherited characteristics concerned with fecundity and persistency, and (c) the relation between persistency and fecundity. From the practical breeding standpoint these considerations are of great im-

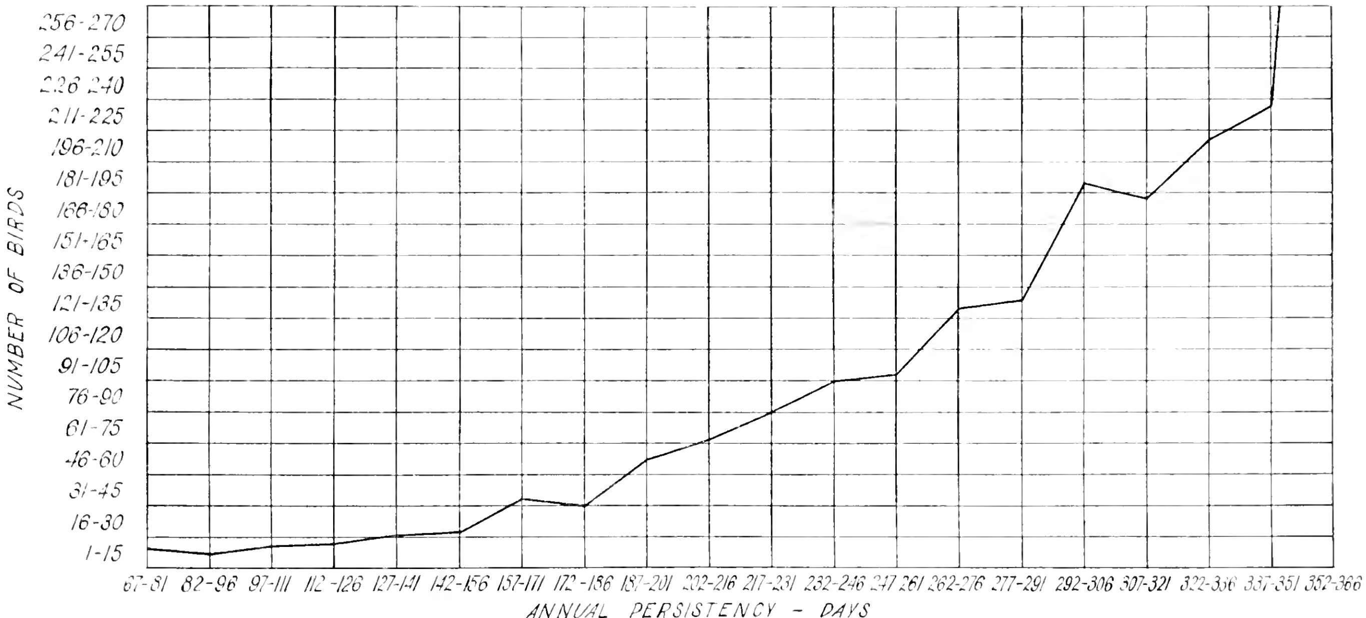

Chart 1. Frequency Distribution of Population with Regard to Persistency.

portance. A knowledge of the part played by environment as well as the part played by inheritance in limiting the manifestation of a desirable characteristic is well worth considering when breeding for egg production. Analytical studies on the complex nature of fecundity should further disclose valuable information that might otherwise be obscured.

Character of Birds Used

The birds used in this study are identical with those used in the two previous reports. Included are the records of all Rhode Island Red females hatched from 1916 to 1924 in the experimental flock upon which pullet-year trapnest records are available. The flock each year is made up of all the daughters of each hen whose progeny was retained. The major portion of the birds belong to the fecundity experiment. There are, however, a limited number of birds bred for non-broodiness, some for intense broodiness, some for hatchability, some for color, and a few inbreds that are included. Pullet-year records are used exclusively in this study.

The Coefficient of Correlation

The simple coefficient of correlation is subject to certain limitations in biological data yet it affords a basis upon which to select groups of breeders and also a basis for predicting future possibilities. The fact is self-evident that the simple coefficient of correlation is not an absolute measure of the degree of association between the variables being studied because each variable may be partially dependent upon other variables. For example, section 3 shows a negative correlation of .6146 ± .0090 between age at first egg and annual persistency. It is a known fact that both the dependent variable, age at first egg, and the independent variable, annual persistency, are dependent upon hatching date and environmental conditions, and that persistency is also dependent upon weight at first egg and possibly upon length of winter pause. The true relation between age at first egg and annual persistency could only be arrived at by making the hatching date and environmental conditions constant, as well as by making weight at first egg and winter pause duration constant. Such procedure necessitates the use of partial coefficients of correlation which require the use of the simple coefficient in their calculation. Both the partial coefficient and the multiple coefficient will be employed in a concluding bulletin of the series. Simple correlations are of very significant practical value to the poultryman, however, in that they show him the relative importance of different environmental influences and inherited characteristics in relation to fecundity, and enable him to formulate his breeding program accordingly.

(A) Relation Between Environmental Conditions and Persistency

The only controllable environmental condition that will be considered in relation to persistency is hatching date. Extreme care has been exercised throughout the experiment to employ the same methods of feeding and brooding. Hatching dates have been kept constant each year, but there have been eight hatches each year at weekly intervals between March 25 and May 15. The range in hatching date thus amounts to 49 days. Hatching date, however, may be controlled at will by the poultryman.

1. *Correlation Between Hatching Date and Annual Persistency.*

The class interval for hatching date is seven days, and the class interval for annual persistency is 15 days with a range of from 67 to 366 days. The following constants were calculated on 2179 birds:

Number of birds	2179
Mean hatching date (April 18)	4.28
Hatching date standard deviation	±2.24
Mean annual persistency	300.47
Persistency standard deviation	±62.64
Coefficient of correlation	—.2208±.0137
Regression hatching date on persistency . .	—.008
Regression persistency on hatching date . .	—6.187

The above constants indicate that, on the average, the birds laid for 300 days before the onset of complete molt. This figure is somewhat lower than it would be if maximum persistency had not been placed at 366 days, because some of the birds laid for a greater time interval. The standard deviation in persistency amounts to almost 63 days and furnishes statistical evidence of very marked variability in persistency.

The coefficient of correlation between hatching date and annual persistency is negative and statistically significant. While this is not an intimate correlation, it does demonstrate a tendency for early-hatched birds to lay longer than late-hatched birds. The fact should be kept in mind, however, that the earliest hatch was taken off each year about March 25 and that this date should not be considered very early in this latitude.

2. *Correlation Between Hatching Date Earlier than the Mean and High Persistency.*

As previously stated, the birds have been divided into two classes with regard to persistency, namely, high and low. All birds are classed as *high* in persistency when they lay for 315 days or more before molting. Birds laying for a shorter period than 315 days are classed as *low* By dividing the population of 2179 birds into these two classes for persistency, and by again classifying these as hatched earlier or later than the population mean, an absolute measure of the correlation between early hatching and high persistency is obtained. The results of this classification follow:

Hatching Date	High Persistency	Low Persistency
Earlier than population mean	720	458
Later than population mean	416	585
Totals	1136	1043

Coefficient of correlation . . . . +.3771±.0124

The division of the population into high and low persistency groups in the above table rests on a possible genetic foundation as already stated. The mean persistency of the entire 2179 birds was found to be about 300 days as section 1 shows. When the point of division between high and low persistency birds is taken between 314 and 315 days, there are 1136 individuals classifying as high and 1043 as low in persistency. The low persistency class ranges from 67 to 314 days while the high persistency class ranges from 315 to 366 days. The wide range for the low class enables them to bring the mean persistency of the population down to 300 days even though there are more high-persistency birds than low-persistency birds in the above classification.

A positive coefficient of correlation .3771±.0124 signifies that early hatching is associated with high persistency. Possibly early hatching better equips the pullet for a long laying year because she begins to lay earlier in the fall and is also able to finish her laying year under more favorable weather conditions than is her late-hatched sister. These data signify, therefore, that hatching before the middle of April tends to increase persistency for the pullet year.

(B) Relation Between Inherited Characteristics Concerned with Fecundity and Annual Persistency.

In the class of inherited characteristics concerned with fecundity the following will be considered in relation to annual persistency: Age at first egg, weight at first egg, winter rate, length of winter pause, and total days broody, all records being based on the pullet year.

3. *Correlation Between Age at First Egg and Annual Persistency.*

Both age at first egg and annual persistency have been found by Goodale and Sanborn (loc. cit.) and by the writers to be of appreciable significance in breeding for egg production. Both early maturity and high persistency are essential in the high producer and for this reason their relation to each other should be known. The identical group of birds studied in section 1 is used to determine the following constants:

Number of birds	2179
Mean age at first egg	208.56
Age standard deviation	±31.28
Mean annual persistency	300.47
Persistency standard deviation	±62.64
Coefficient of correlation	—.6146±.0090
Regression age on persistency	—.307
Regression persistency on age	—1.231

Mean age at first egg is about 209 days, which is a figure falling within the limits of genetic early maturity. Age at first egg is a characteristic that fluctuates widely, and in this particular population the extremes are 140 and 379 days, respectively. Class intervals of ten days for age have been used in these correlation studies.

The mean annual persistency of the population is about 300 days. The

extremes are 67 and 366 days, respectively. The standard deviation for persistency is very large and indicates that a number of factors is concerned.

The negative coefficient of correlation is of such magnitude as to suggest an intimate relation between age at first egg and annual persistency. Those pullets that lay at an early age appear to be much more persistent layers than those maturing later. Herein lies a partial explanation of the significant relation between early maturing and high annual production. These studies point to age at first egg as a criterion of importance for predicting persistency.

4. *Correlation Between Age at First Egg Below the Mean and High Persistency.*

The population has again been divided into the two possible genetically different classes for persistency as in section 2. These classes have been tabulated against age below the mean and age above the mean as follows:

Age at First Egg	High Persistency	Low Persistency
Below population mean	860	387
Above population mean	276	656
Totals	1136	1043

Coefficient of correlation . . . . . . +.6816±.0077

A very intimate correlation is shown by the above coefficient between early sexual maturity and high persistency. This relationship is very significant to the breeder, disclosing possible genetic linkage between two desirable inherited traits that may later be cleared up on a factorial basis.

5. *Correlation Between Weight at First Egg and Annual Persistency.*

Body weight is a convenient standard to use for selection purposes. Weight in poultry is inherited on a multiple factor basis according to Punnett and Bailey (1914). If weight should prove a criterion of persistency, its value for culling purposes soon after pullets begin to lay is very evident. Weight records are available on 2125 of the birds being studied, and when correlated with persistency give the following constants:

Number of birds	2125
Mean weight at first egg	5.58
Weight standard deviation	±.75
Mean annual persistency	302.64
Persistency standard deviation	±58.00
Coefficient of correlation	—.3225±.0131
Regression weight on persistency	—.004
Regression persistency on weight	—25.002

This group of birds averaged about five and one-half pounds at first egg and the extremes are 3 and 9.5 pounds, respectively. Class intervals of .5 pound were used in making these studies. Weight shows a coefficient of variability of about 13 per cent.

The coefficient of correlation exhibits something of a tendency for light weight and high persistency to move together. Such a coefficient might have been anticipated from the fact that weight and age at first egg are positively correlated (Hays, Sanborn and James, 1924), and because hatching date and weight at first egg are negatively correlated (Hays, Sanborn, and James, loc. cit.). In view of these facts, it is doubtful if weight at first egg is a true criterion of persistency.

6. *Correlation Between Body Weight at First Egg Below the Mean and High Persistency.*

Weight at First Egg	High Persistency	Low Persistency
Below population mean	714	468
Above population mean	417	526
Totals	1131	994

Coefficient of correlation . . . . . . +.3161±.0132

The above table presents the absolute correlation between weight at first egg below the population mean and high persistency. Those birds weighing less at first egg than the mean of the whole population may be considered small while the high persistency class includes only those individuals laying for 315 days or more before molting.

The coefficient of correlation is positive and of statistical significance. There is a tendency for the persistent class to weigh less at first egg than does the low persistency class. Although the correlation is significant, it is not pronounced and probably does not imply that factors for rapid growth are inimical to high persistency.

7. *Correlation Between Net Winter Rate and Annual Persistency.*

In order to discover if there is any association between the net rate of laying throughout the winter season and persistency of laying the following fall, a correlation table was made between winter rate and persistency, using the 2147 birds with records for both characteristics. The constants are as follows:

Number of birds	2147
Mean winter rate	67.44
Winter rate standard deviation	±8.87
Mean annual persistency	302.98
Persistency standard deviation	±59.03
Coefficient of correlation	+.1835±.0141
Regression winter rate on persistency	+.028
Regression persistency on winter rate	+1.222

A slight but significant correlation is found to exist between winter rate of laying and persistency. This correlation indicates that, in general, there is some tendency for the more intense winter layers to persist in laying later in the fall than do less intense layers.

8. *Correlation Between Winter Rate Greater than the Mean and High Persistency.*

By classifying all birds with higher winter rates than the mean of the whole population as high for rate, and by classing as highly persistent all individuals laying for 315 days or more, the following table gives the correlation between high winter rate and the presence of possible genetically high persistency:

Winter Rate	High Persistency	Low Persistency
Above population mean	654	466
Below population mean	481	546
Totals	1135	1012

Coefficient of correlation . . . . . +.2236±.0138

The above tabulation presents a moderate degree of positive correlation between two inherited characteristics concerned in high fecundity. The very significant fact is brought to light that high winter rate and high persistency are partially complementary, and there is no evidence of antagonism between the two.

9. *Correlation Between Length of Winter Pause and Annual Persistency.*

The presence or absence of winter pause has been shown by Hays (1924) to depend upon genetic factors. The duration of the pause, however, may depend upon environment as well as inheritance. Most environmental forces affecting the duration of pause are probably beyond control of the breeder and may not properly be considered in this report. This section is devoted to a study of the correlation between length of pause and persistency as has already been done by Hays and Sanborn (1926b). In the population being studied there were 1318 birds with winter pause records which were divided into ten-day class intervals and the following constants arrived at:

Number of birds	1318
Mean length of winter pause	32.39
Pause standard deviation	±21.77
Mean annual persistency	309.03
Persistency standard deviation	±54.89
Coefficient of correlation	+.1017±.0182
Regression persistency on pause	+.256
Regression pause on persistency	+.040

Winter pause duration is subject to extreme fluctuations. Its range extends from 4 to 130 days. The magnitude of its standard deviation indicates that its duration is affected by a considerable number of variables.

The above coefficient of correlation gives a statistically significant yet far from pronounced correlation between length of winter pause and annual persistency. There is but a very slight tendency for long-pause birds to persist longer than do short-pause birds.

10. *Correlation Between Annual Persistency Above the Mean and the Presence of Winter Pause.*

This section is devoted to a consideration of the presenee of winter pause and annual persistency above the population mean of 303.20 days. Such a correlation will bring out any possible association between the heritable characteristic, winter pause, and high persistency which, in this instance, means persistency greater than the mean of the population studied. The classification follows:

Annual Persistency	Pause	Non-Pause
Above population mean	855	423
Below population mean	493	378
Totals	1348	801

Coefficient of correlation . . . . . . +.2156±.0139

The correlation coefficient is significant though of only moderate magnitude. Possibly winter pause birds tend to lay for a slightly longer period than do non-pause birds because the former are more likely to be early-hatched (Hays and Sanborn 1926b), and early-hatched birds tend to be more persistent than late-hatched birds. The exact relation between pause and persistency can only be discovered through the partial coefficient of correlation and will be reported in a later publication.

11. *Correlation Between Total Days Broody and Annual Persistency.*

The heritable trait, broodiness, will next be considered in so far as its intensity as measured by total days broody is correlated with persistency. Only the pullets that exhibited broodiness during their first laying year are used to obtain the constants below:

Number of birds	1037
Mean total days broody	12.69
Broody standard deviation	±27.33
Mean annual persistency	294.05
Persistency standard deviation	±64.82
Coefficient of correlation	+.0532±.0209
Regression days broody on persistency	+.022
Regression persistency on days broody	+.126

Intensity of broodiness, as measured by the total days spent in broodiness during the pullet year is subject to wide fluctuations. Its standard deviation shows marked variability in the population. In view of this fact, it seems probable that intensity of broodiness depends on a number of variables.

The small and statistically insignificant coefficient of correlation indicates practical independence between degree of broodiness and annual persistency.

12. *Correlation Between Annual Persistency Above the Mean and the Presence of Broodiness.*

This section deals with the absolute relation between the presence of the inherited characteristic, broodiness, and persistency greater than the mean of the population studied. Herein lies a definite basis for selection which may or may not be useful in breeding for high persistency. The following results appear:

Annual Persistency	Broody	Non-broody
Above population mean	566	715
Below population mean	471	390
Totals	1037	1105

Coefficient of correlation . . . . —.2081±.0139

The above negative correlation coefficient is statistically significant though it does not reveal an intimate correlation. Eliminating the broody characteristic should in some measure increase annual persistency.

(C) The Relation Between Persistency and Fecundity

Since high annual persistency appears to be a desirable characteristic to develop from several standpoints, it is highly desirable that its relation to both winter and annual egg record be ascertained. Both relations may be considered first from the quantitative standpoint and then from the qualitative standpoint by use of long and short correlation tables, respectively.

13. *Correlation Between Winter Production and Annual Persistency.*

Winter production during the pullet year is represented by the number of eggs laid from first egg to the end of February. It has already been pointed out by many workers as a valuable criterion of annual production. Class intervals of ten have been used to make the correlation table for the 2151 birds with winter records. Constants computed follow:

Number of birds	2151
Mean winter production	62.19
Winter production standard deviation . . .	±25.44
Mean annual persistency	302.82
Persistency standard deviation	±59.32
Coefficient of correlation	+.4551±.0115
Regression production on persistency . . .	+.195
Regression persistency on production . . .	+1.061

The degree of correlation between winter production and annual persistency is positive and of appreciable magnitude. Selection for persistency based on winter records should be of considerable value. Such a condition might be anticipated in view of the high correlation between early maturity and winter production and between early maturity and persistency.

14. *Correlation Between Winter Production Greater Than the Mean and high Persistency.*

In the tabulation below the population is classified into four qualitative groups, namely, high winter producers, low winter producers, possible genetically highly persistent, and possible genetically low persistent. The correlation is then determined between production above the mean and high persistency.

Winter Production	High Persistency	Low Persistency
Above population mean	712	345
Below population mean	424	670
Totals	1136	1015

Coefficient of correlation . . . . +.5306±.0104

This coefficient of correlation demonstrates a positive relation between high winter egg record and high persistency. In other words, selection based on winter records greater than the average should increase the percentage of late-molting or persistent birds.

15. *Correlation Between Annual Production and Annual Persistency.*

Other conditions being equal, any increase in persistency should be accompanied by an increase in annual egg yield. These are purely quantitative relations and in this manner some information concerning the value of high persistency from the fecundity standpoint may be ascertained. The same population of 2179 individuals is tabulated, using ten-day classes for production, to obtain the following constants:

Number of birds	2179
Mean annual production	177.46
Production standard deviation	±44.73
Mean annual persistency	300.47
Persistency standard deviation	±62.64
Coefficient of correlation	+.7082±.0072
Regression production on persistency	+.506
Regression persistency on production	+.992

The above coefficient reveals an intimate correlation between annual egg yield and annual persistency or the length of the laying year. These data furnish definite evidence to commend the practice of emphasizing late molting in breeding for high fecundity. On the average, any increase in persistency within the limits of the pullet laying year is advantageous.

16. *Correlation Between Annual Production Above the Mean and High Persistency.*

By classifying all birds as high producers if they laid more eggs than the population mean of 177.46, and as high in persistency those birds that laid for not less than 315 days before molting, a definite relation between high production and high persistency may be established.

Annual Production	High Persistency	Low Persistency
Above population mean	872	280
Below population mean	264	763
Totals	1136	1043
Coefficient of correlation		+.8000±.0052

The above coefficient of correlation establishes a very intimate relation between the presence of possible genetic high persistency and annual egg yield above the average of the total population. This fact points to high persistency as being closely associated with high annual fecundity. High persistency must, therefore, be classed as a trait of vital importance in breeding for fecundity and one that should be stressed greatly by the breeder. Should high persistency breed as a true recessive, it would be a comparatively simple matter to establish the characteristic in the laying flock.

Discussion and Summary.

Annual persistency is a characteristic bearing a vital relationship to fecundity. Its duration is affected by environmental influences and by inherited traits concerned in fecundity. No conclusive evidence is presented in this report to indicate that high persistency behaves as a simple recessive in inheritance as has been suggested by Hurst (loc. cit.). In this climate persistency may be increased to some extent by hatching before April 15, with such birds as are studied here. Early sexual maturity, non-broodiness and high winter rate probably show some linkage with high persistency. At any rate, there is no evidence of antagonism in attempting to combine these desirable traits in the same individual. Valuable information for selection purposes has been disclosed by these studies. Partial correlation coefficients will be used in a later publication to remove some complications.

The general relation of persistency to winter and annual production for the whole population studied is shown in the following table:

Character of Birds	Winter Production	Annual Production
Persistency above population mean	69.84	198.59
Persistency below population mean	51.57	145.67
Persistency of 315 days or more (Mean 347 days)	71.13	201.98
Persistency below 315 days (Mean 249 days)	52.83	150.75

Mean Persistency by Years

Year	Number of Birds	Average Persistency
1916	278	247.53
1917	347	280.74
1918	194	285.49
1920	125	325.29
1921	314	301.00
1922	379	329.67
1923	317	316.33
1924	225	320.47
Total and average	2179	300.19

The chief findings of this report may be summed up as follows:

1. Early hatching is moderately correlated with high annual persistency.

2. Age at first egg is very intimately negatively correlated with high persistency.

3. Weight at first egg shows significant negative correlation to persistency.

4. Winter rate of laying is moderately correlated with persistency. The two traits appear to be partially complementary.

5. Length of winter pause is but slightly positively correlated with persistency.

6. Total days broody is not significantly correlated with persistency.

7. The presence of broodiness shows a fair negative correlation to high persistency.

8. Winter production and persistency are rather significantly positively correlated.

9. Annual production is pronouncedly correlated with persistency.

10. Persistency behaves as a trait much to be desired from the production standpoint.

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AGRICULTURAL EXPERIMENT STATION

TECHNICAL BULLETIN No. 10 NOVEMBER, 1926

THE THERAPEUTIC EFFICIENCY OF AVIAN DIPHTHERIA, ROUP, AND BIRD POX VACCINES AND BACTERINS

By Norman J. Pyle

Avian diptheria, roup and bird pox cause serious loss to Massachusetts poultrymen by decreasing egg production during the season when eggs are bringing the highest prices. In this bulletin the Department of Veterinary Science and Animal Pathology reports results of their study of the problem. A filtrable virus was found to be the cause of all three types of the disease. None of the commercial vaccines produced immunity, neither did they effect a cure when the disease was present, although they caused a slight improvement in the general condition of the birds. Autogenous bacterins, when used in the early stages of the disease, caused an improvement in the general health of the birds, but were not of sufficient value to make their use economically profitable.

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THE THERAPEUTIC EFFICIENCY OF AVIAN DIPHTHERIA, ROUP, AND BIRD POX VACCINES AND BACTERINS

By NORMAN J. PYLE

Introduction

Avian diphtheria, roup, and bird pox have caused serious financial losses to the poultry industry of Massachusetts during the fall and winter months of past years. Diphtheritic roup has been the predominating form of the disease. It has not been attended with great mortality, but has become of grave economic importance because it has caused a decrease in production, occurring at a time of the year when poultry products bring maximum prices.

Two biological products, a powdered pox virus vaccine and an avian mixed infection bacterin, have been used extensively in an attempt to control the disease. The results obtained following the use of the preparations have been confusing. Some reports claim the vaccine to be 100 per cent efficient, while others claim it to be an absolute failure.

Many factors contribute towards the efficiency of the vaccine and bacterin. It is of primary importance to ascertain the nature of the causative microorganism or virus and whether it is incorporated in either of the preparations. When this is accomplished, it is assured that either the vaccine or bacterin is the logical product to develop specific antibodies against the disease.

It is also necessary to determine whether avian diphtheria, roup, and bird pox are separate etiological entities or various manifestations of a common cause. On the answer to this problem depends the need for one common vaccine or bacterin or for separate ones for each entity.

Historical.

Moore (1), a pioneer American worker on avian diphtheria and roup, isolated a non-motile, pathogenic bacillus from lesions of the disease. He claimed that this organism was "apparently the etiological factor". He was unable, however, to determine its specificity for the affection. Harrison and Streit (2) demonstrated that *Bacillus pyocyaneus* would produce typical lesions of avian diphtheria and roup. These authors also found a second virulent bacterium associated with the diseases, which they called the roup bacillus or *B. cacosmos.* Hausser (3), Bordet and Fally (4), Beach, Lothe and Halpin (5), and Crofton (6) have all added specific organisms to the long list of causative factors.

Bird pox or contagious epithelioma has not been studied from the standpoint of its etiology to the extent that has avian diphtheria. Marx and Sticker (7) reported investigations wherein they found a filtrable virus to be the cause of bird pox. Schmid (8) and Sigwart (9) confirmed this work. V. Betegh (10), De Blieck and V. Heelsbergen (11), and others advanced the theory that all forms of the disease are caused by one and the same virus.

Several references in the early literature maintain that the various poxes, skin eruptions, and variola affecting animal life are all caused by a common virus, which adapts itself over a period of successive generations to a specific host. If this were true, vaccinia or cowpox would have an etiological relationship to bird pox.

Lowenthal, Kadowaki, and Kondo (12) were able to transmit vaccinia to the fowl through five successive generations, but the affections became less and less pronounced and finally died out. Fowls recovering from vaccinia were immune to vaccinia, and those recovering from bird pox were immune to bird pox. They were unable to produce a neutral or combination immunity and concluded that the causes of vaccinia and bird pox were very different.

EXPERIMENTAL DATA ON THE ETIOLOGY OF AVIAN DIPHTHERIA ROUP, AND BIRD POX.

Bacteriological examinations of diphtheritic patches were made and many organisms were isolated, the majority of which were contaminating invaders. In order to avoid this the patches were aseptically removed and the bacteriological examination made directly from the underlying, denuded surface. The same technic was employed in the pox form of the disease; that is, bacteriological cultures were made from the pitted areas after removal of the pox scabs.

Pseudomonas aeruginosa (*Bacillus pyocyaneus*) was found associated with pox and diphtheritic lesions. This organism has been previously observed in diphtheritic roup by Harrison and Streit (2), Hausser (3), Jackley (13), Kaupp (14), and others. Various other pyogenic bacteria were isolated, namely, *Staphylococcus aureus*, *Gaffkya* (*Staphylococcus*) *tetragena*, and *Staphylococcus albus*. A *Pasteurella avicida*-like organism was isolated from infected birds suffering with avian diphtheria, also one similar to the roup bacillus or *Bacillus cacosmus* of Harrison and Streit (2).

These organisms are at least prominent secondary invaders, but their ability to cause diphtheritic roup is in doubt. *Pseudomonas aeruginosa*, when found in an infected flock, was readily isolated from the heart blood, liver, and spleen of those birds dead of the disease. The organism was injected into the wing veins of several healthy birds and death ensued in from fifty-six to eighty-four hours. The germ was recovered from the dead fowls, especially from exudates in the nasal passages, indicating that the organism was associated with roup. Other experiments with the organism, such as injection beneath the skin and application to scarified wounds of the comb, wattles, and membranes of the mouth, failed to produce any type of the disease.

Fresh pox scabs obtained from a Massachusetts infected flock were dried, passed through a coffee mill, and finally pulverized in a ball mill. One gram of this powdered virus was macerated for twelve hours and afterwards triturated in 100 cc. of physiological salt solution. It was then passed through a controlled Berkefeld filter of medium porosity. The filtrate was vigorously rubbed into the scarified comb and wattles of healthy birds and failed to reproduce the disease in forty-three days. These birds were susceptible to avian diphtheria and bird pox for they later succumbed to inoculation with the unfiltered virus. The experiment was repeated, using scab virus from two other States, and again using a filter of medium porosity. The results were the same. The experiments were controlled by the respective unfiltered scab viruses which produced typical pox lesions in the usual incubation period.

It is known that the filtrable virus of smallpox will not pass through a filter of fine porosity, but will pass through one of coarser porosity. Accordingly, Berkefeld filters No. V (coarse) were next used and the results recorded in the following table. The filtrate proved "sterile" upon cultural examination.

TABLE 1. Filterability of Pox Virus with a Coarse Berkefeld Filter.

Bird	December 17	December 23	December 29	January 4	January 12	January 28	January 30
No. 1 (healthy)	Comb and wattles scarified. Filtrate applied by vigorous rubbing.	No pox	Several well formed pox nodules.	Pox nodules markedly developed.	Lesions still persistent.	Condition same.	Destroyed.
No. 2 (slight cold)	Comb and wattles scarified. Filtrate applied by vigorous rubbing.	No pox	No pox	No pox	Few well defined "cankers" roof of mouth.	Condition same.	Destroyed.
No. 3 (healthy)	Comb and wattles scarified. Filtrate applied by vigorous rubbing.	No pox	No pox	Small pox nodules on comb and eyelids. 1st stage of roup in evidence	Pox nodules still present. Few "cankers" on membranes of pharynx.	Condition same.	Destroyed.
No. 4 (healthy)	Comb and wattles scarified. Filtrate applied by vigorous rubbing.	No pox	No pox	Several small pox nodules on comb. 1st stages of roup in evidence.	Pox and roup clearing up.	Condition same.	Destroyed.
Control No. 5 (healthy)	Comb and wattles scarified. Unfiltered virus applied by vigorous rubbing	Pox well developed.	Pronounced pox production.	Maximum pox development.	Pox nodules mature.	Condition same.	Destroyed.
Control No. 6 (healthy)	Comb and wattles scarified. Unfiltered virus applied by vigorous rubbing	Pox well developed.	Pronounced pox production.	Maximum pox production.	Pox nodules mature.	Condition same	Destroyed.

Interpretation

1. The filtered virus produced pox, roup, and avian diphtheria, indicating that one and the same virus is capable of causing all forms of the disease.
2. Bird No. 2 had a simple catarrh when inoculated. This evidently lowered the resistance of mucous membrance surfaces and avian diphtheria followed.
3. The incubation period of the filtered virus was from twelve to eighteen days, while in the case of the unfiltered virus it ranged from seven to nine days. The filtered virus also produced a less pronounced form of the infection than did the unfiltered virus. These latter two points confirm the work of Schmid (8) in 1909.

A bacteriological examination of the unfiltered powdered virus revealed several secondary invaders, such as *Pseudomonas aeruginosa* and various Staphylococci. These organisms undoubtedly assisted the unfiltered virus in causing a shorter period of incubation and a more pronounced form of the disease.

THE UNIFORMITY OF THE VIRULENCE OF COMMERCIAL VIRUSES.

Before studying the efficiency of the powdered pox virus vaccines, it was desirable to ascertain the strength of the viruses which make up these commercial products.

Four groups of birds were inoculated on October 5th with four different strains of powdered pox virus. The course of the disease subsequent to the inoculation is represented by the respective lines A, B, C, and D in the following graph. Virus A was obtained on October 1 from a natural infection in Massachusetts and viruses B, C, and D were of commercial origin.

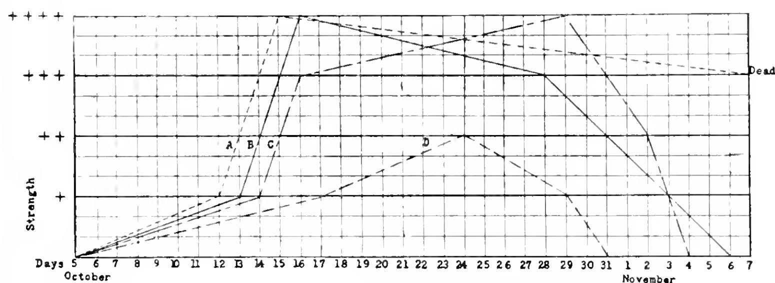

CHART I. Variance in Strength of Powdered Pox Viruses.

-|- Period of incubation.
-|--|- Appearance of a few or several well formed pox nodules.
-|--|--|- Appearance of many pox nodules of mature development.
-|--|--|--|- Maximum development of pox nodules.
Downward curves—Periods of recovery.

Virus A showed the greatest potency. The period of incubation was seven days, the disease reaching its maximum development three days later, and death following within twenty-three days with no appreciable evidence of recovery.

Virus B showed the greatest potency of the three commercial stock viruses. The period of incubation was eight days, maximum development four days later, and complete recovery in twenty-two days more.

Virus C presented an incubation period of nine days, maximum development fifteen days later, and complete recovery within an additional six days.

Virus D was very weak. The period of incubation was twelve days. There was practically no further development of pox and recovery soon took place.

Interpretation.

The degree of efficiency of the powdered pox virus vaccine depends upon the potency and antigenicity of the virus of which it is composed. The following conclusions are then evident:—

1. The viruses, being non-uniform in potency, would produce vaccines of varying efficiency.

2. A method of standardizing the virus and vaccine, which is lacking at the present time, would be essential to the efficiency of the vaccine.

3. An autogenous virus would produce a vaccine of greater value than one composed of a stock virus.

The Efficiency of Powdered Pox Virus Vaccines.

The powdered pox virus vaccine was first used by Manteufel (15) and by Hadley and Beach (16). The vaccine as commercially distributed to-day is a development of the original methods of these workers.

Scabs collected from pox nodules are the source of the virus. In order to produce large quantities of scabs it is necessary to maintain a flock of young cockerels, preferably white leghorns. The combs and wattles are scarified and the powdered scab virus after being "emulsified" in physiological salt solution is vigorously rubbed into the wounded areas. Typical pox scabs will develop and mature on susceptible birds in from seven to twelve days. The scabs are then collected, thoroughly dried, passed through a coffee mill, and finally pulverized in a ball mill. The product is stored away as the stock virus.

The vaccine is made by taking 1 gram of the powdered virus and thoroughly triturating it in 100 cc. of physiological salt solution. It is then attenuated at 55° C. for one hour in a water bath. Finally it is filtered through sterile cheesecloth into vaccine bottles, and after cooling is ready for use. The entire procedure should be handled in as sterile a manner as possible. The vaccine should be used within ten to fifteen days after it is manufactured because it deteriorates rapidly.

In the following experiments having to do with the efficiency of the powdered pox virus vaccines each bird was housed in a separate compartment. The final conclusions are based on a repetition of experiments and the average reaction of a group of birds. The vaccine used was manufactured as described by J. R. Beach (17), a brief description of which is given above.

Experiment 1.

Part A.

A freshly made vaccine, composed of virus B, was administered subcutaneously to a group of six healthy birds, 1 cc. being given to each bird beneath the skin of the breast under the right thigh. The group was divided into three lots of two birds each.

Lot 1. Fourteen days after vaccination both birds were inoculated on comb

and wattles with virus B. Pox nodules developed eight days later and reached a maximum growth in an additional ten days.

Lot 2. Twenty-six days after vaccination both birds were inoculated on comb and wattles with virus B. A mild pox developed eight days later, but soon disappeared without further development.

Lot 3. Forty-two days after vaccination both birds were inoculated on comb and wattles with virus B. Pox was pronounced eight days later, one bird showing diphtheritic patches in mouth as well as pox.

Control: two non-vaccinated birds inoculated with virus B. Incubation period of eight days, maximum development four days later.

Part B.

Two injections of a virus B vaccine were given a second group of six birds in the same manner. The second injection was given six days after the first. The group was likewise divided into three lots of two birds each, and inoculated with virus B fifteen, thirty, and forty-two days respectively after the second injection.

Lot 1. Incubation period of ten days, pox becoming pronounced five days later.

Lot 2. Slight pox developed in eight days in only one bird, clearing up within the next seven days. Second bird showed no evidence of the disease.

Lot 3. Pox developed in ten days, persisting for three weeks in a mild form.

Control: two non-vaccinated, healthy birds inoculated with virus B. Pox developed in eight days, reaching a maximum development four days later.

Part C.

Three injections of a virus B vaccine were given a third group of six birds at intervals of six days. The group was again divided into three lots of two birds each, and inoculated with virus B sixteen, thirty-one, and forty-two days respectively after the third injection.

Lot 1. Pox developed in eleven days and persisted in mild form.

Lot 2. Pox developed in eight days and became pronounced in another week.

Lot 3. Pox developed in twelve days and persisted in mild form for three weeks.

Control: two non-vaccinated, healthy birds inoculated with virus B. Pox developed in eight days, reaching a maximum development three days later.

Result.

One, two, and three injections of the vaccine failed to produce an absolute protection against artificial infection with homologous virus B.

Experiment 2.

Part A.

This experiment was similar to Experiment 1, except that the vaccine was made of virus C and the check inoculations were made with virus B. The first group of six birds was given a 1 cc. injection of the vaccine, divided into three lots of two birds each, and inoculated with virus B seventeen, twenty-six, and forty days respectively after the vaccine injection.

Lot 1. Pox developed nine days later, grew worse, and death followed in one bird.

Lot 2. Pox developed within eight days, but in weak form, and cleared up in two weeks.

Lot 3. Pox developed within eight days in one bird and diphtheritic roup within ten days in the other.

Control: two non-vaccinated, healthy birds inoculated with virus B. Pox developed in nine days, reaching a maximum development four days later.

Part B.

A second group of six healthy birds was given two injections of a virus C vaccine of 1 cc. each at five day intervals. The group was divided into lots 1, 2, and 3 and inoculated with virus B sixteen, thirty, and forty days respectively after the second vaccine injection.

Lot 1. Pox developed in ten days and persisted in a mild form.

Lot 2. Pox developed in eight days in both birds and avian diphtheria in one bird of the lot.

Lot 3. Pox developed in eight days, becoming pronounced, and complicated with roup.

Control: two non-vaccinated, healthy birds inoculated with virus B. Pox developed in eight days and reached a maximum development three days later.

Part C.

A third group of six healthy birds was given three injections of a virus C vaccine of 1 cc. each at five day intervals. The group was divided into lots 1, 2, and 3 and inoculated with virus B fifteen, thirty, and forty-one days respectively after the third vaccine injection.

Lot 1. Pox developed in ten days and persisted in severe form for three weeks.

Lot 2. A slight pox developed in eight days, persisting in a mild form.

Lot 3. Pox developed in eight days, becoming severe and persisting as such.

Control: two non-vaccinated, healthy birds inoculated with virus B. Pox developed in eight days and reached a maximum development four days later.

Result.

One, two, and three injections of the vaccine failed to produce an absolute protection against artificial infection with heterologous virus B.

Other vaccine and virus combinations were used, such as a vaccine made of virus B, and its immunizing ability checked with virus C. The results were comparable to experiments 1 and 2.

Infection by Contact.

A healthy, young cockerel was added to each lot of the foregoing experiments after the disease developed in the supposedly immune birds. This addition of a strange bird to each lot of birds instigated fights, and minor wounds of the comb followed. This allowed a point of entrance for the virus which contaminated the food, water, and litter. Pox developed in about 50 per cent of those birds in contact with the diseased ones. The infection persisted in a mild form, never reaching the severity evidenced in those birds with which they were in contact.

Experiment 3.

An effort was made to determine the curative value of the vaccine. A group of twelve birds was inoculated with virus C. Pox nodules appeared in nine days and a moderate degree of development, which proved to be the maximum, followed in seven days. The group was then divided into two lots of six birds each and placed in separate pens. A virus C vaccine, in a 1 cc. dose was administered to each bird of one lot, the other lot being used as the control. No apparent decrease in number and severity of the pox nodules followed the injection of the vaccine. The injected lot, however, appeared brighter and more active, and loss of flesh was arrested after seven days following the injection. The non-injected lot steadily lost flesh for two weeks, but from then on gained in general appearance and physical conditions.

Results.

The use of the vaccine as a curative measure resulted in a slight improvement in the general condition of the treated birds, but did not cause any diminution in number or extent of the lesions.

One Attack of Bird Pox Confers an Immunity.

All birds recovering from the infection during the experiments were held over for future use. Approximately fifty days following complete recovery from both types of the disease, a group of such birds was inoculated wtih viruses B and C. Lesions of the disease failed to develop, indicating that one attack of the disease, whether of avian pox or diphtheritic type, confers an immunity of at least fifty days' duration. Four healthy birds which served as controls developed pox in eight days with virus B, and in nine days with virus C.

This actively acquired immunity is undoubtedly of greater duration than that demonstrated by the above experimental data. Evidence indicates that it lasts from two months to two years, depending upon the virulence of the infection among the birds which acquire this protection.

The Efficiency of Bacterins.

Several infected flocks were available during the fall and winter of the past year for treatment with bacterins. Autogenous bacterins were resorted to for the control of the outbreaks. Eleven different organisms, aside from the common *Subtilis* group, etc. contaminators, were isolated from diseased birds obtained from five outbreaks of bird pox and avian diphtheria. These organisms were not constantly present in all cases of the disease, and as has been previously stated, they are secondary invaders only. It appears, therefore, that an autogenous bacterin is indicated in preference to a stock bacterin. Also, such a preparation is limited to the control of secondary complications of the disease.

Commercial avian mixed infection bacterins were not used. Their bacterial content does not correspond to the specific bacteria isolated from lesions of birds affected with the disease as it exists in Massachusetts. McNutt (18) in referring to experimental data on the use of such a biologic concludes, "In every case the death loss among the treated equaled or exceeded the loss among the untreated. Usually the loss was greater among the treated."

Flock 1.

A pen, consisting of 112 birds affected with both pox and avian diphtheria, the latter predominating, was treated with an autogenous bacterin. Several of the worst cases of both forms of the disease were examined bacteriologically and the following organisms isolated: *Staphylococci aureus* and *albus*, *Gaffkya* (*Staphylococcus*) *tetragena*, and an unknown, gram negative, short, rod-shaped organism of the colon group. The bacterin, composed of these organisms, was standardized so that one dose of 1 cc. contained 2,000,000,000 organisms.

An initial injection of 1 cc. was given to 80 birds of the pen, selected promiscuously, and 32 birds remained uninjected as controls. All birds were laying well prior to the outbreak of the infection. Both the injected and control groups averaged 43 per cent production at the time the first symptoms of the disease were noticed. Three days prior to the first injection the egg production of both groups dropped to 18 per cent. Fifty per cent of the total number of birds showed symptoms of one or more forms of the disease. There was no appreciable decrease in number or extent of lesions or increase in egg production of both groups during the next few days. The infection appeared to be arrested, however. Nine days following the first injection, a second one of the same dose was given. Three days afterward the injected group of layers improved in general condition and the egg production began to increase gradually. The condition and production of the control group remained at a standstill. These results were evident in the same proportions for the following two weeks, at the end of which time the last reading was taken. The injected group had reached 41 per cent egg production and the control group averaged 35 per cent. Lesions of the disease persisted, however, in all birds, but were somewhat less extensive in type.

In estimating the percentage of egg production care was taken to consider factors other than disease, which would tend to influence it.

Results.

The administration of the autogenous bacterin was followed by an improvement in the general condition and production of the injected group. No diminution in number or extent of the lesions was noted. Local treatment of the lesions would probably have served the purpose.

Flock 2.

An autogenous bacterin was administered to a second flock affected with avian diphtheria. The following organisms, which were used to make the bacterin, were isolated from typical cases of the affection: *Staphylococci aureus* and *albus*, *Gaffkya (Staphylococcus) tetragena*, a gram negative, short rod, bi-polar staining bacillus, and an organism of the *Escherichia* group, typical of *Escherichia schaefferi*.

A severe infection of a similar nature had existed in these same pens during the previous season. At the time of the injection a moderate degree of the infection was present in the birds of houses 1, 2, and 3. One injection of 1 cc. of the bacterin, having a concentration of 2,000,000,000 organisms per cc., was given each bird. Previous to the treatment the egg production had dropped to 40 per cent. From four to six weeks later when final readings were made the production had increased to 66 per cent. House 1 contained 2.8 per cent injected birds showing mild symptoms of the disease as opposed to 11 per cent of the non-injected birds or controls in the same condition. House 2 showed 12.3 per cent infection in injected birds and 30 per cent infection in the controls. House 3 showed 5.4 per cent infection in injected birds and 41.7 per cent infection in the controls. No attempt was made to treat the symptoms of the disease.

Results.

One injection of the autogenous bacterin arrested the course of the infection and brought about an increase in egg production.

Flock 3.

A third flock of 2,000 birds was injected, each bird receiving 1 cc. of an autogenous bacterin consisting of *Staphylococci aureus* and *albus*, and *Pseudomonas aeruginosa*. The bacterial concentration in this instance was but 500,000 organisms per cc. Complete data on the results of the treatment were

not obtainable. An early report from the owner showed an improvement in egg production and but a few mild cases of the disease among the treated birds. It was questionable, however, whether the increase in production was due to recovery from the disease or from an existing neck moult. No data were available concerning the controls. No conclusion can be drawn from the use of the bacterin in this instance.

Flock 4.

A fourth flock of 300 cockerels was injected with an autogenous bacterin composed of *Staphylococcus aureus*, a *Pasteurella avicida*-like organism, and a bacillus similar to the roup bacillus or *B. cacosmus* of Harrison and Streit (2). The infection had practically run its course at the time of the treatment. Two injections were given, the first of 0.5 cc. containing 500,000 organisms, and a second six days later of 1 cc. containing 1,000,000 organisms. The disease entirely cleared up during the following three weeks. No difference was noted between the injected and control groups.

Summary.

1. Several organisms were isolated from the lesions of avian diphtheria, diphtheritic roup, and pox. They proved to be of no causative significance, but were prominent secondary invaders. A filtrable virus was found to be the common cause of all types of the disease.

2. Commercial stock powdered pox viruses varied markedly in ability to produce the disease. The need of a method of standardizing the virus and vaccine was indicated.

3. One, two, and three injections of the powdered pox virus vaccines failed to produce an absolute protection against artificial infection with homologous and heterologous viruses.

4. Infection by contact occurred in 50 per cent of all cases.

5. The powdered pox virus vaccine caused a slight improvement in the general condition of diseased birds when administered as a means of bringing about recovery from the infection.

6. One attack of either or both types of the disease conferred an immunity of at least fifty days' duration against both types.

7. Autogenous bacterins, when administered in the early stages of the disease, caused an improvement in the general health of the birds. As avian diphtheria and pox advance in severity the egg production of hens decreases. With the injections of these bacterins, data at hand indicate that the egg production is increased. While all these observations are interesting and point to a certain degree of therapeutic efficiency; the time consumed in the manufacture, standardization, and administration of these bacterins would work against their use as an economic practice.

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INTENSITY OR RATE OF LAYING IN RELATION TO FECUNDITY

By F. A. HAYS and RUBY SANBORN

This bulletin continues the series dealing with inherited traits in relation to fecundity in the Rhode Island Red breed of domestic fowl. Intensity is an inherited trait which vitally affects fecundity. In this study four measures of intensity have been used: first sixty-day egg record, mean size of winter clutch, net winter rate of laying, and annual rate of laying. From the standpoint of the breeder, mean size of winter clutch is the most satisfactory criterion of intensity because it can be accurately determined and because it is inherited.

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INTENSITY OR RATE OF LAYING IN RELATION TO FECUNDITY

By F. A. Hays and Ruby Sanborn

INTRODUCTION

Intensity or rate of laying was first cited by Goodale and Sanborn (1922) as of vital importance in relation to total number of eggs laid. The significance of intensity in relation to winter fecundity has been further stressed by Hays (1924) and a theory concerning inheritance of winter intensity proposed. The probabilities are that high winter rate behaves as a dominant in inheritance and that two Mendelian factors are concerned. Possible measures of intensity are numerous and diverse. A number of such measures will first be considered.

Measures of Intensity.

Since rate can only be obtained on a time basis, it becomes necessary to make use of some definite time interval. Furthermore, the time element must be based either on calendar months or upon a specific period in the laying year of the individual bird. From the biological standpoint the second method of assigning time in calculating rate might be preferable, yet diverse weather conditions throughout the year may partially nullify effects of biological differences.

Goodale and Sanborn (loc. cit.) suggest as time units the month, the initial cycle, the inter-broody periods, the summer period and the spring period. These workers also mention length of clutch as a possible measure and make the statement that the calendar month may be employed for flock comparisons though unsuitable for individual comparisons.

A rather common measure of intensity is the greatest number of eggs laid by a bird during any calendar month of the pullet year. Such a criterion of intensity is certainly very crude, as Harris and Goodale (1922) have shown for Rhode Island Reds. Probably a long time interval, governed in part by the individual date of first pullet egg and terminated at a definite calendar date, offers the most reliable period for calculating intensity.

In these studies four measures of intensity have been used:

1. *First sixty days' production.*

The number of eggs laid from the first pullet egg for a period of sixty days was tabulated. This figure represents a definite interval in the laying year and furnishes a clue as to the rate at which the birds begin laying. In this instance the actual calendar date varies both with hatching date and with age at which sexual maturity is attained. In other words, the production of the first sixty days is not comparable with production for two specific calendar months.

2. *Mean winter clutch size.*

The term clutch represents the number of eggs laid on successive days. The size of clutch varies widely, with a mean of from 1 to 21 eggs in the population studies. The total number of clutches from first egg to March first was tabulated for each pullet. The number of eggs up to March first was then divided by the number of clutches to obtain the mean winter clutch size. In this manner a measure of intensity was arrived at without employing the time interval. Moreover, it offers an index to the capacity of the reproductive organs for elaborating eggs on successive days. An objectionable feature is apparent in that no evidence is obtained relative to the ability of the birds to continue producing a definite clutch size.

3. *Net winter rate.*

Net winter rate is determined by dividing the total number of eggs from first pullet egg to March first by the number of days from first egg to March first less all pauses of four or more days from November first to March first. This method expresses the rate of laying excluding winter pause and an occasional broody pause before March first. Compared with mean clutch length, net winter rate is probably a superior measure of intensity because it covers a definite time limit.

4. *Annual rate.*

Annual rate is indicative of the gross rate of laying from first pullet egg to the onset of complete molt. This rate is obtained by dividing the total eggs laid during the period by the number of days from first egg to the beginning of complete molt in the late summer or fall if within 364 days from the date of first pullet egg. In cases where molting does not begin until after the 365-day period, time is figured at 365 days. The rate thus obtained does not make allowance for time lost in winter pause or for nonproduction during broody periods. Such a rate is, therefore, but a crude approximation of annual intensity.

Character of Birds Used.

The birds used in these studies are identical with those reported on in Massachusetts Agricultural Experiment Station Technical Bulletins 7, 8 and 9, except that data on the flock hatched in 1925 are added. All records were made during the pullet year and all birds are pure bred Rhode Island Reds. The flocks are somewhat heterogeneous in character including, in addition to the major portion bred for high fecundity, a smaller proportion bred each year for non-broodiness, intense broodiness, high hatchability, good color, and inbreeding studies.

Scope of This Report.

As previously stated, four measures of intensity are made use of here. Attention is given to some prominent environmental influences and to inherited traits that may affect intensity. Major consideration is given to the relation of intensity to winter and annual production. The report is divided into sections A, B, C and D on the basis of the four criteria of intensity employed.

A. FIRST SIXTY DAYS' PRODUCTION.

In dealing with a complex biological and physiological problem like fecundity, strict attention must be given both to inherited and to environmental factors. Strictly speaking the first sixty days' egg record of two or more pullets would only be comparable if the birds were hatched the same day, began laying the same day and at all times were fed and managed identically. Under such restrictions numbers would be so limited as to be of questionable worth. In studying the relation of the first sixty days' production to fecundity, the same productive period is used for each bird even though the actual calendar time varies with hatching date and age of sexual maturity. For these reasons, this should be a superior measure to that employing one or two calendar months for all birds.

1. *Correlation Between First Sixty Days' Production and Subsequent Winter Production.*

A population of 3542 birds was used to discover the correlation between the first sixty days' record and production for the rest of the winter period. In this study only birds that had laid for sixty days by March first could be included. The correlation coefficient shows any tendency of production during the first sixty days and subsequent production up to March first to move together. The following constants were obtained:

Number of birds	3542
Mean sixty-day record	37.83
Sixty-day record standard deviation	±9.95
Mean subsequent winter record	30.65
Subsequent winter record standard deviation	±17.96
Coefficient of correlation	+.3445 ±.0100

The fact should be observed that the average length of laying period up to March first for this population was 123 days. The first sixty-day record therefore covers about half of the winter laying period. It is interesting to note that the mean number of eggs laid during the first sixty days is 37.83 while the mean number laid during the next sixty-three days was 30.65. The variability in production during the second half of the winter period is also much greater than during the first half. Since winter pause is more frequent in January and February than during previous months, lower production during these months might be anticipated.

The coefficient of correlation between first sixty-day record and subsequent winter record is significant, and indicates that the pullets which lay the most during the first sixty days of their year tend to continue at a higher rate than do those of less intensity during the first sixty days. The absolute magnitude of the coefficient is, however, scarcely adequate for selection purposes when maximum winter records are desired.

2. *Correlation Between First Sixty Days' Production and Subsequent Annual Production.*

Accurate methods for predicting probable annual egg yield are wanting. The discovery of reliable criteria in advance would mark an important step in progress and would be of outstanding value in selecting pullets for

egg-laying contests as well as in making up breeding flocks and commercial production flocks. The value of early sexual maturity in flock selection has already been pointed out (Hays and Bennett, 1923), yet sexual maturity is inadequate as the sole guide of the breeder in his selections. If some short-time test can be discovered, its practical worth is self-evident.

A population of 2560 birds is studied over the period from 1916 to 1925. The sixty-day egg record of each individual is tabulated against her production for the remainder of the year, a time interval of 305 days. Constants obtained follow:

Number of birds	2560
Mean sixty-day record	37.86
Sixty-day record standard deviation	±9.91
Mean subsequent annual record	144.27
Subsequent annual production standard deviation	±40.04
Coefficient of correlation	+.3082 ±.0121

Comparing the variability in sixty-day record with the variability in subsequent annual record, the coefficients of variability are 26 per cent and 28 per cent, respectively. Theoretically, the standard deviation in annual record should be five times as great as the standard deviation in sixty-day record because the time interval of the former is ten months, and of the latter two months. In reality, the two constants are about the same, showing that egg production fluctuates most during the early months of the pullet laying year.

A positive coefficient of correlation of .3082 ±.0121 indicates a significant tendency for heavy sixty-day production to be associated with heavy production for the remainder of the year. The degree of correlation is somewhat less than the −.4380 ±.0134 reported by Hays and Bennett (loc. cit.) between age at first egg and annual egg yield. The probabilities are that selection upon first sixty days' record as a partial measure of intensity, and upon age at first egg as another valuable criterion, will increase fecundity.

B. MEAN WINTER CLUTCH SIZE.

An expression for the clutch size of a pullet furnishes information relative to her ability to elaborate few or many eggs on successive days. In other words, it is an index to functional capacity. Mean clutch size throughout the winter season offers possibilities as a measure of intensity over a considerable period of time. Moreover, clutch size can also be definitely measured for each individual and measurable characteristics are most useful in biological studies.

Behavior of Clutch Size in Inheritance.

A frequency distribution of any of the flocks included in this report with regard to clutch size clearly presents a bimodal aspect. A more exact classification of each individual bird for clutch size places the modes at a clutch size of 2 and 2.2, respectively. There is a very pronounced depression in the frequency graph at the clutch size of 2.1. The frequency distribution for clutch sizes from 1 to 2 rather closely approaches a straight line with a positive slope not far from 1. On the other hand, frequency distribution for clutches greater than 2 is less regular and if fitted to a

straight line gives a less abrupt negative slope. An examination of these frequency distributions has brought to light valuable information concerning the inheritance of winter clutch size.

Goodale (1918) recognized the fact that characteristic types of rhythm in laying exist in Rhode Island Reds. He applied the time element and classed hens as one-half, two-thirds, three-fourths, etc. with respect to rhythm. He fully recognized the importance of rhythm in egg laying.

Riddle (1925) presents data to show that the common pigeon lays the greatest percentage of single eggs during January and February and the smallest percentage during July and August. Since the characteristic clutch of the pigeon consists of two eggs, it seems probable that adverse weather conditions tend to reduce the rate of laying and in consequence the mean clutch size.

Daily egg records of the wild ancestors of the domestic fowl are not available for study in comparison with the records of improved flocks. Information on the question of clutch size must for this reason be obtained on flocks of domestic fowl largely unimproved in fecundity and from some information on wild species of birds.

The mean winter clutch size of the foundation birds hatched in 1912, from which the flocks reported upon are descended, is *1.9* for the *119* birds. The mean for the 276 birds hatched in 1916 is 2.5, and for the 541 birds hatched in 1925, 3.1. The normal clutch size for the common pigeon is 2. but this normal may be modified by weather conditions as Riddle (loc.cit.) shows. Data on the Massachusetts flocks of Rhode Island Reds hatched from 1916 to 1925 indicate the existence of two modal classes for winter clutch size and probably a third modal class higher than these two. The first mode occurs at a clutch size of 2 and is very distinctly separated from the second mode, which is about 2.2. The third mode probably is at the 2.6 class. What then is the behavior of clutch size in inheritance?

Proposed Theory.

Extensive studies of available data on Rhode Island Reds indicate that the normal clutch size for the domestic fowl is two and that adverse weather conditions may operate to reduce some of the clutches to one. Further bearing on this point is the fact that the hen not infrequently liberates two ova almost simultaneously making a double-yolk egg, but that the occurrence of more than two ova in the same shell is an extremely rare phenomenon. It seems probable, therefore, that the hen ordinarily ovulates twice either on the same day or on successive days, and that a greater length of clutch than two represents a modification of the normal.

The first modal class appears to consist of the normal individuals that ovulate twice in favorable environmental conditions and thus have a characteristic clutch size of two or less than two. The second modal class in the flocks studied is made up of birds modified for clutch size, so that their mean is greater than two. The third modal class occurs at a clutch size of 2.6.

On a Mendelian factor basis the following seems warranted after very extensive studies of clutch size in families of sisters: That the normal unimproved hen is a recessive for clutch size. That a gene I added to normal gives a clutch size greater than 2. That a second gene I' makes a clutch size of 2.6 or more possible. That genes and I and I' together give the

greatest clutch size—more than 2.6. Both genes are autosomal and no linkage has been observed to date. The four general classes of hens with regard to clutch size are: i i i' i' individuals with a clutch size of 2 or less; I I i' i' individuals with a clutch size of 2.1 to 2.5; i i I' I' individuals with a clutch size of 2.6 or more; and I I I' I' individuals with a clutch size greater than 2.6, and possibly as great as 21 or more for the winter season.

3. *Correlation Between Hatching Date and Mean Clutch Size.*

Hatching date is a controllable environmental condition. It may be varied at the will of the breeder. In the series of years covered by these studies the hatching dates have been kept on the same calendar dates. The first hatch came off each year on March 25 and there was one hatch each week thereafter until May 15, or a total of eight hatches per year over a period of 49 days. If time of hatching is associated with size of winter clutch, it may be discovered by means of the coefficient of correlation. The population consists of 3867 birds upon which constants were calculated as follows:

Number of birds	3867
Mean hatching date (Apr. 19)	4.35
Hatching date standard deviation	±2.28
Mean size of winter clutch	2.64
Winter clutch standard deviation	±1.29
Coefficient of correlation	—.0167 ±.0108

Clutch size exhibits a variability of 49 per cent as shown by dividing its standard deviation by the mean clutch size. This striking lack of uniformity in clutch size is in no small measure responsible for great variability in winter egg records of these flocks. No correlation is shown between hatching date and clutch size.

4. *Correlation Between Age at First Egg and Mean Clutch Size.*

In this section the relation between two inherited traits is brought to light. Both are of significant importance in breeding for fecundity and any linkage relation should be understood. The constants calculated for the population follow:

Number of birds	3867
Mean age at first egg	206.18
Age standard deviation	±29.52
Mean size of winter clutch	2.64
Winter clutch standard deviation	±1.29
Coefficient of correlation	—.2273 ±.0103

Age at first egg shows a moderate negative correlation to winter clutch size. In other words, there is something of a tendency for early-maturing pullets to lay larger clutches than do later-maturing birds. Here then is one of the reasons for the pronounced negative correlation between age at first egg and winter egg record (Hays and Bennett, 1923). There appears to be a significant linkage between early sexual maturity and

large winter clutch size that may be employed advantageously in breeding for egg production.

5. *Correlation Between Weight at First Egg and Mean Clutch Size.*

Because of the importance both of body weight and of clutch size in breeding for fecundity, it is very desirable that their relation to each other be ascertained. The correlation between weight at first egg and mean clutch size was calculated with the following constants:

Number of birds	3797
Mean weight	5.53
Weight standard deviation	±.72
Mean size of winter clutch	2.65
Winter clutch standard deviation	±1.29
Coefficient of correlation	—.1714 ±.0106

The coefficient of correlation is found to be negative and its absolute magnitude is not very great. Statistically, however, the correlation is significant and suggests something of a tendency for smaller birds to exhibit larger clutches. For purposes of prediction or selection the relation is not sufficiently pronounced to be of value.

6. *Correlation Between Winter Production and Mean Clutch Size.*

Heavy winter egg production is important both genetically and economically. Genetically, winter egg record depends on seven pairs of Mendelian factors as has been shown by Hays (1924), and winter production is also known to be intimately correlated with annual production (Hays, Sanborn and James, 1924). Economically, the number of winter eggs is of no small value in affecting the net income per bird. In breeding for high fecundity it is necessary to recognize both environmental influences and hereditary factors that are concerned in winter egg yield. By tabulation of the entire population for clutch size and winter record the constants below were secured:

Number of birds	3867
Mean winter production	62.92
Winter production standard deviation	±25.95
Mean size of winter clutch	2.61
Winter clutch standard deviation	±1.29
Coefficient of correlation	+.4727 ±.0084

The above coefficient of correlation suggests a rather intimate positive relation between clutch size and winter record. This constant may be considered a true measure of correlation and indicates an important relation between clutch size and total egg yield for the winter. In view of this fact large clutch size should be considered as a vital factor in breeding for maximum winter egg records.

7. *Correlation Between Annual Record and Mean Clutch Size.*

Clutch size appears to behave in Mendelian fashion in inheritance. It

has been shown to be rather significantly related to winter fecundity. Probably a more important consideration is the relation of clutch size to annual production. The entire population with annual records has been tabulated in a correlation table and the constants obtained follow:

Number of birds	2532
Mean annual production	182.85
Annual production standard deviation	±42.94
Mean size of winter clutch	2.70
Winter clutch standard deviation	±1.29
Coefficient of correlation	+.3544 ±.0117

On the studies reported herein egg production over a period of 365 days from first egg is taken as the standard for measuring fecundity. The coefficient of correlation is positive and certainly significant so that winter clutch size may be employed as a valuable criterion in the selection of prospective heavy annual egg producers. Since winter clutch size appears to be an inherited trait, there is opportunity for increasing fecundity by breeding for greater mean winter clutch size.

C. NET WINTER RATE

Net winter rate is an expression for the rate of laying throughout the winter season after deducting time spent in winter pause. It represents the rate of laying for an average time interval of about 120 days in the population studied. Net winter rate is considered first in relation to date of hatching.

8. *Correlation Between Hatching Date and Net Winter Rate.*

The population for the ten-year period (1916-1925) is considered, and the following constants are derived:

Number of birds	3863
Mean hatching date (Apr. 18)	4.35
Hatching date standard deviation	±2.28
Mean winter rate	67.79
Winter rate standard deviation	±8.86
Coefficient of correlation	+.0100 ±.0109

Judged by the magnitude of the coefficient of correlation, there is no relation between hatching date and net winter rate of laying. This fully agrees with the findings set forth in section 3 where hatching date and winter clutch size are found to be independent.

9. *Correlation Between Age at First Egg and Net Winter Rate.*

Age at first egg is an inherited characteristic which has been shown by the writer as well as by many other workers to be intimately correlated with winter and annual production. In this report an attempt is made to discover the relation of intensity to fecundity as well as to other characteristics concerned in fecundity. Age at first egg has therefore been tabulated against winter rate to derive the constants below:

Number of birds	3863
Mean age at first egg	206.14
Age at first egg standard deviation	±29.47
Mean net winter rate	67.79
Winter rate standard deviation	±8.86
Coefficient of correlation	—.2274 ±.0103

A moderate degree of negative correlation is found between age at first egg and winter rate of laying. Evidently there is some tendency for the early maturing pullets to lay somewhat more intensely than do the late maturing individuals. This difference is no doubt due to larger clutch size in the early maturing birds as has been pointed out in section 4. The fact should be noted here that the degree of correlation is identical between age and clutch size and between age and net winter rate.

10. *Correlation Between Weight at First Egg and Net Winter Rate.*

Mature body weight in poultry is inherited on a Mendelian basis according to Punnett (1923). Weight at first egg has been shown by Hays, Sanborn and James (1924) to depend both upon hatching date and upon age at first egg. If there is any relation between weight at first egg and winter rate of laying, body weight might be used as a partial criterion in selection. To gain this information the constants below were determined:

Number of birds	3794
Mean weight at first egg	5.53
Weight standard deviation	±.72
Mean net winter rate	67.81
Winter rate standard deviation	±8.82
Coefficient of correlation	—.1756 ±.0106

The above coefficient of correlation is of the same magnitude as was the coefficient of correlation reported in section 5 between weight at first egg and winter clutch size. Again the correlation coefficient is statistically significant but of little practical value for prediction and selection purposes.

11. *Correlation Between Winter Egg Production and Net Winter Rate.*

To discover the relation between net winter rate and winter egg record, the population of 3863 birds is studied. The following constants appear:

Number of birds	3863
Mean winter production	62.95
Winter production standard deviation	±25.92
Mean net winter rate	67.79
Winter rate standard deviation	±8.86
Coefficient of correlation	+.5444 ±.0076

The above constants indicate an intimate positive correlation between winter rate and winter production. This correlation points rather conclusively to the importance of winter rate as a factor in winter egg production. Since winter rate is inherited (Hays, 1924), it becomes evident that one important means of securing high winter production lies in the development of a high winter intensity strain.

12. *Correlation Between Annual Egg Record and Net Winter Rate.*

Net winter rate has been considered one measure of intensity or rate of laying. There yet remains to be studied the relation between winter rate and annual egg yield. A correlation table has been constructed for the entire population and the following constants have been arrived at:

Number of birds	2528
Mean annual egg record	182.96
Annual egg record standard deviation	±42.83
Mean net winter rate	68.27
Winter rate standard deviation	±8.81
Coefficient of correlation	+.4769 ±.0104

A rather intimate correlation exists between net winter rate and annual production. As a criterion for predicting annual egg production, net winter rate is superior to any other thus far considered. This fact would seem to indicate that the rate of laying up to March first is an index to the probable rate throughout the year.

D. ANNUAL RATE OF LAYING

Annual rate is taken as a long-time measure of intensity. The time interval employed in calculating the rate for each individual is the persistency interval or period from first pullet egg to complete molt. The mean time interval for the 1916 flock is 248 days, and the maximum time interval was in the 1922 flock with 330 days. For mean persistency of other flocks, see Massachusetts Agricultural Experiment Station Technical Bul. 9. Annual rate is a gross representation of the percentage of time in the production year that each bird actually laid. The relation of annual rate to hatching date, age at first egg and weight at first egg is first considered, and finally the relation of annual rate to annual production.

13. *Correlation Between Hatching Date and Annual Rate.*

Date of hatching is known to be of considerable practical importance. In previous publications it has been shown to influence age at sexual maturity, winter pause, and annual persistency, as well as rate of growth. It is therefore desirable to know if hatching date shows any influence upon annual rate. The population over the ten-year period was tabulated in a correlation table which gave the constants below:

Number of birds	2560
Mean hatching date (Apr. 18)	4.29
Hatching date standard deviation	±2.26
Mean annual rate	57.57
Annual rate standard deviation	±9.57
Coefficient of correlation	+.0318 ±.0133

The mean annual rate above shows that the birds averaged to lay 57.57 per cent of the time between first egg and the onset of complete molt. This population of 2560 individuals had a mean annual egg production of 181.59. Simple calculation of the time interval shows it to be 315 days.

It is possible that annual rate might be increased by proper methods of breeding.

The coefficient of correlation between hatching date and annual rate is of very small magnitude and not statistically significant. It can only be interpreted as meaning that annual rate is independent of hatching date.

14. *Correlation Between Age at First Egg and Annual Rate.*

Age at first egg was shown in section 5 to be significantly correlated with winter clutch size, and in section 9 to be correlated to a similar degree with net winter rate. Of importance now is a consideration of the relation between age at first egg and annual rate. All of the birds with annual records are included in the correlation table to derive the constants:

Number of birds	2560
Mean age at first egg	205.37
Age standard deviation	±30.56
Mean annual rate	57.57
Annual rate standard deviation	±9.57
Coefficient of correlation	-.0657 ±.0133

The magnitude of the coefficient of correlation given above is insufficient to establish any relation between age at first egg and annual rate of laying. The assumption therefore seems warranted that age at first egg and annual intensity are independent of each other.

15. *Correlation Between Weight at First Egg and Annual Rate.*

Body weight at the beginning of the laying year might be thought of as a crude measure of capacity for food consumption and as such body weight might be correlated with annual intensity. The entire population was assembled in a correlation table to discover possible relations. The constants calculated are as follows:

Number of birds	2504
Mean weight at first egg	5.54
Weight standard deviation	±.73
Mean annual rate	57.62
Annual rate standard deviation	±9.57
Coefficient of correlation	−.1174 ±.0133

The degree of correlation shown between body weight at first egg and annual intensity is small but statistically significant. There is but a slight tendency for smaller birds to exhibit higher annual rates. This correlation may be attributed to the somewhat greater intensity of early maturing pullets and such pullets would normally show lower body weights than later maturing pullets because of the time element. The degree of correlation formed above is of no practical importance, as it is too slight to be used for prediction or selection purposes.

16. *Correlation Between Annual Egg Record and Annual Rate.*

In the concluding section of these studies, the correlation between annual rate and annual production is considered. Such studies will bring to light something of the importance of intensity measured over a long period of time as a factor in annual egg yield. In making up the correlation table the annual record of each individual was tabulated against her intensity record. The constants appear below:

Number of birds	2560
Mean annual production	181.59
Annual production standard deviation	±44.39
Mean annual rate	57.57
Annual rate standard deviation	±9.57
Coefficient correlation	+.7106 ±.0066

The correlation between annual rate and annual production is positive and decidedly intimate. As a criterion of annual production, annual rate is of outstanding value. Only one other criterion of annual production, namely, annual persistency (Hays and Sanborn, 1926 b), shows a similar degree of correlation to yearly production. Annual intensity, therefore, should be adequately stressed in a program of breeding for fecundity.

The Measures of Intensity Compared.

In these studies four measures of intensity have been considered in relation to winter and annual egg production. The measures of intensity employed are: (a) first sixty-day record; (b) mean size of winter clutch; (c) net winter rate of laying; and (d) annual rate of laying. Based on the intimacy of correlation with winter production three criteria rank as follows: net winter rate, mean winter clutch size, and first sixty-day production. On the intimacy of correlation with yearly production the rank is annual rate, net winter rate, mean winter clutch size, and first sixty-day record.

As short-time measures of intensity net winter rate and mean winter clutch size are superior to first sixty-day egg record. Judged by the intimacy of correlation with both winter and annual egg records, net winter rate is somewhat superior to mean clutch size. Both show the same relation to hatching date, age at first egg and weight at first egg. Mean clutch size is more definite than is net winter rate because short pause intervals affect net winter rate and apparently do not affect clutch size. Mean clutch size has been shown to behave as an inherited trait and to depend upon two autosomal genes. From the breeding standpoint, therefore, winter clutch size may be considered the best measure of intensity thus far developed, since annual rate is valueless for prediction purposes and is known only after the first laying year closes.

RELATION OF AGE AT FIRST EGG, BROODINESS, AND INTENSITY

Age at first egg has previously been shown to be an inherited trait (Hays, 1924), and to be intimately correlated with fecundity (Hays and Bennett, 1923) in the Rhode Island Reds being studied. It is desirable to discover if age at first egg and degree of broodiness are dependent or independent.

17. *Correlation Between Age at First Egg and Total Days Broody.*

The broody population for the ten-year period has been tabulated in a correlation table for age at first egg and total days broody during the pullet year. Constants obtained are as follows:

Number of birds	1207
Mean age at first egg	207.76
Age standard deviation	±31.52
Mean total days broody	42.81
Days broody standard deviation	±27.41
Coefficient of correlation	+.0062 ±.0194

The magnitude of the coefficient correlation between age at first egg and total days broody is insufficient to indicate any correlation between age at first egg and degree of broodiness. Because of this fact it is very probable that these two inherited characteristics are in no way linked in inheritance.

Age at first egg has been shown in sections 4 and 6 of this report to be rather intimately correlated with winter intensity. Apparently there is linkage between the genes E and E' for early maturity and genes I and I' for large clutch size. The degree of linkage has not been determined as yet.

18. *Correlation Between Total Days Broody and Mean Winter Clutch Size.*

Hays and Sanborn (1926a) report no significant correlation between degree of broodiness in the broody population and net winter rate of laying. Since mean winter clutch size is a useful measure of intensity, it is desirable to ascertain if the degree of broodiness is correlated with mean winter clutch size. The following constants have been calculated for the broody population over the ten-year period:

Number of birds	1188
Mean total days broody	42.89
Days broody standard deviation	±27.54
Mean winter clutch size	2.96
Clutch size standard deviation	±1.38
Coefficient of correlation	+.2205 ±.0186

In section 3 the mean winter clutch size of both broody and non-broody birds is 2.64. The mean winter clutch size of the broody birds alone is found to be 2.96. This fact suggests that, on the average, broody birds tend to lay in larger clutches during the winter than do non-broody birds.

The coefficient of correlation between total days broody and mean winter clutch size is statistically significant, and suggests that degree of

broodiness is in a measure positively correlated with winter clutch size. From the standpoint of annual fecundity, however, the broody trait should be eliminated as Hays and Sanborn (loc. cit.) show.

GENERAL SUMMARY.

Four measures of intensity have been considered in relation to fecundity; namely, first sixty-day egg record, mean size of winter clutch, net winter rate, and annual rate. The data have been secured over a ten-year period on succeeding flocks from the same foundation with reasonably constant environmental conditions. From the standpoint of the breeder, mean size of winter clutch is the most satisfactory criterion of intensity because it can be accurately determined and because it is inherited in Mendelian fashion.

In studying the correlation between these four measures of intensity and different environmental conditions and inherited traits affecting fecundity as well as their correlation to winter and annual egg production, the following facts appear:

1. There is a positive correlation between first sixty-day egg record and subsequent winter record of .3445 ±.0100. Such a correlation shows something of a tendency of production for the first sixty days to be associated with a somewhat similar production for the next two months. But production for both the first and last half of the winter period is often reduced by the onset of inherited winter pause, making the absolute number of eggs laid during any part of the winter season an unreliable criterion of intensity.

2. The number of eggs that a pullet lays during the first sixty days of laying is correlated with the number she lays for the remainder of the year; yet the degree of correlation is less than with some other measures of intensity.

3. Size of winter clutch is inherited on a two-factor Mendelian basis.

4. Size of winter clutch is not affected by hatching date.

5. Factors for early sexual maturity indicated by age at first egg are linked with factors for large clutch size.

6. Body weight at first egg and mean winter clutch size are negatively correlated to a moderate degree.

7. Winter egg production is intimately correlated with mean winter clutch size making clutch size a valuable criterion of winter intensity.

8. The correlation between annual egg record and mean winter clutch size is positive and of sufficient magnitude to establish winter clutch size as a good measure of intensity.

9. Net winter rate is very similar to mean winter clutch size in relation to hatching date, age at first egg, and weight at first egg.

10. Net winter rate is somewhat more intimately correlated with winter egg yield than is mean winter clutch size, but the former is a less specific measure of intensity alone because of winter pause.

11. Net winter rate shows a coefficient of correlation of +.4769 ±.0104 with annual egg record compared with a coefficient of +.3514 ±.0117 between mean winter clutch size and annual record; but the former is not as valuable a criterion of intensity as the latter because of winter pause disturbances.

12. Annual rate of laying is not affected by date of hatching.

13. Annual rate is but very slightly correlated with age at first egg in a negative direction.

14. Annual rate shows but a small negative correlation with body weight at first egg.

15. Annual rate is most intimately correlated with annual egg record, the constant being +.7106 ±.0066 for the population studied.

16. Annual rate is valueless for prediction purposes during the pullet year and is but a gross approximation of rate for the entire pullet laying year.

17. Age at first egg and degree of broodiness are independent.

18. A positive correlation between total days broody and mean winter clutch size suggests that birds carrying factors for broodiness show a tendency to lay in larger clutches during winter than do non-broody birds The degree of correlation, however, is not sufficient to indicate that non-broody birds may not carry high intensity.

19. In general, intensity or rate has been shown to vitally affect fecundity; and as a short-time measure of intensity, mean winter clutch size has been suggested because it is definitely measurable and because it is inherited.

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MASSACHUSETTS
AGRICULTURAL EXPERIMENT STATION

Technical Bulletin No. 12 December, 1927

NET CORRELATIONS OF CHARACTERS CONCERNED IN FECUNDITY

By F. A. HAYS and RUBY SANBORN

This bulletin completes the series dealing with the five inherited traits concerned with fecundity in the Rhode Island Red breed of domestic fowl. Net correlations are presented, which more adequately portray the relative importance of the several characters than do the simple correlations previously used. Annual egg production is shown to be entirely independent of age at first egg; to be dependent to an important and substantially equal degree upon length of winter pause, intensity as measured by winter clutch size, and degree of broodiness; but to be most intimately affected by annual persistency. The multiple correlation of +.8642 shows that the five characters here considered largely control the annual egg yield.

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NET CORRELATIONS OF CHARACTERS CONCERNED IN FECUNDITY

By F. A. Hays and Ruby Sanborn

Annual egg production has been found by several workers to depend upon the characteristics early sexual maturity, non-pause, high intensity, non-broodiness, and high persistency. All five of these traits have been shown to be inherited in Mendelian fashion (Hays, 1924 and 1927). In order to obtain maximum annual egg yield in the domestic hen, a combination of the above five characteristics in the same individual is necessary, as well as very favorable environmental conditions. Familiarity with the mode of inheritance of any one of the above five traits clearly indicates the difficulties in combining all in the same individual and serves to explain the marked variability occurring in the egg production of individual hens in the same flock. Even when hens that carry all five of the desirable traits are used as breeders, there will not be marked uniformity in production of the daughters of such matings unless the breeding hens are genetically homozygous for the fecundity characteristics and are mated to males of like genetic composition. Too much stress cannot be laid upon the importance of selecting as breeders both males and females that are homozygous for the characteristics sought.

In previous reports (Technical Bulletins 7, 8, 9, and 11 of the Massachusetts Agricultural Experiment Station), the relation of the five fecundity traits to production has been fully considered by means of the simple coefficient of correlation and the relative importance of each trait as a selection unit has been studied. There yet remains to be considered in this concluding report the net relation of each of the five traits to each other as well as to annual production when all characteristics except the two under consideration are made constant. These results are obtained by use of partial correlation coefficients as calculated by standard formulae from the simple correlation coefficients. For illustration: in calculating the partial correlation between age at first egg and annual production; winter pause, intensity, broodiness, and persistency are made constant. The use of partial correlation coefficients here accomplishes a two-fold purpose. First, it brings out any possible linkage between traits—an invaluable item of information to the breeder. Second, it shows clearly the relative importance of the five characteristics to fecundity so that each may be properly stressed in the breeding program.

The concluding section makes use of multiple correlation to discover the exact degree in which annual production depends on the combined influence of the five traits under consideration, and also whether other factors are concerned in fecundity.

Birds Used

All of these studies are based on pullet-year records on Rhode Island Reds bred by the Massachusetts Agricultural Experiment Station from 1916 to 1925. The major portion of the birds was bred for fecundity, but each year there has been included a limited number bred for broodiness and in inbreeding studies.

Relation of Characteristics Concerned in Fecundity to Each Other

1. Age at First Egg and Length of Winter Pause.

Age at first egg may be definitely recorded and has been used extensively as a criterion of future productive ability. The simple correlation between age at first egg and annual egg yield in these flocks is —.4380±.0134 (Hays and Bennett 1923), but this apparent correlation may be due to linkage relations between early sexual maturity and other high fecundity traits. By making intensity, days broody, and annual persistency constant, the net or partial correlation between age at first egg and length of winter pause is —.2236±.0139. Here is a significant correlation which suggests some linkage between early sexual maturity and long winter pause. The relationship is far from intimate, however, and probably does not signify that genetically early-maturing birds need be handicapped by winter pause.

2. Age at First Egg and Mean Size of Winter Clutch.

The mean size of winter clutch is obtained by dividing the total eggs laid from first egg to March first by the number of clutches involved. Clutch size has been shown by Hays and Sanborn, (1927) to be a good measure of intensity and behaves in inheritance on a two-factor basis. The simple correlation between mean winter clutch size and annual egg yield is +.3544±.0117 (Hays and Sanborn, loc. cit.). If early sexual maturity is linked with large winter clutch size, there should exist a significant net correlation between the two. The net correlation between age at first egg and mean winter clutch size, after making the length of winter pause, total days broody, and annual persistency constant, is —.1879±.0105. This is a statistically significant constant, but its magnitude does not suggest that early sexual maturity is intimately linked with high intensity.

3. Age at First Egg and Total Days Broody.

Total days broody during the pullet year is a good measure of degree of broodiness (Hays and Sanborn 1926a). These workers also show in the same report that degree of broodiness as well as the presence or absence of broodiness during the pullet year must be considered in the breeding program. The simple correlation between total days broody and age at first egg is+.0062 ±.0194, a constant of magnitude insufficient to indicate any relation between early sexual maturity and days broody. By calculating the partial correlation between age at first egg and total days broody where winter pause, intensity and persistency remain constant, the true relation between sexual maturity and degree of broodiness is arrived at. The partial correlation coefficient is found to be +.0473±.0194, which establishes independence between age at sexual maturity and degree of broodiness.

4. Age at First Egg and Annual Persistency.

Persistency or long laying period at the close of the pullet laying year is of great significance as affecting annual egg production. Hays and Sanborn (1926c) report the simple correlation between annual persistency and annual

production as +.7082±.0072. The simple correlation between age at first egg and annual persistency is reported as —.6146±.0090. There appears to be an important relation between age at first egg and persistency, and this may be accurately determined by the partial correlation coefficient where winter pause, intensity and days broody are made constant. The calculations give —.5956±.0093 as the partial correlation coefficient. This constant indicates an important linkage between heritable factors for early maturity and for high persistency as has been previously pointed out by Hays (1927). Therefore, by the use of breeding females that carry the early maturity factor that is linked with the persistency factor, it is entirely possible to combine the two desirable traits in the same individual bird.

5. Length of Winter Pause and Mean Winter Clutch Size.

The simple correlation between length of winter pause and winter clutch size is —.0674±.0145 (Hays and Sanborn, 1926b). This constant does not suggest a significant relationship. By applying the method of partial correlation where age at first egg, total days broody, and annual persistency are made constant, the coefficient of correlation between length of winter pause and winter clutch size is —.0874±.0145. This constant is of very small magnitude and probably indicates no significant linkage between length of pause and winter clutch size.

6. Length of Winter Pause and Total Days Broody.

The simple correlation between length of winter pause and total days broody is —.1832±.0243 (Hays and Sanborn, unpublished data). Such a constant would indicate a tendency for intensely broody birds to exhibit shorter winter pause than do less intensely broody individuals. Possibly a short winter pause is compensated for the following summer either by longer broody periods or by a greater number of broody periods. By means of the partial correlation coefficient the correlation between the two characteristics may be calculated when age at first egg, winter clutch size, and annual persistency are made constant. The partial or net correlation between length of pause and total days broody is —.1609±.0245. This constant is statistically significant and indicates a slight tendency for intensely broody birds to pause for short periods in winter. There is apparently no linkage relation between the dominant genes for winter pause duration and the genes that intensify broodiness, but rather a tendency for short winter pause to be associated with a long period of broodiness.

7. Length of Winter Pause and Annual Persistency.

The simple correlation between length of winter pause and annual persistency is +.1017±.0182. The magnitude of this constant does not warrant the assumption of an important relation between length of pause and annual persistency.

The partial or net correlation of winter pause and annual persistency, when age at first egg, winter clutch size, and days broody are made constant, gives the true relation of winter pause to persistency. The partial correlation

coefficient is —.0393±.0183 and indicates complete independence between duration of winter pause and annual persistency.

8. Mean Winter Clutch Size and Total Days Broody.

Broody birds exhibit some tendency to lay in longer clutches during winter than do non-broody birds. Stated differently, there is a somewhat higher winter intensity in broody than in non-broody individuals (Hays and Sanborn 1927).

The net correlation between winter clutch size and total days broody for the pullet year is +.2079±.0187. There are, however, many non-broody individuals showing large clutch size and these birds should be used as breeders in the production flock.

9. Mean Winter Clutch Size and Persistency.

Both large clutch size and high persistency are desirable from the standpoint of fecundity. They should be combined in the same individual to secure maximum egg production. The net correlation between winter clutch size and persistency when age at first egg, winter pause duration and total days broody are made constant is +.0190±.0134. Thus complete independence is established between intensity and persistency.

10. Total Days Broody and Annual Persistency.

Degree of broodiness may be measured by the total days spent in broody behavior during the pullet laying year. The loss of production during broody periods has a pronounced effect in lowering annual egg records. On the other hand, high persistency is associated with large annual records. The net correlation between total days broody and annual persistency where age at first egg, intensity and winter pause are made constant is +.0579±.0209. Thus there is shown to be no significant linkage between degree of broodiness and annual persistency.

Relation of Characteristics Concerned in Fecundity to Annual Production

In this final study of the relation of characteristics concerned in fecundity to annual production the relative net correlation of each of the five traits with annual egg record is calculated. In this manner the true value of each trait as a selection unit may be discovered, as the method of partial correlation eliminates any possible effects from interrelation of characteristics concerned.

11. Age at First Egg and Annual Production

Age at first egg is a good measure of early or late sexual maturity in the pullet and has been used rather extensively as a selection unit in making up both laying and breeding flocks. The reason why early sexual maturity is desirable cannot be discovered without a knowledge of the relation of sexual maturity to the other fecundity traits as presented in sections 1, 2, 3, and 4 of this report. The possibility also exists that pullets that begin laying at an early age are able to complete their annual record under more favorable weather conditions than are later maturing pullets. Since the last two or

three months of the pullet laying year mark her greatest susceptibility to adverse environmental influences, it is entirely probable that early maturity may enable the bird to persist late, as pointed out in section 4.

By applying the method of partial correlation, winter pause duration, intensity, degree of broodiness and persistency are made constant and the net correlation between age at first egg and annual egg production arrived at. The net correlation between age at first egg and annual production is .0238 ±.0177. This insignificant constant clearly discloses that age in itself is not associated with annual production. Sections 2 and 4 make clear the fact that early maturing pullets may carry slightly greater intensity and that they beyond question tend to exhibit greater persistency than late maturing pullets. Thus the high yearly producer must be early maturing not because early maturity itself is of importance but because early maturity has some linkage with high intensity and very intimate linkage with persistency.

12. Length of Winter Pause and Annual Production.

The probabilities are that the domestic hen possesses functional capacity to lay a rather definite number of eggs previous to the time when she must cease laying and renew her reserve of materials necessary in the complex physiological processes of egg production. The mean length of the winter cycle for the flocks studied is 52.26 days (Hays and Sanborn, 1926b) and the mean length of winter pause is 32.26 days. The standard deviation in winter cycle is ±34.23 days, showing great variability in the length of time the birds may lay before the onset of winter pause. With these facts in mind, the negative correlation between age at first egg and length of pause observed in section 4 may be understood. Furthermore, there exists an appreciable negative correlation between early hatching and duration of pause as might be anticipated. The tendency to pause in itself is governed by a dominant inherited factor (Hays, 1924).

The net correlation of length of winter pause with annual egg record is important and is determined by making age at first egg, clutch size, total days broody and annual persistency constant. This constant is −.5487±.0128. Here is shown a rather intimate negative association between length of winter pause and annual egg yield. In the breeding program hereditary pause should be eliminated by the constant use of tested breeding males and females, and environmental pause should be controlled by time of hatching and methods of management.

13. Average Size of Winter Clutch and Annual Production.

The mean size of winter clutch has already been shown to be specific and a workable measure of intensity. In order to discover the association between mean clutch size and annual production the method of partial correlation is applied. In this instance age at first egg, length of pause, days broody and annual persistency are made constant. The net correlation between winter clutch size and annual production is +.1944±.0101. This constant is of sufficient magnitude to demonstrate that intensity as measured by winter clutch size is a significant characteristic in relation to annual production and of about the same importance as duration of winter pause.

14. Total Days Broody and Annual Production.

Degree of broodiness may be measured by the total broody days during the pullet laying year. The degree of broodiness is affected by inherited factors so that it may be reduced. The presence of broodiness in any degree has been shown to be inimical to annual egg production (Hays and Sanborn, 1926a) and the broody trait itself has been shown to be inherited (Hays, 1924).

To discover the true relation of degree of broodiness to annual production, the method of partial correlation is applied with age at first egg, winter pause, winter clutch size and persistency made constant. The net correlation between total days broody and annual egg production is $-.5630 \pm .0097$. This is an important relation and makes clear that degree of broodiness is a vital factor in annual egg yield. Degree of broodiness may be placed on a par with winter pause and intensity as a characteristic affecting annual production (see sections 12 and 13).

The simple correlation between degree of broodiness and annual production is $-.1964 \pm .0194$ and the simple correlation between broodiness and higher than mean annual production is $-.2640 \pm .0132$ (Hays and Sanborn, 1926a). This latter constant does not show the presence of broodiness to be as inimical to annual egg yield as is the presence of winter pause. From these observations it appears that a very important step has been the reduction in degree of broodiness and that the increased annual egg record from non-broody birds is only significantly greater than that from birds broody but once in the pullet year.

15. Annual Persistency and Annual Egg Production.

Annual persistency in laying (long laying period) at the end of the pullet year has been shown to be very important in relation to annual egg record. The true relation of persistency to egg record may best be arrived at by using the method of partial correlation where age at first egg, length of winter pause, winter clutch size and total days broody are made constant. The net correlation between persistency and annual egg yield is $+.7501 \pm .0063$. This is a very intimate correlation and places annual persistency as the greatest single characteristic affecting annual production.

A breeding program should therefore lay special stress on the high persistency characteristic which is intimately linked with early sexual maturity (Hays, 1927).

16. Multiple Correlation Between Five Fecundity Traits and Annual Egg Production.

Theoretically, if all influences affecting annual egg yield were brought together and correlated with egg yield, the correlation should be perfect. In this report five of the most important inherited traits are considered. No account is taken of various environmental influences that operate to affect fecundity, because such influences are not breeding problems but rather problems of management. If by this method a high total correlation is discovered, it will be an indication that the breeding program has been directed along constructive lines.

By means of multiple correlation the characteristics age at first egg, length of winter pause, winter clutch size, total days broody and annual persistency have collectively been correlated with annual egg production. The constant obtained is R = +.8642. This constant shows that the five traits considered show a high degree of correlation with annual production and that they are very largely responsible for annual production.

Summary

Relation to Each Other of Characters Concerned in Fecundity

Characters Compared		Simple Correlation	Net Correlation
Age at first egg	Length of winter pause	.2329±.0138	-.2236±.0139
Age at first egg	Winter clutch size	-.2273±.0103	-.1879±.0105
Age at first egg	Total days broody	+.0062±.0194	+.0473±.0194
Age at first egg	Annual persistency	-.6116±.0090	-.5956±.0093
Length of winter pause	Winter clutch size	-.0674±.0145	-.0874±.0145
Length of winter pause	Total days broody	.1832±.0243	.1609±.0245
Length of winter pause	Annual persistency	+.1017±.0182	-.0393±.0183
Winter clutch size	Total days broody	+.2205±.0186	+.2079±.0187
Winter clutch size	Annual persistency	+.1692±.0130	+.0190±.0134
Total days broody	Annual persistency	+.0532±.0209	+.0579±.0209

Relation to Annual Production of Characters Concerned in Fecundity

Age at first egg	Annual production	-.4380±.0134	-.0238±.0177
Length of winter pause	Annual production	-.2107±.0176	-.5487±.0128
Winter clutch size	Annual production	+.3544±.0117	+.4944±.0104
Total days broody	Annual production	-.1964±.0194	-.5630±.0097
Annual persistency	Annual production	+.7082±.0072	+.7501±.0063

The partial or net correlations tabulated above indicate the following relationships:

1. Age at first egg and winter pause are significantly but not intimately negatively correlated.
2. Age at first egg and mean winter clutch size are negatively correlated to a significant but not intimate degree.
3. Age at first egg and total days broody are independent.
4. Age at first egg and annual persistency are intimately correlated and early sexual maturity is linked with high persistency.
5. Length of winter pause and winter clutch size are independent.
6. Length of winter pause and total days broody are negatively correlated in a minor degree.
7. Length of winter pause and annual persistency are independent.
8. Mean winter clutch size and total days broody are positively correlated to a moderate degree.
9. Winter clutch size and annual persistency are independent.
10. Total days broody and annual persistency are independent.

11. Age at first egg and annual production are independent.

12. Length of winter pause and annual production are negatively correlated in an important degree.

13. Winter clutch size and annual production are positively correlated in about the same degree as days broody and annual production are negatively correlated.

14. Total days broody and annual production are negatively correlated and days broody is of about the same importance as length of winter pause and clutch size in relation to production.

15. Annual persistency and annual production are very intimately correlated with each other. The degree of correlation places persistency as the most important characteristic affecting fecundity in the flocks studied.

16. The multiple correlation of age at first egg, length of pause, winter clutch size, days broody, and persistency with annual egg record is +.8642.

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MASSACHUSETTS
AGRICULTURAL EXPERIMENTAL STATION

Technical Bulletin No. 13 May, 1928

Washing Powders For Dairy Use

By A. W. Phillips, M. J. Mack and J. H. Frandsen

The importance of cleanliness in the production and handling of dairy products is very generally recognized, and as a consequence there is much interest in the use and manufacture of suitable cleaning compounds. It is recognized that under our present system of advertising the use of a product may not necessarily be proportional to its merit. It was in an attempt to determine what constitutes merit in a cleaning powder that this study of composition and properties was undertaken.

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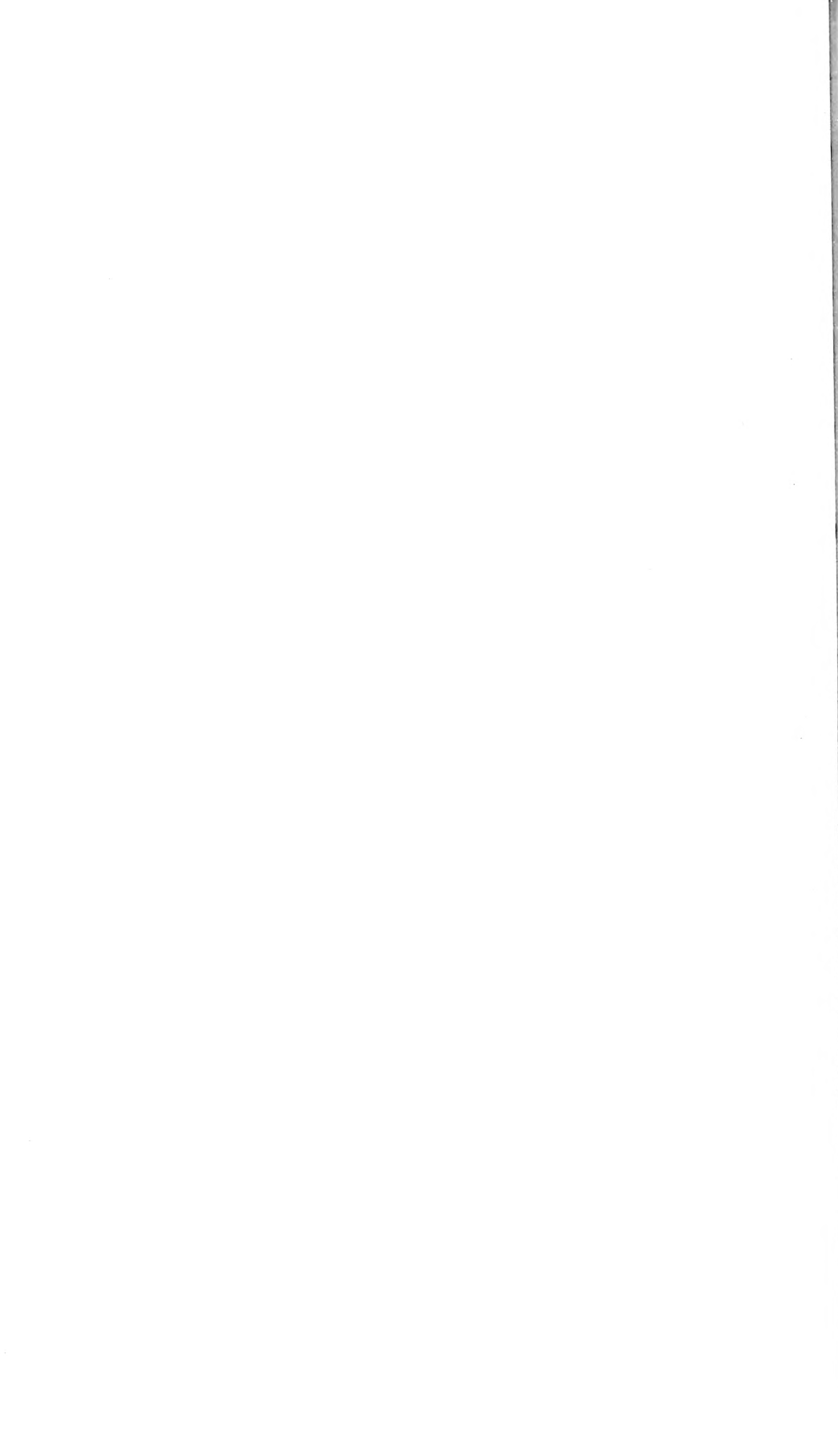

WASHING POWDERS FOR DAIRY USE

By A. W. Phillips, Assistant Research Professor of Dairying, M. J. Mack, Assistant Professor of Dairying, and J. H. Frandsen, Professor of Animal and Dairy Husbandry[1]

Introduction

The most important factor connected with any food supply is cleanliness. This is particularly the case in the handling of dairy products because, owing to their greasiness and solid particle content, these products adhere very tenaciously to all utensils with which they come in contact, and, being very good food upon which many micro-organisms can live, offer excellent breeding grounds for bacteria. Therefore, it is extremely important that all milk utensils should receive a thorough cleaning after each use.

This paper reports a study of the cleansing of dairy utensils and how it may be accomplished satisfactorily. There are two phases of the problem due to the complexity of the milk products themselves: first, the cleaning of the utensils from adhering particles of the dairy product; and second, the sterilization of the cleaned surface. The cleaning operation is by far the more important step, for without thorough cleaning sterilization would be extremely difficult if not impossible. Also, sterilization is accomplished to a certain degree during the cleaning process.

Chemical Changes in Cleaning

Milk and the products manufactured from milk are of a very complex physico-chemical nature. In the handling of dairy products we are dealing with a very delicately balanced system of emulsions, colloids, and solutions which, even when unaltered by any treatment, possess great adhesive properties for surfaces with which they come in contact. When the products are altered by intrinsic agencies such as souring or by outside agencies such as heat, then the original systems of emulsions and colloids are changed and the solid and liquid materials thus thrown out are found to adhere even more tenaciously, and the difficulties of the cleaning operation are therefore greatly increased. The problem in cleaning is, then, to re-emulsify these deposits or bring them back into the colloidal state.

The fat or oily ingredients in dairy products can be separated from the surfaces to which they adhere by the action of some emulsifying agent provided they are not bound there by some other ingredient. This emulsifying action is not, as is popularly believed, due to neutralization of fatty acids and saponification. If the fatty substance is not rancid there is scarcely any free acid present and, moreover, saponification does not take place under conditions existing in the washing process.

An alkali is capable of assisting in the formation of a surface layer by reacting with the free fatty acid in the grease to be removed. The "surface activity" of the detergent is increased by adding alkali (1). The alkali lowers the interfacial tension between oil and water but does not saponify the oil to any extent. In order to maintain a constant alkali strength buffer salts are needed in the detergent.

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[1] Acknowledgement is made to Professor H. F. Judkins, former head of the Dairy Department, who originally proposed this problem.

(1) Shorter: Proc. Roy. Soc. London **A92**:231 (1916).

The cleaning of dairy utensils is not merely "cutting the grease" with alkali. Milk proteins are soluble in alkali. But once coagulated or dried, solution is very slow even with a strong concentration of alkali. Therefore, something besides alkali must be used in cleaning. The cleaning solution must possess deflocculating or emulsifying power so that coalescence of the oily substances into droplets is impossible. This is prevented by the formation of a surface layer of the deflocculant around the fat globules. Surface activity is therefore a vital function in the cleaning solution. The detergent must go into true solution, not colloidal or crystalloidal, before it can become oriented and adsorbed upon the surface of the particle. Deflocculation occurs when a certain minimum concentration of "absorbed oriented molecules" of the detergent upon the dirt particle surface has taken place (2).

Methods of Comparing Washing Powders

The first step in this investigation was logically the chemical analysis of the various washing powders found on the market. However, the evaluation of complex and variable materials such as most cleansers are cannot be based upon chemical analysis alone. A properly designed test for performance often affords data of greater practical usefulness.

No test yet proposed for determining the cleansing efficiency of detergents has received general acceptance, and chemists still depend almost entirely upon data of composition for evaluating the respective merits of competitive samples. Such dependence is justified, once the effect of each ingredient has been studied in relation to its use alone and with other ingredients in varying proportions.

In making laboratory tests, no objections can be raised to tests which closely parallel service conditions provided they are not impractical. Such tests may actually prove capable of distinguishing between various samples with sufficient precision for ordinary comparisons. The tests devised for this work, which are described in detail below, may be considered practical in every respect.

Tests based upon the determination of surface tension or interfacial tension appear not entirely satisfactory (3). Such tests show wetting power but this is not necessarily vitally connected with detergent action. The cleaning compound must wet the dirt surface, but it must do more than this. It must have the powers of emulsification and deflocculation.

The test of greatest importance is of course the washing power of the powder. Yet this was the hardest test to conduct. Other investigators (4) have found that when using a specific detergent results varied on the average by ten per cent when all conditions insofar as possible were kept constant. With milk in particular the cleaning action is quite varied, depending upon the depth, hardness, et cetera, of the dried or gummed deposit.

Chemical Analyses of Powders

In order to ascertain the types and differences in washing powders on the market, thirty-six brands of powders recommended for dairy use and on sale in Massachusetts were collected. These powders were subjected to chemical analysis to determine the kind and amount of ingredients present. Then the

(2) Chapin: Ind. and Eng. Chem. 17:1187 (1925).
(3) Chapin: Ind. and Eng. Chem. 17:461 (1925).
(4) Luksch: Seifenseeder. Ztg. 40:413.

powders were subjected to laboratory and plant tests for efficiency of performance in the various cleaning operations.

The results of the chemical analyses are listed below. If the figures given do not total 100 per cent the reader must bear in mind that the water content may be very high in some cases (15 per cent or over), and many ingredients not determined but listed later may have been present. Chapin in some of his work,(3) leaves undetermined quantities as high as 26 per cent.

Analyses of Washing Powders.

Sample	Total Alkali as NaOH %	Sodium carbonate %	Sodium hydroxide %	Tri-sodium phosphate $12H_2O$ %	Soaps as fatty acids %	Remarks
1	57.8	61.5	0.0	37.8	0.0	Chlorine
2	31.0	0.0	2.0	98.0	0.0	Trace insol.
3	57.2	61.5	0.0	38.1	Little	
4	55.7	63.7	7.7	0.0	Little	
5	47.9	45.5	8.8	14.8	27.7	
6	15.5	4.5	12.1	0.0	Little	Much grit
7	89.3	60.5	40.8	0.0	0.0	Trace insol
8	60.7	85.5	0.0	0.0	0.0	Trace insol
9	71.8	52.3	32.4	0.0	8.0	
10	39.6	38.6	10.5	0.0	19.8	Sulfates
11	34.0	3.6	3.0	95.0	0.0	
12	60.8	88.6	0.0	0.0	0.0	
13	53.5	63.7	5.5	0.0	17.9	
14	88.3	55.5	46.4	0.0	0.0	
15	58.5	66.0	0.0	32.7	0.0	
16	58.5	55.5	0.0	45.0	0.0	
17	59.7	61.5	7.3	18.7	Little	
18	61.3	68.2	0.6	29.1	0.0	
19	38.6	18.2	0.0	80.0	0.0	
20	54.7	66.8	4.2	0.0	12.6	
21	64.5	76.0	7.2	0.0	0.0	
22	33.6	1.0	5.0	94.0	0.0	
23	25.0	0.0	0.0	89.0	0.0	Trace insol.
24	34.6	7.7	0.0	93.0	0.0	
25	63.8	66.0	14.0	0.0	Little	
26	53.2	56.0	10.9	0.0	24.1	
27	33.6	0.9	5.1	94.2	0.0	
28	27.2	0.0	0.0	100.3	0.0	Trace insol.
29	62.0	94.6	0.0	0.0	0.0	
30	93.0	46.8	54.6	0.0	0.0	
31	60.7	95.5	0.0	0.0	0.0	
32	75.0	56.8	32.2	0.0	0.0	
33	76.8	34.0	51.2	0.0	0.0	
34	58.6	65.0	9.6	0.0	13.6	
35	64.0	72.8	6.8	7.0	Little	Grit
36	62.7	95.5	0.0	0.0	0.0	
A	100.0	0.0	100.0	0.0	0.0	Pure sodium hydroxide
B	81.2	100.0	0.0	0.0	0.0	Pure sodium carbonate
C	26.8	0.0	0.0	100.2	0.0	Pure tri-sodium phosphate
D	14.3	0.0	14.3	0.0	85.1	Pure castile soap

It may be observed from this table that many of the powders were of very nearly identical composition, yet great variations are also shown. The powders may be classified into four main groups: those containing pure carbonate, those containing tri-sodium phosphate, those containing free caustic

and those containing soap. The roles played by the various ingredients will be discussed later after the practical tests have been described.

The water content of the powders varied considerably. No determinations of moisture content are given because this factor would tend to vary. Powders high in sodium hydroxide would tend to absorb water more rapidly than others. All the powders when weighed were of a dry, somewhat dusty nature, and not in the least gummy or sticky.

The ease of solution of the powders followed a general trend depending upon composition. Those containing tri-sodium phosphate were all very slow to dissolve. Also, the soapy powders were somewhat slower to enter solution and often lumped. The carbonate and hydroxide powders dissolved quite readily although there was a tendency for the hydroxide powders to lump. Those high in sodium hydroxide tended to heat considerably upon dissolving and those high in tri-sodium phosphate tended to cool.

Practical Tests

In this part of the investigation it was aimed to duplicate actual working conditions in the laboratory and then to conduct practical tests in the plant. The tests used and the method of procedure for each are given below.

The strength of solutions employed in these tests was standard 0.6 per cent based on the dry powder. This was found to be the average concentration recommended by the various manufacturers. A few recommend a weaker or stronger solution, but in order to study the efficiency of the powders a standard had to be adopted. This concentration corresponds to five pounds of powder to one hundred gallons of water.

The water softening power of the powders was determined by treating 100cc portions of the samples with 50cc quantities of standard hard water and then adding standard soap solution until permanent bubbles appeared.

The washing power of the powders was determined by tests on uniformly dirty bottles. Five cubic centimeter quantities of milk were run into bottles and allowed to dry. While drying, the bottles were occasionally tilted and rotated so as to wet the sides. The action of 100cc amounts of the cleaning solutions upon these dirty bottles was then observed.

The emulsifying power was tested by shaking a 100cc portion of the powder solution with 1.0cc of butterfat, the whole contained in a tall cylinder. The degree of emulsification was determined by the whiteness of the emulsion and by the length of time the emulsion persisted.

The ease of rinsing of each solution was tested by moistening the fingers with the solution and then counting the number of half seconds the slippery feel lasted while the fingers were held under a flow of water and gently rubbed twice per second. The flow was from a faucet and was regulated to give a stream one-fourth inch in diameter. This test was found to be satisfactory and gave consistent and reproduceable results. The average time of three experiments was taken in each case.

The action of each washing powder solution upon the metals aluminum, copper, nickel, tin and zinc was also studied. Strips of these metals were cleaned and allowed to stand in solutions of the various powders. These tests were run for a considerable length of time in order to determine the effect of continued use of the powders.

All results are tabulated below. In case the figures given seem to show some discrepancy the reader is reminded that the tests are not absolute, and the previous workers on similar tests for washing efficiency obtained values varying as much as 10 per cent.

Results of Practical Tests on Washing Powders.

Sample	Water Softening Power*	Washing Test	Emulsification Test	Rinsing Test†
1	200	Good	Good	9
2	216	Good	Poor	12
3	28	Good	Fair	7
4	237	Good	Very good	15
5	194	Fair	Very good	17
6	28	Poor	Very good	2
7	181	Fair	Good	15
8	157	Poor	Good	5
9	231	Good	Very good	27
10	241	Good	Very good	25
11	75	Fair	Poor	15
12	237	Poor	Poor	1
13	231	Good	Good	7
14	178	Fair	Good	48
15	72	Poor	Poor	8
16	71	Poor	Poor	9
17	84	Fair	Very good	23
18	124	Good	Good	18
19	183	Good	Good	25
20	202	Poor	Very good	11
21	150	Poor	Good	7
22	27	Good	Poor	12
23	31	Fair	Good	13
24	52	Poor	Poor	12
25	250	Poor	Very good	10
26	276	Good	Good	10
27	143	Poor	Poor	14
28	112	Fair	Good	14
29	237	Fair	Very good	3
30	135	Fair	Fair	17
31	178	Fair	Fair	4
32	162	Fair	Good	40
33	94	Fair	Fair	15
34	243	Poor	Good	10
35	235	Fair	Good	4
36	234	Good	Poor	1
A	31	Very good	Fair	28
B	175	Very good	Good	9
C	153	Poor	Good	10
D	250 plus	Fair	Very good	8

* The figures in this column give the cubic centimeters of standard hard water (containing the equivalent of 0.2 grams of calcium carbonate per liter) softened by 100 cc of the standard solutions of the powders.

† The figures in this column give the number of half seconds the slippery feel lasted when tested as outlined above. These figures are all comparable, the low ones indicating rapid rinsing.

Further tests were conducted, which concerned the action of the typical powders numbered 1, 2, 3, 6, 7, 8, 20, 23, 28, 31, A, B, and C, upon the metals aluminum, copper, nickel, tin and zinc. The action of the powders upon the metals was carefully watched during the first day, and then observed daily thereafter. Strong alkali powders reacted immediately upon aluminum. Other combinations reacted less readily and in reverse proportion to their caustic strengths. Powders high in soap content tended to blacken and tarnish the metals. The tests were all allowed to stand for fifteen days. A summary of the results is listed below:

The Action of Washing Powders on Metals.

Sample	Aluminum	Copper	Nickel	Tin	Zinc
1	P	G	E	F	E
2	P	E	E	B	E
3	B	F	E	F	E
6	F	F	E	G	F
7	P	B	E	B	F
8	P	B	E	E	E
20	P	F	E	F	F
23	B	E	E	P	E
28	P	E	E	P	E
31	P	B	E	G	E
A	B	B	E	P	E
B	B	P	E	F	E
C	F	E	E	F	E

Key

E—Excellent condition
G—Good condition
F—Fair condition
P—Poor condition
B—Bad condition

Tests of the germicidal action of solutions of the above samples showed that in solutions of 0.6 per cent strength, dirty, milky water was sterilized after standing for thirty minutes in all tests at room temperature.

Plant tests were conducted upon representative powders by using them in the daily bottle and can washing operations. The results in these cases substantiated those obtained from the laboratory tests.

Summarizing, we may place the ingredients in the order of effectiveness as follows:

Water Softening Powers:	Carbonate, phosphate, soap, hydroxide
Washing Powers:	Carbonate, hydroxide, soap, phosphate
Emulsifying Powers:	Soap, phosphate, carbonate, hydroxide
Ease of Rinsing:	Carbonate, phosphate, soap, hydroxide
Action on Metals:	Hydroxide attacks aluminum, copper and tin Carbonate attacks aluminum, copper and tin Phosphate attacks aluminum
Disinfecting Values:	All powders in strength ordinarily employed act as disinfectants to such a degree as to make the washing solution sterile.

The Role of Constituents Used in Cleaning

Reviewing and correlating the data observed in the above experiments, it is possible to draw somewhat trustworthy conclusions as to the roles played by each particular ingredient in the powder and their action when combinations are used.

Sodium Carbonate has very good softening power, greatly aids washing mechanism, is a poor emulsifying agent, rinses very easily, has slow action on tin and very mild action on hands, and neutralizes odors.

Sodium Hydroxide is a very poor water softener, with good washing power and poor emulsifying effect. It is very difficult to rinse, acts on metals, has a severe action on hands, is a strong caustic, and will pit cans.

Tri-Sodium Phosphate is an excellent water softener, with poor washing power and excellent emulsifying properties. It is easy to rinse, has very mild action on metals and hands, is a good solvent for casein, and gives buffer effect which keeps caustic strength uniform.

Soap has poor softening power, poor washing power and excellent emulsifying properties. It is hard to rinse, has mild action on metals and hands, and is apt to leave scum in bottles.

Other Ingredients are often added for specific purposes. *Hypochlorites* are added in some instances because of their germicidal action. *Sulfates* are occasionally added, but have no desirable qualities. *Silicates* of some metals are added to render protection to the machinery parts in contact with the solution. *Sodium zincate* and *aluminate* are sometimes added for protection of zinc and aluminum parts respectively. *Resinates* and *gums* are also occasionally found in the powders and are usually added to protect copper fittings. *Grit* is added to some to clean metals. *Borax*, although not often found, has certain advantages of emulsification and disinfecting powers.

Thus it may be seen that no one ingredient is a perfect washing compound of itself, and for efficient washing a mixture should be used.

General Considerations

Soap, owing to the difficulty of rinsing which is due in large measure to suds formation, and owing to the dangers from odor, is not considered a wise, nor is it an essential ingredient for washing powders for dairy use. Also it is more expensive and will deteriorate upon long standing. Powders containing soap are light in weight and thus often deceive the purchaser as to the quantity he is obtaining.

For hand washing any free caustic should be avoided because such solutions readily attack the skin of the operator. It also attacks metals and in general its use is not justified, for other ingredients of less drastic action are even more efficient in the actual cleaning operation. Caustic lacks many of the essential qualities of a good cleaning agent. It is very difficult to rinse off and thus will cause pitting and darkening of the cans.

For machine washing strong caustic may be used and is efficient in removing grease. Although it acts upon metals and glass the action is not drastic and is noticed only after continued use. The mechanical washer is perhaps the most efficient because of the possibility of using stronger solutions and hotter water. A 3 per cent caustic at 140° F. can be used with the mechanical washer.

Tri-sodium phosphate is a particularly valuable constituent because of its remarkable emulsifying and softening power. In softening water containing calcium sulfate, calcium, magnesium and iron silicates and calcium carbonate the action is to throw all these metals out of solution as phosphates while the acid radicals will become united with sodium and remain in solution where they will cause no harm. The phosphate also has a strong buffer effect which keeps the caustic strength of the cleaning solution uniform by supplying more caustic as soon as some is neutralized. The caustic strength of the phosphate is only about one-tenth that of an equal amount of hydroxide and therefore no great caustic concentration is necessary at the start of operations in order to have the solution strength maintained during the washing when the phosphate is used. In this respect, using phosphate, added in quantities at the start, gives the same effect as adding a small amount of hydroxide every little while.

Casein is soluble in alkali in proportion to the caustic strength. However, much of the precipitated or baked-on casein is in the form of calcium caseinate which is insoluble in alkali. Here tri-sodium phosphate acts to a much greater advantage than the other components. Calcium in the water supply is more efficiently removed by sodium phosphate. Casein is soluble in borax although this substance is scarcely ever used in dairy washing powders. It would aid the disinfecting properties but it has only a mild cleansing action. Borax is easily rinsed and forms a good emulsion with milk fats.

The action of the various powders upon glass is rather severe over long periods of time. With bottles, the length of life is so short that the corrosive effect does not need to be considered. However, with the new glass-lined vats the problem might become important. In the order of their severity of action upon glass the several ingredients may be listed: hydroxide, phosphate, carbonate and soap.

Alkalies of any kind attack paint and so no strong cleaning solution should be allowed to remain on painted surfaces for any length of time.

Cleaners containing abrasives should be used sparingly, and only when absolutely necessary. Any abrasive will wear away the surface over which it is rubbed. If the surface is plated, the under metal will soon be exposed and form an electrolytic cell with the metal used in plating. This causes increased corrosive action. Also the grit present is very hard to rinse off.

On the average, five to six pounds of powder to a hundred gallons of water may be regarded as the proper strength solution for ordinary hand washing. This of course will vary with the particular powder used and the hardness of the water. Smaller amounts of any powder are not to be recommended even though the sales agent may speak very highly of his product and what it will do. The majority of the powders on the market may be classified under three heads having nearly the same composition, and a high price does not necessarily infer any greater efficiency. Prices quoted different consumers vary greatly even for the same product from the same manufacturer and, of course, vary with the size of the order. The current prices for the basic ingredients are as follows:

Sodium carbonate — \$0.90-\$1.30 cwt. depending upon purity.

> The unrefined material is satisfactory. There are several grades depending upon the water content: as soda ash, Na_2CO_3; crystal carbonate, $Na_2CO_3.H_2O$; and soda crystals or washing soda, $Na_2CO_3.10H_2O$. Soda crystals contain 63 per cent water. One ton of crystal carbonate contains as much alkali as 48 cwts. of soda crystals and occupies only half as much space. The soda ash is highly concentrated and is liable to cake. It is higher priced and has no advantages.

Sodium bicarbonate	\$2.00 cwt.
Sodium hydroxide	3.00 cwt.
Tri-sodium phosphate	4.00 cwt.

The 60 per cent carbonate—40 per cent phosphate (12 H_2O) recommended in the following pages would cost \$2.20 per cwt., or a little over 2 cents per pound for raw products. The cost of mixing would be negligible.

Soap prices are omitted because they would vary greatly depending upon the purity of the product, and because soap is not considered advisable for use.

Thus it is evident that users of large amounts of washing powder would find it profitable to buy their own stock and mix their own powders. Smaller dealers might not be sufficiently reimbursed for the trouble. Great care is necessary to protect the eyes from alkali dusts. Also the dust is irritating to the throat and nose. Therefore the mixing should be done in an enclosed room, and the man doing the mixing should be protected by a sponge through which to breathe, and his eyes protected by goggles.

The temperature of the washing solution should not much exceed 140° F. or 60° C. because, if allowed to do so, the precipitated casein and gummy

deposits will bake on harder. For hand washing this temperature is too severe and a lower temperature must be resorted to. However, up to about 140° F. the hotter the better as regards the cleaning action. The bactericidal action is markedly reduced below 35° C. or 95° F.

From a survey of the powders used by a number of dairies in Massachusetts the outstanding feature is that the majority of changes from powders previously used has been for the adoption of powders containing tri-sodium phosphate. The tri-sodium phosphate powders are held in high favor by those using them and these powders are proving very desirable. There is a tendency to avoid the use of strong caustic powders except for very special uses.

A Suggested Composition for Washing Powders

It may be safe to say that the best powder for dairy cleansing should have no soap and no free caustic for hand washing, and little free caustic for machine washing. A satisfactory powder should analyze approximately as follows:

Sodium carbonate (anhydrous)	60%
Tri-sodium phosphate ($12H_2O$)	40%
Total alkali as NaOH about	58%
Sodium hydroxide	None (except what may occur free in the carbonate)
Soap	None

This combination has been found to give good results in all laboratory tests as well as in all plant tests. It was found to be efficient in its cleansing action and to possess all the desirable qualities of a good powder. This percentage composition could be obtained from various mixtures of the commercial chemical products which should be analyzed and mixed accordingly. Commercial tri-sodium phosphate ordinarily contains 12 molecules of water.

For machine washing it may be deemed advisable to increase the total alkali content by the addition of a little sodium hydroxide.

Summary and Conclusions

1. Analyses of many washing powders on the market show four general classes, containing carbonate, caustic, phosphate and soap respectively.
2. There is very slight variation in those powders which come in the same class.
3. Laboratory and plant tests on these powders, on other mixtures, and on the pure ingredients have demonstrated the specific roles played by each ingredient.
4. A desirable composition for general dairy use has been indicated to be 60 per cent sodium carbonate and 40 per cent tri-sodium phosphate.
5. By buying the commercial chemicals the price per pound of cleaner may be reduced to close to 2 cents as compared with from 8 to 16 cents now paid for a similar grade of product.
6. The washing efficiency of the powders increased up to about 140° F. Below 95° F. the bactericidal action is greatly reduced.
7. All powders showed disinfecting powers in 0.6 per cent solution, by rendering the wash water sterile.

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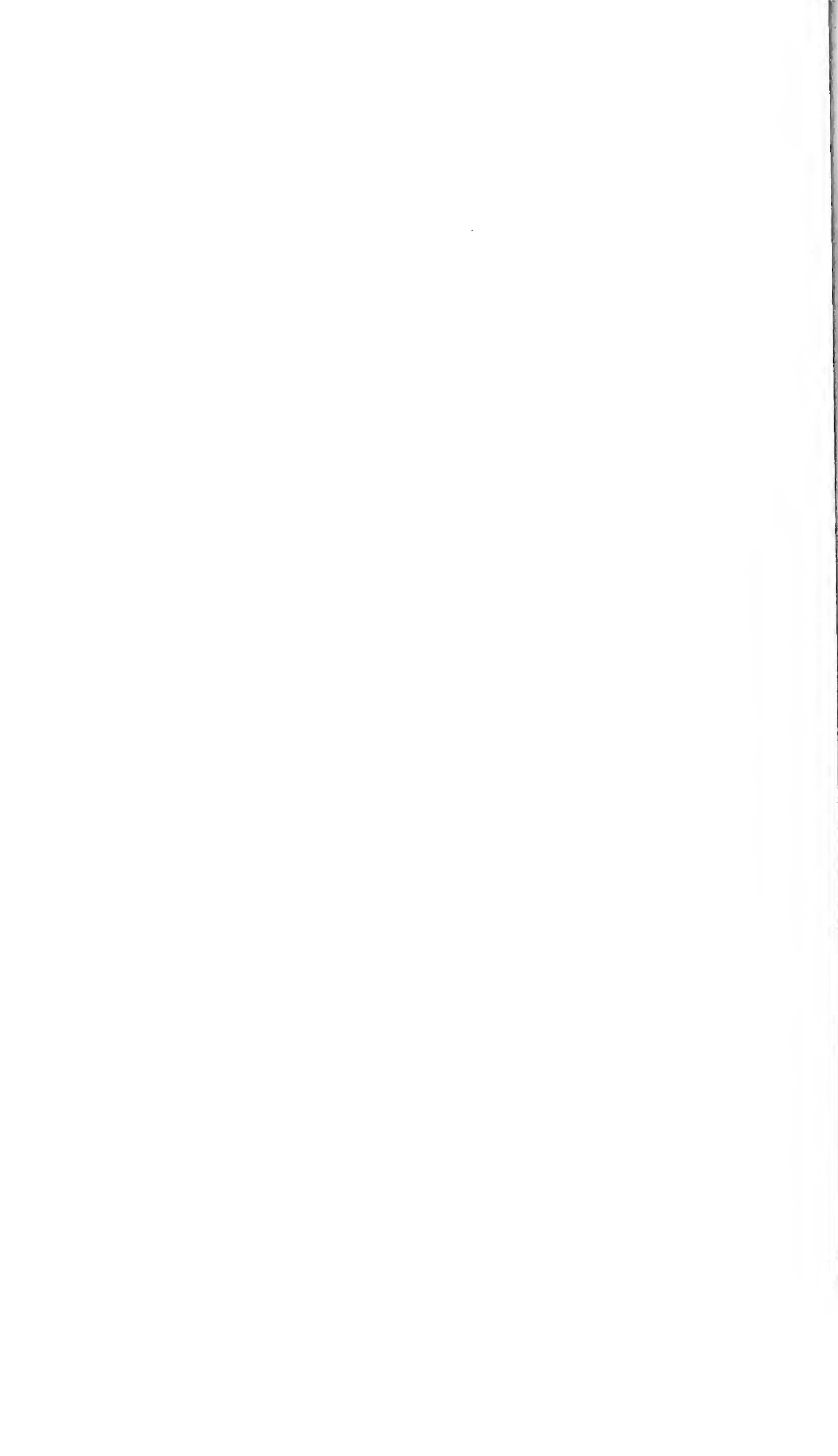

MASSACHUSETTS
AGRICULTURAL EXPERIMENT STATION

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Cutaneous Immunity in Relation to Contagious Epithelioma

By Norman J. Pyle

The investigation of contagious epithelioma at this Station has for its purpose the determination of an efficient preventive and curative treatment for the disease. This is of vital importance to the Massachusetts poultry industry because the disturbance causes serious loss by decreasing egg production during the season when eggs are highest priced. A serological study of immune birds was made for the purpose of standardizing the vaccine already extensively used. As this work progressed it became evident that immunity against the disease was not of a general nature, and therefore a study of cutaneous immunity was undertaken in its stead. Results of this latter investigation are reported in this bulletin.

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AGRICULTURAL EXPERIMENT STATION
AMHERST, MASS.

CUTANEOUS IMMUNITY IN RELATION TO CONTAGIOUS EPITHELIOMA.

By Norman J. Pyle, Assistant Research Professor of Avian Pathology.

Contagious epithelioma, a disease of poultry commonly known as chicken pox, has caused serious loss to Massachusetts poultrymen by decreasing egg production during the season when eggs are bringing the highest prices. The treatment for the disease has not been satisfactory. For this purpose a powdered pox virus vaccine, as recommended by Beach (1), was used quite extensively up until the winter of 1926-1927. In November, 1926, the writer (2) published a report on the experimental use of this and other vaccines, wherein it was shown that the vaccine did produce a slight degree of immunity, but not enough to protect the bird fully against the disease under all conditions. It was thought at the time that if a method of standardizing the vaccine could be determined, its therapeutic efficiency would be considerably enhanced. Accordingly, further investigations were undertaken with that objective in mind.

The logical method of attacking such a problem was to study immune birds serologically and attempt to produce a unit value of antiserum against which the virus or vaccine might be standardized. It was naturally assumed that immunity to contagious epithelioma was of a general nature; that is, due to a specific antibody concentration in the blood serum. As the work progressed antibodies were found, but they were present in the sera of immune birds in such a low concentration that it was evident that a protective force other than a general immunity was operating against the disease. Because of this conclusion and due to the fact that varying complement-fixation reactions prevented the determination of a unit value of antiserum, the entire aspect of the problem was changed.

The data herein reported include:

1. Serological studies on normal and immune birds which demonstrate that a specific antibody concentration in the blood serum of immune fowls is not the sole protective force against contagious epithelioma.
2. Immunity experiments which demonstrate that a cutaneous immunity is the chief protective force against the disease.
3. Experiments with local or cutaneous vaccines and their standardization.

SEROLOGICAL STUDIES

A review of the literature on the serological study of contagious epithelioma of the domestic fowl reveals very few references on the subject. Beach (1) mentions that the vaccine cannot be standardized by complement-fixation methods, but he does not describe the technic used in the investigation. Sweet (3) has published an interesting complement-fixation study of the serum of fowls affected with the disease. He found that hemolysis was complete in serum from normal birds, while in serum from birds in which the disease was operating he obtained fixation in varying degrees, or to use his own words, "There was considerable evidence of a specific antibody".

Source of Experimental Birds

White Leghorn cockerels and Rhode Island Red pullets and cockerels were used in the following experiments and were obtained from the poultry plant of the Massachusetts Agricultural College. They were progeny of birds which were free from *Salmonella pullorum* infection, as determined by the agglutination test. The birds were reared on a clean grass range which had not been used for this purpose for three years. At no time were they subjected to any infectious disease.

Technic of Bleeding Fowls.

The pin feathers are plucked from the inner surface of the wing over the triceps region and the area is cleansed with a suitable antiseptic, preferably alcohol. The brachial vein is selected for bleeding. It is found subcutaneously in a muscular groove formed by the bodies of the two major triceps muscles. A Luer syringe of 10 cc. capacity with a 20 gauge needle, 1½ inches long, is used for aspiration. Aseptic precautions are used during the bleeding process. From 20 to 25 cc. of blood can be taken from a mature bird in good physical condition without any apparent ill results. Approximately 150 birds were bled during the course of this investigation, and an average of 20 cc. of blood was taken from each individual without a single loss. Frequently desirable birds were bled a second and third time at weekly intervals. When more than 10 cc. of blood are required, as was the case in this investigation, a second syringe and the brachial vein of the other wing are used. After bleeding, the needle is removed from the syringe and the blood gently forced into a sterile test tube (6/8 by 6 inches). The tube is slanted until the blood clots, and it is then placed in the ice box over night, during which time the serum separates.

Source of "Immune" Serum.

The first serum subjected to serological study was obtained from White Leghorn cockerels, four to five months of age, which had been used for virus production and had fully recovered from the operation. Later, serum was obtained from yearlings that had recovered from either avian diphtheria or bird pox, or both, during their pullet and cockerel year. Serum was also taken from birds while they were in the active stages of contagious epithelioma. There was no doubt whatever that the first two groups of birds were immune to the disease. One might question the immunity of the yearling group, but this was determined by check inoculation of combs with active virus. Lesions of the disease did not appear. If specific antibodies were associated with immunity, they should be found in one or more of these three groups of sera.

In addition to the above, attempts were made to produce sera containing specific antibodies by using various vaccine combinations of the active virus. Healthy young White Leghorn cockerels were used in the experiments. It was determined that the subcutaneous administration of 30 milligrams of virus per dose, suspended in a 10 per cent glycerol-physiological saline solution, fully protected the birds, in the majority of cases, when they were check inoculated on the comb with virus for immunity determination. However, scablike lesions, which were found to contain pox virus, invariably appeared at the point of inoculation, and in several instances pox nodules also ap-

peared on the comb and eyelids. Those birds, in particular, which developed local and generalized reactions after the vaccine injection, demonstrated a complete immunity after check inoculation on the comb with the virus.

This vaccine was labeled the triple strength vaccine because it contained three times as much virus per dose as that recommended by Beach (1). Unattenuated vaccines of 10, 15, 20, and 25 milligrams of virus per dose were also used. They failed to produce the same degree of immunity as did the triple strength vaccine.

Daily rectal temperatures and weight readings of the birds in the triple strength vaccine experiment were taken. It was found that a prevaccinating average normal temperature of 106.6° F. to 107° F. was increased, beginning late on the day of vaccination and reaching a crisis of 108.6° F. to 109.5° F. three days later, then decreasing until it had regained normal temperature on the ninth day after the injection. A noticeable effect on the weight of the birds occurred. The cockerels were from five to six months old and gaining in weight on the average of from 10 to 20 grams per day prior to the vaccination. After the vaccination this gain in weight was either retarded for eleven to twelve days or decreased from an average of 1480 grams to 1420 grams during the same period. Thereafter the birds regained the average of 1480 grams and steadily increased in weight

These reactions on the part of the body to the vaccine injection indicated that the body was developing its protective forces against the disease and from all probabilities these forces were specific antibodies.

Complement-fixation Test.

Antigen.

Sweet (3) concluded, in his work with the complement-fixation test in contagious epithelioma, that the antigen which he was using lacked marked antigenic properties. He did not attempt to improve his antigen in this respect.

In this investigation a polyvalent, active virus was always used. An antigen composed of 1 gram of the virus, thoroughly triturated in 100 cc. of physiological saline solution and passed first through infusorial earth and next through filter paper, was but slightly antigenic. Alcoholic extracts were prepared from pulverized pox scabs and diphtheritic membranes, from pulverized liver from pox infected birds, and from pulverized normal chicken hearts. They were found to be without value as antigens.

The antigen finally selected as most suitable and perhaps the only possible reagent for this purpose, was 1.5 grams of powdered pox virus, thoroughly triturated in 100 cc. of 0.5 per cent phenolized physiological saline solution and passed through ordinary filter paper. Its hydrogen ion concentration was varied in an attempt to improve the antigenic properties. The original concentration of approximately 5.8 to 6.0 was the most satisfactory. Prior to its titration for antigenic and anticomplementary properties, it was heated at 60° C. for forty-five minutes.

A known four-plus positive serum was, of course, not available for use in titrating the antigen for its antigenic properties. Varying doses of serum and dilutions of antigen, and finally undiluted antigen, were used and a degree of fixation obtained. It was necessary to use a two-plus serum in the titration as a substitute for a four-plus serum. The reason for this will be apparent when the results of the complement-fixation test are discussed. In the antigenic titration of the antigen, 0.1 cc. of the undiluted antigen was found to be the titre or unit dose. Four units of the undiluted antigen were used as a working dose in the test.

Complement.

Normal guinea pig serum was used as complement. It was freshly collected once a week by bleeding directly from the heart of the guinea pigs. The undiluted complement was preserved with a 12 per cent solution of sodium acetate in the proportion of 1 part sodium acetate solution to 2 parts undiluted complement. A 1 to 4 dilution of the preserved complement was used for the preliminary complement titration. One and a half times the complement titre was used as the working dose.

Hemolytic amboceptor.

Sheep hemolytic amboceptor was used. In the routine titration of the amboceptor it was determined that the titre was .05 cc. of a 1-600 dilution. Five units or .25 cc. were used as the working dose.

Sheep cells.

Freshly drawn sheep blood was agitated in a 2 per cent solution of sodium citrate to prevent clotting. For this purpose from 5 to 10 cc. of the citrate were used to about 80 cc. of sheep blood. The red blood corpuscles were washed three times and a 2 per cent suspension of cells used in the test, 0.5 cc. being added to each tube.

Suspected or "immune" serum.

Difficulties were encountered in obtaining serum separation from the avian blood. In the first place the amount of serum obtained from the blood was small, considerably less than the usual 40 per cent. This is characteristic of cockerels' blood. It oftentimes "jellied" and in many cases was anticomplementary. It appeared that by exercising aseptic precautions in handling the serum, a great deal of this anticomplementary action was eliminated. The serum in many instances would also "precipitate", or present a flocculent appearance during or immediately after inactivation, especially if the temperature of the inactivating bath rose above 56.5° C. The suspected serum was tested in doses of 0.4 cc., 0.3 cc., 0.2 cc., 0.1 cc., and 0.05 cc.

Results.

With normal fowl serum, aside from the usual number of "jellied" and anticomplementary samples, hemolysis was complete. Tabulated results of the complement-fixation studies on the "immune" sera are too lengthy to be reported herein. It is sufficient to say that about one hundred and fifty such examinations have been made and in no instance was it possible to obtain complete fixation. Fifty per cent of the sera presented on the average a two-plus fixation of complement with 0.4 cc. of serum. An occasional three-plus fixation was noted in the same amount of serum. The remainder demonstrated a one-plus fixation, a mere inhibition of hemolysis, or were entirely negative (complete hemolysis).

Precipitin Test.

The antigens used in this test contained varying amounts of powdered pox virus, ranging from 0.5 gram to 1.5 grams, suspended in 50 cc. of 0.5 per cent phenolized physiological saline solution, and passed through a Seitz filter. Sera from a group of fifteen birds were subjected to the test. These birds had recovered from a severe experimental contagious epithelioma infection and had demonstrated a complete immunity after reinoculation with the virus.

TABLE 1.—Protocol of complement-fixation test.
Suspected serum

Tube No.		1	2	3	4	5
Serum	cc.	.1	.3	.2	.1	.05
Antigen	units	4	4	4	4	4
Complement	units	1.5	1.5	1.5	1.5	1.5
Saline	cc.	1.5	1.5	1.5	1.5	1.5
Water bath fixation 1 hour at 37.5° C.						
Amboceptor	units	5	5	5	5	5
2% sheep R. B. C.	cc.	.5	.5	.5	.5	.5
Water bath for 2 hours at 37.5° C.						

Controls

		Negative Serum				Suspected Serum									Undiluted Antigen		
Tube No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Serum	cc.	.4	.3	.2	.1	.4	.3	.2	.1	.05	.4	.2	.4	.2			
Antigen	units	4	4	4	4										4	6	8
Complement	units	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5					1.5	1.5	1.5
Saline	cc.	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Water bath fixation for 1 hour at 37.5° C.																	
Amboceptor	units	5	5	5	5	5	5	5	5	5			5	5	5	5	5
2% sheep R. B. C.	cc.	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5

Water bath for 2 hours at 37.5° C.

Seven of the birds were given a dose of the triple strength vaccine in order to make the immunity more intense, if such a thing were possible. The sera were tested both undiluted and diluted. Normal fowl serum was used as a control. All tubes were placed in a water bath at 37.5° C. for one hour and thereafter placed in the incubator over night.

TABLE 2. Protocol of precipitin test.

Rack No. 1

Tube 1.	Anti-pox serum	0.5 cc. + 1 cc. virus filtrate.
Tube 2.	Normal serum	0.5 cc. + 1 cc. virus filtrate (control).
Tube 3.	Anti-pox serum	0.5 cc. + 1 cc. saline (control).
Tube 4.	Saline	0.5 cc. + 1 cc. virus filtrate (control).

Rack No. 2

Tube 1.	0.2 cc. serum (undiluted)	+ 0.2 cc. virus filtrate.
Tube 2.	0.2 cc. serum (1-5 dilution)	+ 0.2 cc. virus filtrate.
Tube 3.	0.2 cc. serum (1-10 dilution)	+ 0.2 cc. virus filtrate.
Tube 4.	0.2 cc. serum (normal)	+ 0.2 cc. virus filtrate (control).
Tube 5.	0.2 cc. serum undiluted	+ 0.2 cc. saline (control).
Tube 6.	0.2 cc. saline	+ 0.2 cc. virus filtrate (control).

Results.

In no case was there any evidence of a precipitin reaction.

Passive Immunity.

Eight Rhode Island Red cockerels were divided into two groups of four birds each. Each bird received three injections of "immune" serum in the manner shown below in Table 3. The serum was obtained from immune birds that had demonstrated a three-plus complement-fixation reaction of their sera at various times. The sera were collected and pooled for injection on the same day that they were administered.

TABLE 3—Degree of protection afforded by the intravenous and subcutaneous injection of "immune" serum

Group and Bird Numbers	Method of Administration	Amount of "Immune" Serum Administered			Check Inoculation	Results of Check Inoculation for Immunity Determination		
		Dec. 13	Dec. 19	Jan. 6	Jan. 13	Jan. 17	Jan. 20	Jan. 28
		cc.	cc.	cc.				
Group 1, (103 to 106)	Intravenously	2	2.5	2.5	Comb and wattles scarified and virus suspension vigorously rubbed into the areas.	+ (*104–)	++ (*104–)	+++ (*104–)
Group 2, (107 to 110)	Subcutaneously	3.5	3.5	5		+	++	+++
(Controls) (111 & 112)						+	++	++++

\+ First evidence of pox nodules, immature and sparse.
++ Appearance of a few or several well formed pox nodules.
+++ Appearance of many pox nodules of mature development.
++++ Maximum development of pox nodules.
– Negative.

Interpretation.

The above results indicated that the intravenous and subcutaneous administration of "immune" serum, for the purpose of producing a passive immunity, failed to protect the birds against artificial inoculation with the virus of contagious epithelioma.

Conclusions.

1. The varying and incomplete complement-fixation reactions of sera from birds immune to contagious epithelioma indicated that the formulation of a unit value of protective antiserum, against which the vaccine or its virus might be standardized, was impossible.
2. The low degree of specific antibody concentration in the sera of birds immune to contagious epithelioma and the failure to produce a passive immunity indicated that a general immunity was not the sole protective force against the disease.
3. The development of lesions containing the virus of the disease at the point of inoculation and on the comb, after the subcutaneous administration of the triple strength vaccine, which was followed by the production of a complete immunity, indicated that the skin probably plays an important part in the immunity against the disease.

CUTANEOUS IMMUNITY.

It appears that there is another immunity factor operating in contagious epithelioma and it is evident that the subcutaneous injection of virus does not consistently produce this factor.

De Blieck and Van Heelsbergen (4) have apparently solved the problem of immunization against contagious epithelioma in European countries by producing a local or cutaneous immunity. They use a vaccine, known as "Antidiphtherin", which is "a thoroughly living vaccination material, the vitality of which has not been decreased, either physically or chemically, which always gives rise to a local pox-eruption, which never generalizes, which immunizes against the experimental as well as against the spontaneous infection, and which is constant for all these properties during all seasons". The vaccine is applied to an area of denuded feather follicles on the leg by means of a vaccinating instrument or small trocar. A swelling (pox eruption) of the follicles results and the investigators claim that birds showing such local or cutaneous reactions are immune to both the experimental and spontaneous infections. Hol (5) claims to have had marked success with "Antidiphtherin" in Holland. Doyle (6), in testing out samples of the vaccine procured on the open market, found them to vary in degree of "attenuation", and to cause a generalized infection in several instances. According to De Blieck (7) these faults of the vaccine have since been overcome.

De Blieck and Van Heelsbergen do not mention their method of producing "Antidiphtherin". Their various publications refer but briefly to a description of the general properties of the vaccine and its method of administration.

Experimental Comb Vaccination.

The early attempts to produce a local or cutaneous immunity (July, 1926)

were modifications of the method of Panisset and Verge (8). Approximately 1 sq. c.m. of comb area was curetted or scraped until lymph was drawn. Care was exercised not to produce a bleeding surface. Virus suspensions, unattenuated and attenuated by moist heat at 55° C. for one hour, were rubbed into the areas. Well formed epitheliomata always developed after the usual incubation period of from four to seven days, but in at least 50 per cent of all cases the lesions spread to contiguous surfaces of the comb, not remaining localized. The virus content of the suspensions was decreased until 200 milligrams of virus in 50 cc. of physiological saline solution were used. The results were about the same. The suspensions were then injected intracutaneously in 0.1 cc. doses, into a barb of the comb. This method of administering the vaccine greatly decreased the number of cases that presented lesions on the comb after the vaccination other than at the point of inoculation.

After the local lesions had fully cleared up in the birds used in these experiments, the combs were lightly scarified and unmodified virus rubbed into the areas to test the degree of immunity production. In all cases where local lesions had developed following vaccination, the immunity was complete or nearly so.

Experimental Skin Vaccination.

At the same time that the above experiments were in progress, various efforts to induce a local or cutaneous immunity by feather follicle vaccination were being made. Young and healthy White Leghorn cockerels, four to eight months old, were used in the experiments.

In the following experiments the place selected for vaccination was on the outside of the right leg just above the tibio-femoral joint. An area of approximately 1 square inch was denuded of feathers, cleansed with sterile physiological saline solution, dried with sterile cotton, and the vaccine applied by rubbing into the follicles with a cotton swab attached to a wooden applicator. The left leg was similarly treated for control purposes, saline solution being used instead of vaccine. The experiments described below differ only in the vaccine used and the method of preparing the feather follicle area prior to the administration of the vaccine.

Experiment 1.

Twelve birds were used. The vaccine was an unattenuated suspension of 0.5 grams of pox virus in 50 cc. of physiological saline solution. The bared feather follicles and interfollicular spaces were lightly scarified and the vaccine applied by rubbing into the area with a cotton swab attached to a wooden applicator. Five days later a definite swelling of the follicles had developed and scab formation over the orifices of the follicles was in progress. On the tenth day after vaccination, the scab formation was fully developed. The infection became generalized, pox lesions appearing on the comb and eyelids, in four of the birds. A systemic reaction, as evidenced by droopiness, lethargy, etc., also occurred in these four birds.

While scab formation was taking place, a local pyogenic inflammation developed, which was undoubtedly due to the *Staphylococcus aureus* and *Pseudomonas aeruginosa* content of the fresh virus (2) used in the vaccine. It was evident that scarification of the skin and feather follicles induced this local inflammation. Scarification was, therefore, contra-indicated.

The feather follicle scabs were removed, dried, pulverized in a mortar, suspended in saline solution and applied to the scarified comb areas of normal

birds. Lesions of contagious epithelioma developed, indicating that the scabs contained the pox virus.

The control legs of the birds showed no reaction.

After complete recovery from the general effects of the vaccination (about 22 days), virus inoculation of the comb proved that there was an apparent immunity, evidently of a cutaneous nature. Three normal birds were inoculated on the comb as controls for the virulence of the virus used to check the immunity. They all developed a heavy infection.

Experiment 2.

Twelve birds were used. In this experiment the vaccine contained the same amount of virus. It was also unattenuated, but passed through several layers of sterile gauze and cotton for the purpose of freeing it of epithelial debris, etc. It was injected into each denuded feather follicle, about 1 drop to each follicle. The purpose of the injection was to penetrate the dermal papilla, not deeply, but very superficially.

The resulting reactions were not confined to the follicle alone, but spread to the adjacent tissue as well. The systemic reaction was not so pronounced as in the preceding experiment, nor did pox nodules appear on the comb and eyelids. Scabs did appear on the orifices of the vaccinated follicles.

All controls were satisfactory and the degree of immunity development, as determined by check virus inoculation, was pronounced.

Experiment 3.

Fourteen birds were used. The vaccine in this case contained but 200 milligrams of virus. It was suspended in 50 cc. of a 40 per cent glycerol-physiological saline solution. Glycerol was used for the same purpose as in the triple strength vaccine; that is, it was an excellent medium for suspending the virus in solution and it was thought that it might have some attenuating action. The vaccine was applied directly to the follicles by rubbing it in well with a cotton swab attached to a wooden applicator. The area was not previously cleansed nor scarified. The birds reacted to the vaccine in a uniform manner and with but slight variation, as follows:

a. Within 4 to 8 days after vaccination, a gradual swelling of follicles occurred.

b. Within 8 to 18 days after vaccination, a gradual appearance of follicular scabs occurred.

c. Within 18 to 31 days after vaccination a gradual disappearance of follicular scabs and swelling occurred, the latter being the last to disappear.

The reactions remained localized. Pox nodules did not appear on the comb. The birds were check inoculated on the comb on the 31st day after vaccination with a suspension of virus. They all showed a complete immunity, while the usual number of controls on the virulence of the virus all presented a heavy comb infection.

Johnson (9) has reported the experimental use of such a vaccine, but found that it caused unfavorable reactions in laying birds and its use was oftentimes attended with mortality. The virus content of his vaccine was slightly higher than that used in Experiment 3. This investigator used a pared down camel's hair brush in applying the vaccine to the feather follicles. This method of application has since been used in duplicating experiments and found to be both efficient and practicable. Gidlow (10) used the vaccine as recommended by Johnson in several flock trials and reports that

the vaccinated birds did not come down with pox during the subsequent months.

Experiment 4.

This experiment was planned to determine if birds after recovery from natural and experimental infection would react to the local or cutaneous vaccination of feather follicles on the leg. A group of 28 White Leghorn cockerels that had recovered from contagious epithelioma was used for this purpose. The control birds of the previous experiments were also used, they having recovered from the experimental infection. The vaccine containing 200 milligrams of virus in 50 cc. of a 40 per cent glycerol-physiological saline solution was applied to the feather follicles in the manner described.

The results were clear cut. Not one bird developed a local or cutaneous reaction (pox eruption) at the point of vaccination.

Experiment 5.

An effort was made to determine what effect the age of the cutaneous vaccine of Experiments 3 and 4 had on its ability to produce a complete immunity against contagious epithelioma.

TABLE 4—Results of ageing of the cutaneous vaccine on the subsequent production of complete immunity

Bird No.	Age of Vaccine	No. of Days After Skin Vaccination			Virus Inoculation of Comb to Determine Degree of Immunity		
	Days	Maximum follicular swelling	Maximum scab formation	Disappearance of scab	6th day after inoculation	10th day after inoculation	20th day after inoculation
V 91	6	5	none	none	negative	negative	negative
V 92	11	6	18	31	negative	negative	negative
V 93	16	8	23	31	negative	negative	negative
V 94	19	9	21	35	negative	negative	negative
V 95	25	10	15	27	negative	negative	negative
V 96	31	10	none	none	light infection	light infection	light infection
V 97	40	10	none	none	negative	light infection	light infection
V 98	46	11	none	none	light infection	light infection	light infection
62	(controls)				(controls)	(controls)	(controls)
64	not				light infection	pronounced infection	heavy infection
67	vaccinated						

Interpretation. The above data indicate that when the vaccine is 25 days, or less, in age at the time it is administered, it produces a complete immunity. When older it does not confer absolute protection against the experimental infection. It cannot be said at the present time whether this attenuation was due to actual ageing of the virus in the vaccine, the action of the glycerol upon it, or some other unsuspected factor. Further experiments are planned for determining this question. The data also show that apparently

the scab formation at the point of inoculation is essential to the production of a complete immunity.

In conjunction with Experiment 5 a group of birds was vaccinated cutaneously with the vaccine used in that experiment. The birds were check inoculated with virus for immunity determination at various periods after the day of vaccination. As near as could be determined from the experimental evidence in this group of birds and the birds of Experiment 5 as well, immunity began to develop on about the 20th day after vaccination, when the follicular scab had reached its maximum development, continuing until a complete immunity had been produced on or about the 29th to 31st day after vaccination, at which time the follicular scab and swelling had practically disappeared.

Experiment 6.

A pen of 100 trapnested, pedigreed Rhode Island Red pullets at the college poultry plant was used in this experiment. The cutaneous vaccine (200 milligrams virus in 50 cc. of 40 per cent glycerol-saline solution) was administered to 70 of the birds, the remaining 30 being left as controls. The chief purpose of this experiment was to determine the effect that the vaccine had on egg production.

Eighteen days after the vaccination, every one of the 70 vaccinated birds demonstrated a well formed feather follicle reaction with scab formation. Three of them showed one or two pox lesions on the comb. It was questionable whether the lesions on the comb were due to generalization of the virus in the vaccine or the result of contact infection. Presumably, they were due to generalization of the virus for such lesions were not found in any birds of the control group.

A close comparison of the trap nest egg records of the control and vaccinated groups for one month prior to vaccination and thereafter for an additional ten weeks, indicated that the percentage of egg production in the vaccinated group was materially decreased. This drop in egg production began 8 days after vaccination, and on the 21st day after vaccination it had reached its lowest point. From then on the production gradually, but slowly, increased to normal.

No opportunity was given to check the immunity production by virus inoculation of the comb.

This experiment demonstrated two important points. First, that the cutaneous vaccine, without exception, always produced a local pox-eruption of the nature of a follicular swelling and scab formation, which apparently is essential to the development of a complete immunity. Second, that its administration was followed by a material decrease of egg production.

Complement-Fixation in Relation to Cutaneous Immunity.

All birds used in Experiments 3, 4 and 5 were bled and the sera obtained for complement-fixation tests prior to the check inoculation of the birds for immunity determination. Prior to subjecting these sera to complement-fixation tests, known three-plus sera were used to determine the antigenic properties of the antigen to be used. In the actual test of the sera from the cutaneously vaccinated birds, positive and negative sera were used as controls.

The usual reaction was complete hemolysis. In two cases a slight anticomplementary action of the sera occurred.

The results indicated that complement-fixing antibodies were not concerned in the immunity acquired following cutaneous vaccination.

Standardization of The Cutaneous Vaccine.

The powdered pox virus used in the above experiments, when applied to the scarified areas of the comb and wattles, always produced pronounced lesions of contagious epithelioma in susceptible birds, within four to seven days after the inoculation. The virus, therefore, had an incubation period of from four to seven days. This variance of three days depended on the age of the bird inoculated, or in other words on the individual resistance of the birds to the virus, and on the age of the virus at the time it was used. Virus over one year of age was eliminated.

By varying the virus content of the cutaneous vaccine it was determined that less than 200 milligrams per 50 cc. of 40 per cent glycerol-physiological saline solution failed to produce local vaccine reactions in all trials, while more than this quantity of virus caused a more pronounced generalized reaction. It was demonstrated in Experiment 5 that the vaccine became attenuated in some manner to such an extent that it failed to produce a complete immunity when more than 25 days old.

Based on this information, a tentative standard has been adopted for the vaccine, until such time as it may be improved upon. The virus should be less than one year old and produce definite lesions of contagious epithelioma in from four to seven days after inoculation. The cutaneous vaccine should contain 200 milligrams of such a virus suspended in 50 cc. of a 40 per cent glycerol-physiological saline solution. It should not be attenuated by heating. It should be used within 25 days after manufacture, preferably within 10 to 15 days. When continuous ice-box storage is not available, 0.5 per cent phenol may be added to the suspension.

Discussion.

It is apparent, from the results of the foregoing experiments, that local or cutaneous vaccination of an area of bared feather follicles, with the proper virus suspension or vaccine, results in the production of a local pox-eruption and the development of a complete immunity.

The theory of cutaneous immunity in relation to contagious epithelioma is comparatively new. Verge (11) refers to it as a "cuti-immunity" and states that it can be obtained by cuti-vaccination. The experimental evidence in this paper confirms this statement. Verge used 1/10 to 1/20 cc. of his cutaneous vaccine injected intracutaneously into the wattle. He further states that "the general immunity is in reality only an immunity of the ectoderm", and claims "that the protection in the cutaneous and mucous membranes isolates the structures remaining sensible and thus creates a refractory state that extends to the whole body".

It will be recalled that while producing "immune" serum for serological study the subcutaneous administration of a triple strength vaccine produced a complete immunity in all cases where scablike lesions containing pox virus developed at the point of inoculation. In many of these cases pox nodules also appeared on the comb. It was evident that the skin was actively concerned in the immunity production. Beach (12) has made similar observations. He used subcutaneous injections of vaccines containing lesion tissues from fowls affected with the disease and noted that the percentage of birds immunized thereby was higher among those that developed scab lesions at the point of inoculation. He, likewise, found these scab lesions to contain pox virus. The evidence is convincing. In order to produce a uniform, con-

sistent, and complete immunity the skin must be vaccinated and a local pox reaction produced at the point of inoculation.

Besredka's (13) explanation of cutaneous immunity, as it is produced in several mammalian diseases, hinges on two propositions, which in the writer's mind are directly applicable to contagious epithelioma:

1. "The susceptibility of the animal is limited principally, if not exclusively, to the cells of the skin.

2. "The immunity of the animal is due to the vaccination of the receptive cells."

The question naturally arises, how does this immunity spread from a localized area on the skin to the entire cutaneous surface? Additional investigation is necessary before the question can be answered. Besredka is inclined to believe that "the immunity is local, but its effect reacts upon the rest of the cutaneous surface, because of the large net of lymphatic vessels interested in the process". Whatever the explanation may be, it has been demonstrated that the entire cutaneous surface is immune to the virus.

SUMMARY.

A triple strength vaccine, containing 30 milligrams of active pox virus per dose, when administered to birds subcutaneously, produced a high degree of immunity to contagious epithelioma. However, scablike lesions and pox nodules developed at the point of inoculation and on the comb and eyelids, respectively, following its administration. These lesions were found to contain pox virus. Those birds showing such skin lesions were completely immune to the disease, as determined by check inoculation of the comb and wattles with the virus.

Blood was taken from these and other immune birds for serological study. It was found that from 20 cc. to 25 cc. of blood could be safely drawn from the brachial vein of a mature bird in good physical condition without any apparent ill results.

Complement-fixation reactions varied and on the average were but a two-plus reading. Occasionally, a three-plus serum was found. Precipitin studies were negative. The attempt to produce a passive immunity was a failure. It was, therefore, concluded that, because of the relatively low concentration of specific antibodies in the sera of immune birds, and the failure to produce a passive immunity, a general immunity was not the sole protective force against contagious epithelioma.

The development of lesions containing pox virus at the point of inoculation and on the comb and eyelids after the subcutaneous administration of the triple strength vaccine, which was followed by a complete immunity, indicated that the skin was actively concerned in the immunity against the disease.

Various vaccines were applied to scarified comb areas and injected into the barbs of the comb; also, to scarified areas of denuded feather follicles and interfollicular skin surface on the leg just above the tibio-femoral joint. Unfavorable reactions followed. The vaccines were then applied to the denuded feather follicles, without previous scarification or cleansing, by rubbing them directly into the follicles with a cotton swab attached to a wooden applicator or a pared down camel's hair brush.

A cutaneous vaccine containing 200 milligrams of virus suspended in 50 cc. of a 40 per cent glycerol-physiological saline solution (2 parts glycerol and 3 parts saline) always caused a swelling of the feather follicles followed by the development of scabs over the orifices of the follicles. This vaccine

always produced a complete immunity of a cutaneous nature after the development of the local pox eruption.

Additional experiments showed that this cutaneous vaccine always produced the local pox eruption, which was essential to the development of a complete immunity, but its administration was followed by a decrease in egg production. It was also determined that complement-fixing antibodies were not produced during the development of cutaneous immunity against contagious epithelioma.

A tentative standard for the cutaneous vaccine was adopted. The virus to be used should have an incubation period of from four to seven days and, therefore, must be less than one year old. The vaccine should contain 200 milligrams of such a virus suspended in 50 cc. of a 40 per cent glycerol-physiological saline solution. The product should not be attenuated by heat. It should be used within 25 days after its manufacture, preferably within 10 to 15 days, because it does not always produce a complete immunity when older. If continuous ice-box storage is not available, 0.5 per cent phenol should be added as a preservative.

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The Extraction of Apple Juices in the Manufacture of Jelly

By Carl R. Fellers

The many different practices used in jelly making at present would in themselves indicate that the choice of a method has only experience as a basis for its selection. This investigation is an attempt to establish certain principles which may lead to greater economy in production, and provide a sounder basis for the housewife and commercial preserver for the manufacture of a uniformly high quality product.

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AGRICULTURAL EXPERIMENT STATION

AMHERST, MASS.

THE EXTRACTION OF APPLE JUICES IN THE MANUFACTURE OF JELLY

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INTRODUCTION

In the manufacture of apple jellies, pectin concentrate, extract, or syrup, it is customary to extract the juice from the fruit with water by the use of heat. Whether the operation be carried on in the home or in the commercial manufacturing plant, the principles involved remain the same. In the past very little attention has been given to the study of extraction methods not only of apples, but of all the common juice or jelly-yielding fruits.

It is the purpose of this bulletin to present data bearing upon this problem. It is not proposed to discuss cold pressed juices such as cider, because the cold press is a distinctly different problem. In the latter case, very little pectin is obtained in the juice, whereas in the heat extracted juice, considerable pectin is found. True, cider jelly without the addition of pectin may be manufactured from cold pressed apple juice, but only by concentration to one-sixth to one-eighth of its original volume. This is necessary because of the small amount of jellifying pectin and sugar which is present in cold pressed apple juice. Heat disintegrates the pectin-rich cell walls of fruits and thus releases the pectin into solution. To a lesser degree freezing (5) accomplishes the same purpose.

Directions for juice extraction are far from standardized in that various ratios of fruit to water, and diverse periods of extraction at various temperatures are recommended. In some cases the fruit is sliced, in others pulped and yet again the use of added acid to aid in the extraction is advocated. Often the directions call for one extraction only, sometimes two, and occasionally three. How is the commercial plant or the farm factory operator or even the housewife to know which methods are best? The literature bearing on this subject is appalling in its diversity of methods and lack of orderly scientific approach.

PLAN OF STUDY

In order to determine the yield of juice as well as the relative amounts of the three recognized jelly essentials, namely sugar, pectin, and acid, which were extracted from apples by the use of various methods of heat extraction, a series of laboratory tests were conducted during 1926 and 1927 on Baldwin, Red Astrachan, Rhode Island Greening, Winesap, McIntosh, Wealthy, King David and Red Siberian Crab varieties. Under standardized conditions, given weights of apples were successively extracted three times at each of the following temperatures, viz. 88°C. (190°F.), 100°C. (212°F.), and 109°C. (228°F.), respectively for 15, 30 and 60 minutes. A 15-minute extraction followed by a standing period of 10 minutes, was also employed. Sliced apples were compared with chopped or pulped fruit. Similarly, the effect upon the extractives of various concentrations of acid added to the apples was considered. The ratios of apple to extraction water, by weight, were varied in this study from 3:2 to 3:4.

Equipment

With the exception of Red Astrachan, Red Siberian Crab and Wealthy varieties, uniform Grade C apples kept in cold storage for from one to three and one-half months were used. Just enough of the fruit was brought to the laboratory each morning for one day's run. Only sound, firm fruit was used. A hand slicing machine adjusted to give slices one-eighth inch in thickness, and a large sized food chopper equipped with medium knives were used to slice or chop the fruit as desired. Pieces not over one-fourth inch in diameter were obtained by the use of the food chopper. The chopped apples were somewhat finer in texture than the press stock usually obtained by the average shredder or mill in a cider factory.

Unchlorinated Amherst tap water (pH 6.9) was used for the extractions. Aluminum stew pans with close-fitting covers were found to be convenient utensils in which to cook the apples with water. Circular gas burners were used as the source of heat except where temperatures above 100°C. were desired. In the latter case steam-heated retorts or autoclaves were used.

Extraction Methods

Three pounds (1.36 kilograms) of either sliced or chopped fruit were placed in the covered aluminum pans, together with the required weight of cold water. The gas flame was turned on fully until the desired temperature was reached. It was then adjusted so as to maintain this temperature for the period of the extraction. Where the ratio of fruit to water was high, as in the 3:2 ratio, the pan contents were stirred with an aluminum spoon when necessary to prevent scorching; otherwise no stirring was done.

Upon completion of the extraction period, the contents were poured into a moist cheese cloth (2 thicknesses), allowed to drain one minute, then well squeezed by wringing both ends of the cloth in the hands for another minute, after which the pulp was returned to the original stew pan, the desired quantity of cold water added, and again extracted over the gas flame. Similarly, a third extraction of the pulp was obtained. Thus for each apple sample there was secured and kept separately, first, second and third extracts, as well as the pulp remaining after separation from the third extract. These various extracts and pulps were at once carefully weighed, placed in glass fruit jars and pasteurized for 30 minutes at 71°C. (160°F.) in case they could not be examined immediately. Both chemical and organoleptic examinations were made. When tartaric acid was used to acidify the fruit, the desired amount in solution was added in the extraction water. None was added except in the first extraction. For the sake of uniformity the same quantity of water was added to the pulps remaining after the first or second extractions, as was originally added to the apples. The several juice extracts as well as the pulp were reserved for chemical examination and for use in preparing the jelly samples.

Method of Preparing Jelly

For the sake of uniformity a given weight of juice, 511 grams (18 ounces), was taken from each extract for conversion into jelly. When it was desired to make a jelly representing the combined first and second extracts, amounts proportional to the yield of each were taken so that the total weight was 511 grams. To this was added enough sugar to total 341 grams (12 ounces),

allowance being made for that present in the juice. The juice was then concentrated to a sheeting, spoon jelly test (104° to 105°C. or 219° to 221°F.). The finishing point was checked by determining the weight of the pan and contents from time to time. Inasmuch as jelly formation usually occurred when the sugar concentration reached 67 to 69 per cent by weight, this furnished a simple and accurate check upon the finishing point. The jelly was weighed in the pan at once and poured through one thickness of medium mesh cheesecloth into straight sided, 2-ounce jelly glasses. After skimming, these were covered with melted paraffin and capped. Jelly strength, chemical, and organoleptic determinations were made after 1 to 3 months storage at 21° to 23°C.

At first, jellies were made from all three extracts individually, together with combinations of the first and second, as well as all three extracts combined. Third extractions produced juice which seldom gave satisfactory jellies unless acid was added. Moreover the pectin content was usually too low to give a firm jelly. Naturally a jelly made from the third extract was of poor color and flavor. Although combinations of all three extracts gave fair to good jellies, it was decided that a combination of the first and second corresponded more closely to home or factory practice and this was the procedure finally adopted.

Chemical Methods

Each sample of juice, i. e., first, second, and third extracts, was examined for total titratable acidity, hydrogen ion concentration, soluble solids by Abbé refractometer, Brix and specific gravity hydrometers, pectin by the alcohol precipitate and centrifugal methods, pectic acid by the A. O. A. C. (2) and centrifugal methods, sterility, and such physical characters as taste, color, turbidity and sediment. Determinations of insoluble solids, soluble solids, alcohol precipitate, pectic acid, total acidity, pH value, and sterility were also performed on the pulp. Determinations of total sugars, reducing sugars and sucrose were made in some cases. The A. O. A. C. methods were used wherever possible.

The hydrogen ion concentration was determined in most cases colorimetrically, though several check determinations with the potentionmeter were made. Notwithstanding the statement of Myers and Baker (8) that the colorimetric method was of no value in the study of fruit juices, it was found to be very useful and economical of time. Occasional checks by the electrometric method showed good agreement in pH values.

In order to prepare samples for pectic acid, acidity, sugar determinations, etc., from the pulp or fresh fruit, 300 grams of the well pulped and mixed samples in a 2-liter beaker with 800 c. c. of water were boiled one hour, the volume being kept constant by the addition of hot water at intervals. The contents were transferred to a 2-liter flask, cooled, diluted to volume, and filtered.

It was found that, in drying the residue for the determination of total solids, losses occurred where a temperature above 60°C. at 25 inches of mercury in the vacuum oven was employed. This was due probably to levulose decomposition. Similarly in drying alcoholic precipitate or pectic acid it was found that decomposition occurred at 100°C., hence a temperature of 90 to 95°C. was used. The absolute necessity of washing these precipitates free from hydrochloric acid was also observed, as charring and loss invariably occurred when this precaution was not fully carried out.

Color, taste, turbidity, and sediment were given ratings on a basis of organoleptic tests only. Four classes were made in each case—for example, the color or taste was considered excellent, good, fair, or poor. Likewise turbidity and sediment were classified as much, moderate, slight, or none.

Sugar in jellies was determined by the Abbé refractometer. In the case of juices a comparison was made of the results obtained by the Brix hydrometer, refractometer and chemical determination of soluble solids. These results are discussed elsewhere in this bulletin.

Centrifugal Method for Pectic Acid

A centrifugal method was developed during the course of the work and will be described more fully in a separate publication. In brief, it consisted of measuring either 5 or 10 c. c. of juice, according to concentration, into 15 c. c. tapered, graduated, glass centrifuge tubes. When 5 c. c. were employed, the juice was always diluted to the 10 c. c. mark before the addition of alkali. One c. c. of a 10 per cent sodium hydroxide solution was added to each tube, the contents mixed by shaking and allowed to stand for 15 minutes. Two c. c. of a 10 per cent hydrochloric acid solution were introduced and thoroughly mixed. The tube was then placed in boiling water for 5 to 8 minutes (or until the gelatinous precipitate was entirely flocculated and freed from air bubbles), removed, cooled to below 25°C. and whirled in a centrifuge 15 minutes at 2600 revolutions per minute on a 14-inch head. It was found within certain limits that the volume of the precipitate could be correlated with the chemical determination of pectic acid. The precipitate was also centrifuged without previous heating, but the readings were much higher than with the heated precipitate and less consistent. It was found that the centrifugal method did not yield reliable results when applied to the alcohol precipitate of juices or pectin extracts.

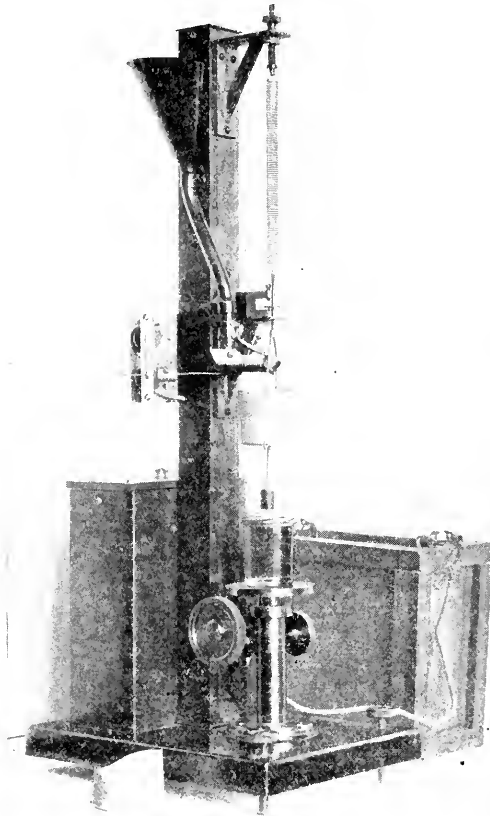
For practical purposes, it was believed that the chemical determination of pectic acid yielded more reliable results than the alcohol precipitate (pectin). It has been proved recently by Nelson (11) that the pectic acid obtained in this manner consisted of a definite stable substance, namely digalacturonic acid. The composition and physical properties of the alcohol precipitate were found to be variable and it was difficult to obtain satisfactory checks by using the method. The ratio of pectin (alcohol precipitate) to pectic acid, A. O. A. C. method, was variable but averaged approximately 1.7:1 for Baldwin apples. Wichmann (19) estimated that this ratio for most varieties of apples was about 2:1.

Jelly Strength Determination

Obviously the jelly strength test commonly employed, i. e., the resistance of the jelly to the fingers, is inaccurate. Paine (12), Sucharipa (16), and Tarr (18) have described the advantages of a suitable jelly-strength tester. Sucharipa (16) devised a tester which would break a layer of jelly of definite thickness by means of air pressure, the latter being registered on a manometer. Later Tarr (18) and Baker (3) modified and simplified Sucharipa's apparatus by using water pressure in place of air pressure.

By substituting a light paper cup in place of the heavier metallic one furnished with the Bloom gelometer (13), a standard instrument used in gelatin and glue testing, the writer found this instrument gave very satisfactory results in determining the jelly strength of fruit or pectin jellies, jams or sauces.

Figure 1. Bloom Gelometer Used to Make Jelly Strength Tests.



The instrument is electrically manipulated and allows fine shot to flow into the pan above the plunger until the latter has penetrated into the jelly a definite depth, usually 5 mm., when the circuit is closed and the flow stopped. The jelly strength is merely the weight in grams of the shot on the pan. Straight sided jelly tumblers containing 56 grams (2 ounces) of jelly, were used in the tests, all of which were conducted at room temperature, 20° to 23°C. (68° to 74° F.). The flow of shot was always regulated so that a definite weight flowed through the outlet in a given time—in other words, the orifice was opened exactly the same distance (regulated on the instrument) for each test. For details regarding the use of the Bloom gelometer for jelly strength determinations in fruit and pectin jellies the paper by Fellers and Griffiths (6) should be consulted.

CHEMICAL COMPOSITION OF THE APPLE VARIETIES USED FOR JUICE EXTRACTION

Before extracting the juice by heat, representative samples of each variety were subjected to chemical examination. In most cases several analyses of

a variety were made and the average taken. Baldwin apples were analyzed at approximately the same degree of maturity for three successive years. All these data are presented in Table 1.*

The composition of the Baldwin apple varied somewhat from year to year though these differences were not striking. In 1925 and 1927 there was a small amount of residual starch even in the mature fruit. It is possible that this may be the cause of the cloudiness which is often associated with Baldwin apple jelly. The pectin is reported as pectic acid, but this figure may be converted readily to pectin (alcohol precipitate) by multiplying by a factor found to vary from 1.5 to 2.3. Inasmuch as the factor is not constant and depends upon the amount of hydrolysis or deesterification that the pectin has undergone as well as upon impurities, the pectic acid values are preferable to the alcohol precipitate.

The ash of the several varieties showed but little variation. Pectin varied from 0.29 per cent in the 1925 crop of Baldwins to 0.62 per cent in Red Siberian Crab. Starch was usually present only as a trace in mature fruits. Total sugars made up approximately 81 per cent of the soluble solids present. The ratio of sucrose to reducing sugar proved extremely variable even in the same variety. The insoluble solids averaged 2.5 per cent in Baldwins and 2.7 per cent in other varieties. The mean acidity for Baldwins was 0.53 per cent as malic acid while other varieties varied considerably. The mean pH of Baldwins was 3.46, the range for other varieties being from 3.18 for Red Astrachan to 3.6 for McIntosh. Shaw (14), Bigelow, Gore and Howard (4) and Alwood, Davidson and Moncure (1) reported analyses of a large number of varieties of apples grown in different localities, at several stages of maturity and during storage. For a more complete discussion of the chemical composition of the apple, reference should be made to those reports.

STUDIES OF THE EXTRACTED JUICE

Yield and Composition of Extracted Juice per Unit Weight of Apples

Although analytical data were obtained on three successive extracts and the residual pulp as well, only two extracts are usually considered here. Ordinarily in jelly making, only two extractions are made. The third extraction usually yields a juice which is too dilute to concentrate and use economically for jelly. Fruit flavors and colors as well as pectins were injured readily or even destroyed completely by prolonged heating, and for this reason it was considered inadvisable to mix the third extract with either the first or second or both.

Table 2 was summarized from 126 tests including Baldwin, Red Astrachan, Red Siberian Crab, Wealthy, McIntosh, King David, Winesap and Rhode Island Greening varieties. Obviously the ratio of fruit to extraction water greatly influenced the amount of juice obtained from a given extraction period. For example the mean yield increase of a 3:3 and 3:4 ratio over a 3:2 was 33 and 91 per cent respectively. Hence the juice from the 3:2 ratio of fruit to water was much more concentrated than the others and required less evaporation to convert it into jelly. Since the pectin and acid content of such a concentrated juice is relatively high, more sugar may be utilized thus greatly increasing the jelly yield. In general, where the juice was concentrated as in the 3:2 extract, the total amount of pectin obtained

*The tables are presented in the appendix at the end of the bulletin.

from a given weight of apple was somewhat less than where greater dilutions were used. On the other hand since longer heating was required for the conversion of a dilute juice into jelly, there was a more or less serious loss in the jellifying power of the pectin. In other words the total amount of pectin may be greater in the more dilute extracted juices, yet because of deesterification (10) of pectin by heat and acid, its jellifying qualities may be impaired. This hydrolysis of the pectin may affect seriously the yield and the quality of the resultant jelly if the extraction period is too extended. This readily became apparent when a 30-minute extraction period was used, and caused irreparable loss in jelly yield and in quality if extended to 60 minutes. (See Table 14).

Due to evaporation during extraction, the yields of juice from the longer extraction periods were less in general than those from shorter periods, though the former contained more solids including pectin. As already stated, this may mean little, because the pectin may be partly demethoxylated and of poorer quality (7, 9, 10). The results here presented indicate clearly that a loss occurred. The least amount of pectin was extracted in the 15-minute extraction period, the most in the 60-minute period. There was little difference in either juice yields or composition in the 15-minute extraction period as compared with a 15-minute extraction period followed by 10 minutes standing removed from the source of heat. An additional column in Table 2 was inserted to show the actual amounts of pectin obtained in the combined first and second extracts from one kilogram of apples. The amount increased with both the length of the extraction period and the widening of the apple-water ratio. The same observation applied though in a different degree in the case of soluble solids and acidity.

Composition of Heat Extracted Apple Juice (8 varieties)

(Extraction Period, 15 Minutes at 100°C.)

Table 3 is largely self-explanatory and was prepared to show the general composition of the juice obtained from the first, second, and third extractions and of the residual marc or pulp. Since different temperatures or periods of extraction showed only minor variations in the composition of the juices, they have not been included. The chopped fruit yielded slightly more concentrated juices than the sliced; similarly the longer the extraction, the more concentrated became the juice, but for practical purposes these differences were insignificant.

The averages reported in Table 3 were found to be very similar to others compiled using different methods of extraction and even distinct apple varieties. Some varieties were better for jelly making than others (see Tables 10 and 11), but as previously shown in Table 1, there were no striking variations in solids among the eight varieties analyzed. The differences in pectin content were marked and merited some attention. The large amount of pectin remaining in the residual pulp was striking. The A. O. A. C. methods (2) of analysis, no doubt, were responsible for a part of this high percentage. The method required boiling 300 grams of the pulp with 800 c. c. of water for 60 minutes, replacing from time to time the water lost by evaporation. Thus, much additional pectin was brought into solution. Moreover, by repeating the operation still more pectin was obtained. The method yielded arbitrary results only, and did not indicate the absolute amount of pectin present in the pulp. Nevertheless it proved of value in comparative work such as this. In general, a slightly higher percentage of pectin was found

(see Table 3) in the residual pulp than was obtained in the first extract from the fruit. However, since the weight of juice obtained in the several extractions was much greater than that of the pulp, the actual amount of pectin remaining in the pulp was much less. This is shown clearly in Tables 10 and 11. An average of 31 tests, where the ratio of fruit to extraction water was 3:2 and the time 15 minutes at 100°C., showed that 25.5 per cent of the pectin remained in the pulp. Where the ratios were 3:3 and 3:4, the percentages of pectin in the pulps were 20.4 and 19.1 respectively.

In Table 4 are found the mean actual amounts of soluble solids, pectin and acid calculated to malic, in the combined first, second and third extracts plus that found in the residual pulp. These figures approximated the composition of the fruit itself. Although some differences existed among the several varieties, there were only slight variations between tests on the same variety. For example, in 91 different tests on Baldwin apples, the mean value for soluble solids was 134.7 grams per kilogram of fruit with an average deviation from the mean of 9.55 grams. Similarly the values for pectin were 4.84 ± 0.50 and for acid calculated as malic, 4.01 ± 0.57. Red Siberian Crab, Winesap, and King David gave the highest yields of soluble solids; Red Astrachan and Wealthy were among the lowest. The Winesap and Baldwin varieties carried the most pectin while McIntosh, Red Astrachan and King David contained the least. Red Siberian Crab, Red Astrachan and King David produced the juice of highest acidity for jelly, while McIntosh and Baldwin yielded juice of low acidity.

Comparison of Brix Hydrometer and Abbé Refractometer for Solids and Sugar Determinations in Apple Juice.

Check determinations of soluble solids (mainly sugar) were made by both the Brix hydrometer and the Abbé refractometer, to ascertain which gave the more accurate results. Percentages of sugar from a direct reading sucrose scale on the refractometer also were compared with the refractive index values as computed from the Tables by Schonrock (2) and with the total sugars as determined gravimetrically. Temperature corrections were made according to Stanek (2), though every effort was used to make readings at 20°C. so that such corrections usually were unnecessary. The A. O. A. C. table (2) for correcting saccharometers for temperature variations was used. The results obtained from the chemical determinations of solids and sugar made according to the A. O. A. C. methods are given for comparison in Table 5.

The sugar constituted an average of only 76.5 per cent of the soluble solids present in the juice. For this reason care must be exercised to report Brix or refractometer readings in terms of solids rather than sugar. The Brix reading was too high consistently as were the values obtained from the direct reading sucrose scale on the refractometer. The latter registered a mean difference of 1.45 per cent over the total sugars actually present. The computation of solids from refractive index gave good results, though they were usually slightly lower than those obtained by use of the gravimetric method. As a result of 263 comparisons between Brix and refractometer readings on total solids in heat extracted Baldwin apple juices, the Brix method gave a mean increase in solids of 0.308 per cent. In 101 tests on other apple varieties the increase was only 0.22 per cent. These results corroborated certain data recently reported by Sherwood (15) on the refractometer analysis of sugar beet juice.

Likewise comparisons were made on jellies between refractive index and direct sucrose readings from the refractometer scale. Here the results checked very well. For example in 66 determinations the sucrose readings averaged only 0.053 per cent more than the solids calculated from the refractive index. The direct reading sucrose scale on the refractometer should not be relied upon in testing apple juices for solids or sugar. The refractometer gave more reliable results than the Brix hydrometer.

Hydrogen Ion Concentration and Titratable Acidity of Extracted Apple Juices and Jellies.

Data collected relative to the titratable acidity and pH of apples are presented in Table 1, while the resulting juices and jellies are considered in Table 6. In general, the hydrogen ion concentration of the juice was slightly lower than that of the fruit itself. The second and third extracts were lower than the first. The resulting jellies made from the combined first and second extracts corresponded closely in pH to the juices. Tarr (17) stated that pH 3.46 was the minimum at which jelly formation occurred with a relatively pure source of pectin. From Table 6 it is evident that some of the jellies made from apple juice exceeded this value. Apparently natural fruit juices did not behave like pure pectin in this regard.

The relation existing between total titratable acidity and hydrogen ion concentration was reasonably constant, i. e., the higher the hydrogen ion concentration the higher the acidity and vice versa. Some varieties appeared to contain more buffer substances than others. The total acidity, calculated to malic, of the finished jelly varied from 0.22 per cent in McIntosh to 0.66 per cent in Red Siberian Crab, while the pH varied from 3.2 in Red Astrachan to 3.62 in Baldwin, 1925 crop.

Recovery of Soluble Solids, Pectin and Acid in Successive Extractions Made under Various Conditions

Data were compiled bearing upon the influence of the following factors upon the percentage composition of the extracts from eight varieties of apples.

1. Successive extractions
2. Sliced and chopped apples
3. Ratio of fruit to water during extraction
4. Time of extraction
5. Temperature of extraction
6. Added acids
7. Yearly variations of Baldwin apples

Tables 7—12 and Charts 1—5 contain condensed analytical data showing the mean percentage of soluble solids and pectin successively extracted from eight varieties of apples by the use of various methods. Weighted averages were used throughout. Due to space limitations, physical and organoleptic observations were omitted. In spite of the number of tests some few inconsistencies occurred, yet on the whole the results were fairly uniform and showed definite trends. Because of the large number of tests with Baldwin apples under controlled conditions, it is believed that considerable significance may be attached to them.

Influence of Successive Extractions

Three successive extracts, together with the pulp obtained in the manner already described, from 91 series comprising in all 364 samples, were examined quantitatively for soluble solids (chiefly sugar), pectin and acid. Knowing the amount of juice recovered at each extraction and keeping the amount of apples used a constant, viz. 3 pounds (1.36 kilograms), it was possible, with the aid of these analytical data, to calculate the percentages of soluble solids, pectin and acid recovered in the several successive extracts and residual pulp.

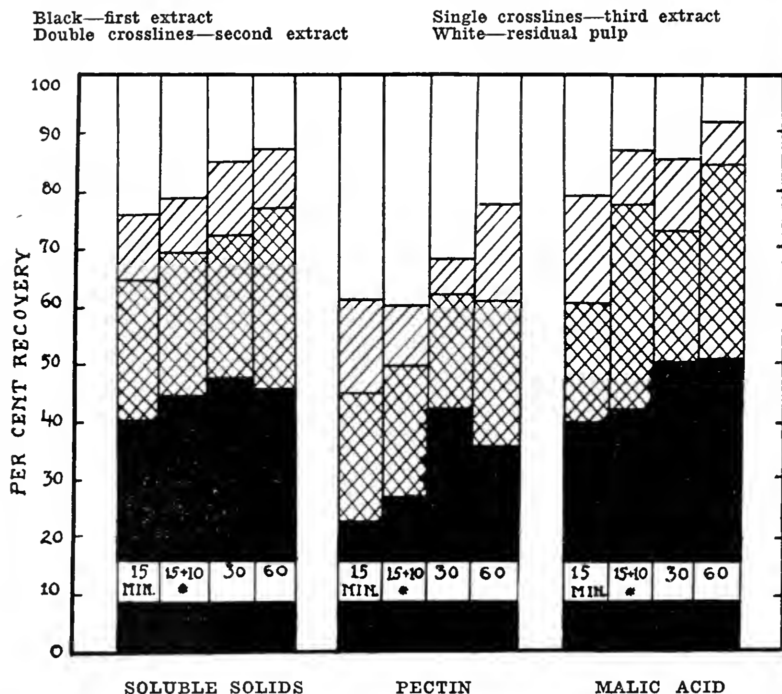
Method of Calculating Results—To illustrate this method of calculation assume that the first, second, and third extractions and the remaining pulp weighed 50, 40, 32, and 16 ounces respectively. The soluble solids were determined to be 10, 6, 3, and 3 per cent while the pectin was 0.20, 0.09, 0.04 and 0.12 per cent respectively, and the acid as malic 0.20, 0.12, 0.06, and 0.07. The total weight of soluble solids in 3 pounds of apples becomes $(50\times.10)+(40\times.06)+(32\times.03)+(16\times.03)=8.84$ ounces. The total amount of pectin and malic acid extracted in each separation was calculated in a similar manner. Knowing the amount and composition of each extract, the exact percentage of the soluble solids, pectin or acid obtained in any of the three extracts or in the remaining pulp was readily calculated. For example, the total solids retained by the pulp after three successive extractions was found to be $(16\times.03)\div8.84=5.43$ per cent.

Discussion of Results. Three successive 15-minute extractions of sliced Baldwin apples with water removed from 80 to 94.4 per cent of the soluble solids and from 63.5 to 88.3 per cent of the pectin, whereas the chopped fruit yielded soluble solids ranging from 85.9 to 95.4 per cent, and pectin from 63.1 to 83. In only two cases did the sliced fruit yield more soluble solids than the chopped. In each of these, the extracting temperature was 109°C. (228° F.). Though more solids were obtained in the juice by chopping the apple, still the actual gain was slight. It was realized that the amounts of solids or pectin obtained from fruit under different methods of extraction tended to become equalized when the totals of the three extracts were considered. Hence the percentages of solids and pectin recovered by one extraction and by two successive extractions showed wider differences. For this reason these results were included in the tables. Three successive extractions with equal weights of water removed from the Baldwin apple all except about 10 per cent of the soluble solids, which remained in the residual pulp. The maximum amount remaining in the pulp was 22 per cent in the case where the ratio of apple to water was 3:2 and the extraction was carried on at temperatures of either 88° or 100°C. The minimum amount of extractable solids remaining in the pulp was only 4.6 per cent. This occurred where the ratio of fruit to water was low, viz. 3:4, and the extraction temperature was 100°C.

In tables 7 to 12 and graphically in Chart 1 data are assembled showing the relative percentage recoveries of soluble solids, pectin and acid which may be expected from one, two, or three successive extractions at varying periods and temperatures. Likewise the influence of acid added to the apples, as well as varying ratios of apple to water, are shown. Charts 1 to 5 graphically portray the effect of a variety of conditions on the chemical composition and percentage yield of the extracted juice.

Chart 1. Relative Amounts of Total Soluble Solids, Pectin and Acid Recovered in each of Three Sucessive Extracts and in the Residual Pulp. Random Tests. Temperature of Extraction 100° C. Ratio of Sliced Apple to Water 3:2. No Added Acid.

Black—first extract
Double crosslines—second extract
Single crosslines—third extract
White—residual pulp



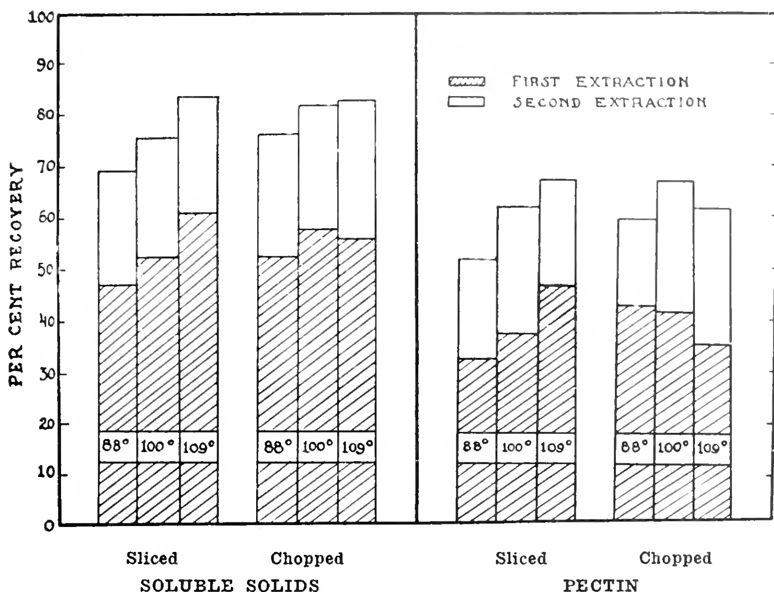
*Boiled 15 minutes and allowed to stand 10 minutes.

Thus Chart 1, constructed from individual tests, pictures the relative percentages of soluble solids, pectin and malic acid obtained in successive extracts under a constant set of conditions, i. e., temperature of extraction, ratio of apple to extraction water and acidity, while only the extraction period was varied. In this manner it was possible to determine the influence of these several factors upon yield and composition of the juice obtained from each successive extraction.

The limits in percentage for soluble solids recovered in the first, second, third extracts and remaining in the pulp were respectively 33.0—66.8, 17.0—31.6, 4.4—19.7, and 3.3—22.9. For pectin, these limits were 21.5—64.0, 10.8—33.2, 6.0—22.2, and 7.9—43.9; and for acid calculated to malic they were 34.9—82.3, 6.1—43.1, 4.5—26.2, and 2.0—31.2.

The relative lack of variation of solids or pectin in the second and third extracts regardless of the treatment is particularly emphasized in Charts 1-5. In fact one of the outstanding results of this investigation was that juice could be extracted successfully from apples by almost any method, though with unlike results. It is fortunate indeed that this is true, because every conceivable method of juice extraction is practiced in the home and factory. The wide limits within which a degree of success may be attained in preparing apple jellies, for example, makes total failure difficult, though improvement in yield and quality always can be secured by the use of correct methods.

Chart 2. Effect of Extraction Temperature upon Yield of Soluble Solids and Pectin in First and Second Extracts of Sliced and Chopped Apples. Ratio of Apple to Water 3:3.

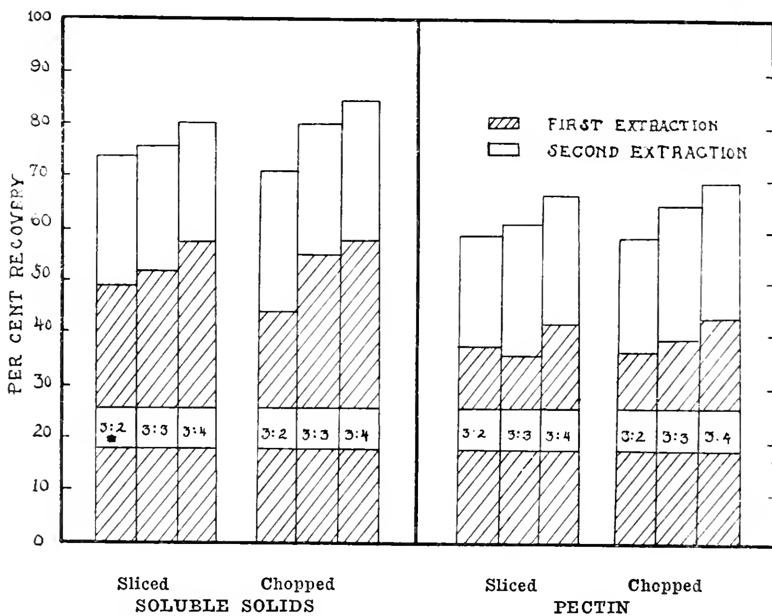


What has just been said relative to Baldwin apples normally held true for Red Astrachan, Red Siberian Crab, Wealthy, McIntosh, King David, Winesap, and Rhode Island Greening. Of course there were differences in ease of extraction. For example, Red Astrachan, King David, and Wealthy gave up their soluble solids, including pectin, more readily than most of the other varieties. A relatively high acidity undoubtedly aided in breaking up the cell walls and liberating the cell contents of the fruit. Referring to Tables 7 and 8, it is evident that the cellular structure of certain varieties was more easily broken down by heat extraction with water than that of others. The Greening, Winesap, and Baldwin were among the varieties most difficult to extract with water, while McIntosh and Red Siberian Crab occupied an intermediate position in this respect. At any rate it appeared that, for all the varieties tested, two successive 15-minute extractions of the sliced apples at the boiling point removed from 58 to 74 per cent of the pectin and 73 to 93 per cent of the soluble solids. For chopped fruit, the recovery was slightly increased, but at the expense of clearness of the juice. The percentage of soluble solids remaining in the pulp after three extractions varied from 2.2 in Wealthy to 14.3 in McIntosh, with a general average of 6.0 for all varieties.

Comparison of Sliced and Chopped Apples for Juice and Jelly.

Influence of Temperature and Ratio of Fruit to Extraction Water. Comparative data are presented in Tables 7 and 8 and in Charts 2 and 3. In general the chopped apples gave a slight increase in yield of soluble solids, pectin, and malic acid over sliced fruit. On the other hand the expressed juice of chopped apples was always more cloudy and turbid than that from sliced fruit. This was true in the case of the first extract alone or of 2 or 3 successive extracts combined, though this increased recovery seldom amounted

Chart 3. Influence of Ratio of Apple to Water on Recovery (Yield) of Soluble Solids and Pectin in Sliced and Chopped Baldwin Apples. Temperature of Extraction 100° C.



*Ratio of apple to water by weight.

to as much as 10 per cent of the total and averaged about 5 per cent. It was greatest where the extraction temperature was low, i. e., 88°C. (190° F.); it was negligible at the boiling temperature and finally became negative at 109°C. (228° F.). The probable cause of the decreased recovery of sugar, acid and pectin at 109°C. was the poor heat conductivity of the chopped apple mass. This resulted in lower temperatures in the mass itself than was indicated by the retort thermometer. This was experimentally proved. It took more than 30 minutes for a retort temperature of 109°C. to bring the whole of the apple pulp to that temperature. For this reason, in all pressure cooker extractions under 30 minutes, the temperature of the chopped pulp mass was below 109°C. This readily explains the poor recoveries obtained at this seemingly high extraction temperature. On the other hand in the pressure cooker, sliced apples allowed ready penetration of heat, largely due to unimpeded convection currents and increased conductivity in the apple—water medium. Where the amount of water used for extraction was large, as in the 3:4 ratio, there was an increase in the time necessary to bring the mass to the desired temperature. After reaching this point, however, the temperature of the mass was relatively constant, due to the large volume of liquid present.

Where the ratio of fruit to water was 3:2 the chopping of the fruit had a slight inimical effect upon yield of soluble solids, acid, and pectin. Where the ratio was 3:3 or 3:4 the chopped fruit usually yielded considerably more of these substances than the sliced. Thus it appeared that where the apples were ground up or chopped, the ratio of fruit to extraction water should be widened over that necessary where the fruit was sliced. For sliced fruit, a

ratio of 3:2 was found to be very suitable, though for chopped fruit the ratio which gave most satisfactory results was 3:3. The ratio 3:4 was uneconomical because of the huge fuel consumption necessary to evaporate the large volume of juice. This objection was not recompensed by a significantly greater yield of solids or pectin in the juice. Furthermore, the juice was inferior in quality for jelly making or pectin manufacturing purposes.

In Table 10 where a 15-minute extraction period at 100°C. was used on eight common apple varieties, the gain in soluble solids of a ratio of fruit to extraction water of 3:3 over 3:2 was 5 per cent. For pectin the difference was still less. Furthermore if two or even three successive extractions were made using ratios of 3:2, 3:3 and 3:4, the yields of total soluble solids, pectin, and acid tended to become equalized, though the greater the amount of water used, the higher the recovery of extractives became. In a 30-minute extraction period (Table 11) the difference in recovery of extractives between these ratios was still further minimized. All eight varieties reacted similarly in this respect.

Comparing the several varieties it is seen that some yielded up their soluble solids including pectin much more readily than others. Besides Tables 7 and 8, additional data bearing upon this point may be found in Tables 10 and 11. For example, Red Astrachan and Wealthy both yielded nearly 70 per cent soluble solids and over 50 per cent pectin in a single 15-minute extraction. King David, McIntosh and Winesap varieties held these substances more tenaciously in the fruit tissues. In most cases the sliced fruit when subjected to 15 minutes at 100°C. liberated slightly less soluble solids including pectin and malic acid than the chopped fruit. The Red Astrachan variety proved to be an exception, while in Greening and Winesap, but minor differences were noted between the two methods. The extracted juice from all varieties gave high grade jelly, though in every case that obtained from the chopped fruit was less clear and therefore of poorer quality than that made from sliced fruit. Red Siberian Crab, King David, Red Astrachan, Winesap and McIntosh were all considered to be first class jelly varieties. Baldwin and Wealthy are inferior to these while Greening yielded an unattractive light colored jelly. For this very reason the latter served as an excellent base for mint jelly.

Other things being equal, the optimum ratio of fruit to water is the one that yields the highest concentration of soluble solids, including acid and jellifying pectin, per unit volume of liquid. However, it should be noted at this time that enough extraction water must be used to prevent scorching, also that if too little liquid is present, especially where chopped fruit is used, satisfactory filtration or separation of the juice from the pulp, is difficult. The resulting juice may also be more cloudy and thus lower the jelly quality.

Effect of Temperature Upon the Recovery of Soluble Solids, Pectin and Acid in Heat Extracted Apple Juice.

In general there was a consistent increase in percentage recovery of soluble solids, pectin and acid with rise in temperature from 88° to 109°C., though the increase was more marked in passing from 88° to 100°C. than from the latter temperature to 109°C. (See Tables 7, 8 and 9). Results were much more consistent with sliced than with chopped apples, especially at the maximum extraction temperature of 109°C. In the first extract using sliced fruit, the average increase in yield of both soluble solids and pectin at 100°C. varied from 4 to 13 per cent over the amounts extracted at 88°C. Smaller

increases were noted where chopped fruit was used. Likewise greater yields were obtained at 100° than at 88°C. if the sum of either two or three successive extracts is considered. For two extracts combined an average maximum increase of 15 per cent was noted, whereas for three extracts, the maximum was only 7 per cent. Though some beneficial effects were gained by extracting at 109°C. as compared with 100°, still these were too slight to be of importance when the difficulties involved in cooking the apple—water mix under steam pressure, are considered. In fact for chopped apples, unless the time of extraction was at least 30 minutes, there was usually no gain in recovery of solids or pectin over the extraction carried on at 100°C.

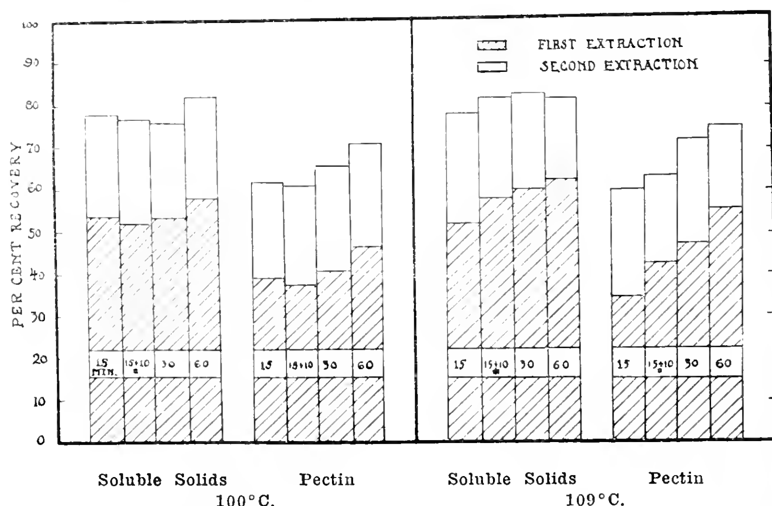
The evidence indicates that any temperature above 88°C. is efficacious in bringing into solution the sugars, acid, and pectin of apples, though on account of the cost and difficulty of cooking under pressure it is probably not desirable to exceed the boiling temperature. For ordinary purposes 100°C. appears to be the most satisfactory extraction temperature though somewhat lower temperatures do not greatly influence the yield of soluble solids, acid or pectin in Baldwin apples. Attention is again called to the greater cloudiness of all juice samples produced from chopped fruit. This makes for a poorer quality jelly or pectin extract and is distinctly objectionable.

Influence of Extraction Period upon the Composition of Apple Juice.

Baldwin Apples. Data bearing upon this question have been assembled in Tables 9, 10 and 11 and Charts 1 and 4.

Chart 4 gives a clear average picture of the influence of period of extraction upon soluble solids and pectin recovered in the juice. For the removal of soluble solids and pectin in one or more extractions the 15-minute cook was practically as efficient as where, in addition to the regular extraction time, a standing period of 10 minutes removed from the source of heat, was

Chart 4. Influence of the Length of the Extraction Period upon Recovery of Soluble Solids and Pectin in Sliced Baldwin Apples at 100° and 109° C.



*Boiled 15 minutes and allowed to stand 10 minutes.

allowed. A slight gain in amount of pectin extracted was found, but this was of little significance particularly when two or more extractions were considered.

It is interesting to note that one 30-minute extraction was not equivalent by any means to two 15-minute extractions. At all three temperatures used, there was an average increase of less than 10 per cent in soluble solids, pectin, or acid in the 30-minute as compared to the single 15-minute extractions. Two successive 15-minute extractions yielded from 50 to 90 per cent more soluble solids, pectin and malic acid than a single 30-minute extraction. Of course other considerations enter here such as a greater volume of liquid to evaporate when two 15-minute extractions are made instead of one 30-minute period, doubled labor in handling the two extracts and a small loss of time. The cost of fuel is practically the same and, all things considered, the data indicate that two short extractions were much more efficient and economical than a single long one.

Although a slight gain in both total solids and pectin was observed in the 60-minute extract, it was far too long to be practical. Furthermore the increased amounts of solids obtained did not repay for the huge fuel consumption, time, and loss in jelly quality. This latter point is very important and in almost every case where apples were extracted for as long as 60 minutes, a serious loss in jelly quality was observed. The long-continued boiling hydrolyzed the pectin and reduced its activity. Usually apples could be boiled 15 minutes without great apparent injury to the pectin or jelly quality though after 30 minutes boiling this was very apparent. A decrease in both jelly yield and jelly strength in juices boiled for 30 minutes showed that even in that short heating period, some pectin decomposition occurred.

Other Varieties. As regards apple varieties other than Baldwins, the same conclusions hold. A comparison of the data in Tables 10 and 11 (15-and 30-minute extractions respectively) shows very little difference in the amounts of soluble solids obtained. Pectin recovery was slightly greater in the 30-minute extraction period, though the increase was not significant. Since considerably more evaporation loss occurred during a 30-minute extraction period than during a 15-minute period, the volume in the former case was considerably less. It was also more difficult to separate the liquid from the pulp where the amount of liquid was small. There was a tendency toward mushiness as the period of extraction increased and this physical condition of the fruit pulp interfered somewhat with the removal of juice from it, that is the pulp retained a higher percentage of the extractives. The data indicate quite clearly that under the conditions of the experiment two 15-minute extraction periods with the ratio of fruit to water 3:2 to 3:3 were best for juice extraction from the common varieties of the apple. It is possible that a shorter period than this is desirable, but none was studied in this experiment. By this procedure from 65 to 85 per cent of the total soluble solids, according to the ratio of apple to water used and the temperature, and from 50 to 70 per cent of the pectin present in the apple can be utilized. Under the average conditions of jelly manufacture a third extraction would be uneconomical.

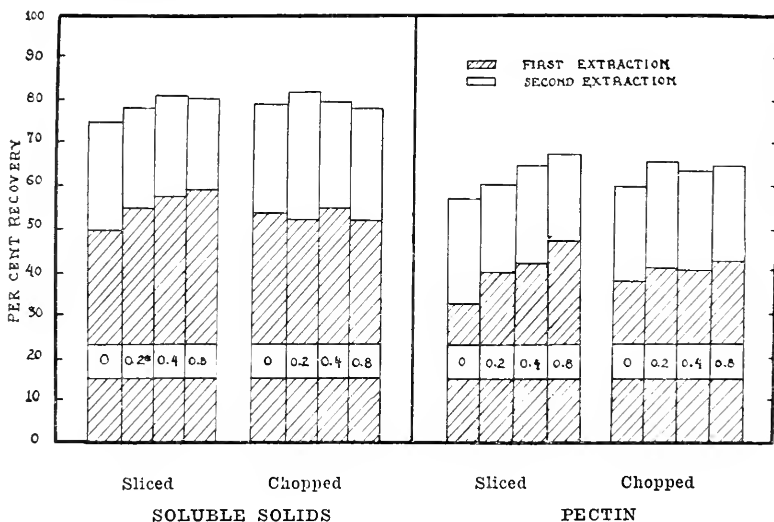
Influence of Added Acid upon the Composition of the Juice.

Tartaric acid was added directly to the sliced or chopped apples in quantities sufficient to produce theoretically acidities of 0.2, 0.4, and 0.8 per cent tartaric acid in the apple-water mixture. This was in addition to the natural

malic acid constantly present in the fruit. No acid was added after the first extraction had been made.

Table 12 and Chart 5 show the effect upon soluble solids and pectin yield of extracting in the presence of increased acidity. In general a slight but uniform increase in the amounts of soluble solids, including pectin and malic acid, was obtained wtih increased acidity. This increase was more marked in the case of sliced than of chopped fruit. An acidity of 0.4 per cent tartaric acid was practically as efficient as double that amount. In other words beyond a certain point little or no advantage accrued from increasing the

Chart 5. Effect of Added Acidity upon the Recovery of Soluble Solids and Pectin in First and Second Extracts of Sliced and Chopped Apples. Ratio of Apple to Water 3:3.



*Per cent added aciidity.

acidity. There was but little effect upon the second or third extracts; the increased yields of solids and pectin being much more pronounced in the first extract. In but few cases did the addition of tartaric acid in order to increase the natural acidity of the fruit, produce more than a 10 per cent increase in yield of solids or pectin even in the first extract. Pectin extraction was aided somewhat more by the acid than was the extraction of soluble solids; in neither case was the increase appreciable, especially if two extractions were considered.

It is questionable whether the addition of organic acids to apples yielded a sufficiently richer juice to pay for the trouble. The resulting jelly was improved in quality, yield, color, and taste by the acid, though of course if sold, a declaration of added acid would be required by State and Federal food laws. However, addition of acid may be advantageous on other grounds inasmuch as Tarr (17) has demonstrated that the hydrogen ion concentration must be at least 3.46 for jelly formation using pure pectin, with the optimum from 2.9 to 3.0. It is likewise true that added acid in many cases appreciably increased jelly yields and improved its physical properties and flavor. For some non-acid varieties of apple a distinct improvement in jelly flavor was effected by the addition of a small amount of acid.

Seasonal Variation of Baldwin Apples

Data were collected for the three years, 1925, 1926, and 1927, on the chemical composition of the fruit as well as the heat-extracted juice. Table 1 shows no striking changes in composition from year to year. Similarly Table 13 presents only minor differences in the heat-extracted juices from apples harvested during three successive years. Despite varying ratios of fruit to extraction water, the range of soluble solids obtained by a single 15-minute extraction was narrow, 47.9 to 59.6, and for the second extraction 22.2 to 27.7. Similarly, the amounts of extracted pectin and also malic acid varied but little. In general, both jelly yields and jelly strengths were higher in the 3:2 ratio than in the 3:3 or 3:4 ratios.

STUDIES OF JELLY

Jelly Yields per Unit Weight of Apples

In Table 14 is summarized the average jelly yields from two extractions at 100°C. for the 8 varieties already mentioned. Additional data also are found in Tables 10, 11 and 13. In interpreting these data, the method of making the jelly must be kept in mind. That is, to 18 ounces (511 grams) of juice composed of proportionate parts of the first and second extracts, was added sufficient sugar in addition to that already present in the juice, to make 12 ounces (341 grams). The juice was then rapidly concentrated until the jelly sheeted or, in the absence of a satisfactory jelly test, until the sugar content reached 69 to 70 per cent. In general jellies containing over 70 or under 64 per cent of sugar were abnormally soft in consistency or were otherwise of poor quality.

Had the sugar been proportioned exactly to the pectin actually present, the increase in jelly yields in columns 3, 8 and 13, Table 14, would not be as marked, though the general trend would have been the same. This may be shown by a simple calculation using the pectin content of the juice or jelly as tabulated respectively in Tables 2 and 3. The pectin content of jellies made from a 3:2 ratio of fruit to extraction water was higher than where the ratio was 3:3 or 3:4.

The effect of pectin content of the juice upon jelly strength is clearly presented in Table 14. Other things being equal, for short extractions the jelly strength increased as the pectin content of the jelly increased. Most of the jellies made from the 3:2 ratio of fruit to extraction water were tough, i. e., over 100 Bloom grams, while those made from a 3:3 ratio were of medium firmness, and those from the 3:4 ratio were considerably softer in consistency. The grade of jelly depended upon several factors such as flavor, color, texture, consistency, stickiness, syneresis and ability to withstand storage.

Two 15-minute extraction periods using equal parts of fruit and water, gave high yields of juice containing sugar, acid and jellifying-pectin in suitable amounts to produce in turn high yields of well flavored, high grade jelly. Normally, this procedure gave best results. In some cases it was possibly more economical to use a ratio of apple to extraction water of 3:2 but the danger of scorching, difficulty of separating the juice from the pulp and the lower yields of solids, pectin and jelly usually outweighed the advantage gained by having only a small amount of relatively concentrated juice. If the sugar was properly proportioned to the amount of jellifying pectin

present in the juice and with due regard to the hydrogen ion concentration of the juice, optimum yield and quality of the jelly resulted and there was no great variation in either jelly yield or quality. The 3:4 ratio of fruit to extraction water, though giving maximum yields of juice, solids, and jelly was found to be impracticable, because of the dilution of the resultant juice, the greater fuel consumption in concentration, and the loss of jelly quality.

Acidity of Apple Jellies

The total titratable acidity as well as hydrogen ion concentration was determined on all jellies. These data are summarized in Table 6. The total acidity calculated as malic acid varied from 0.22 per cent in McIntosh to 0.66 per cent in Red Siberian Crab. The pH value varied between 3.62 for the 1925 crop of Baldwins and 3.20 for Red Astrachans. Inasmuch as jelly yields were dependent to some degree on the pH of the juice, the lower this value, the better was the yield of jelly. Most people prefer an acid or subacid jelly, hence those varieties possessing high total acidity and hydrogen ion concentration are to be preferred for use in jelly making.

Sugar Content of Jellies.

The sugar content of all jellies was determined by the Abbé refractometer. On 101 samples of Baldwin jellies the sugar ranged from 61 to 72 per cent with an average of 65 per cent for jellies classed as marketable, i. e., Grade 1 or 2. The soft jellies very often contained over 70 per cent of sugar and crystallized badly during storage. The sugar content of high grade jellies made from Red Astrachan, Crab Apple, Rhode Island Greening, Winesap and King David averaged 67.5 per cent. McIntosh and Wealthy gave jellies with a sugar content of approximately 65.5 per cent.

Most directions or recipes for making jellies proportion the amount of added sugar to the juice. This is satisfactory only when extractions are made in exactly the same way, i. e., time and temperature of extraction, number of successive extractions, ratio of fruit to water and still other factors must remain nearly constant. Othewise there is danger of using too little or too much sugar for maximum yields of good quality jelly.

It is far better to proportion the added sugar to the weight of fruit originally taken, viz., pound for pound or some other ratio as experience may indicate. This basis is much more scientific and makes for a better product. Each apple variety differs from the other in suitability for jelly manufacture (Tables 10 and 11.) For example, Red Astrachan, King David and Red Siberian Crab produce highly colored and well flavored jellies. Other varieties such as Greening give very light colored jellies; still others are low in acidity, or give jellies which are cloudy or of poor consistency. The variety is important if a product possessing certain known qualities is desired. On the other hand nearly all varieties will yield a fair amount of good edible jelly.

Maturity is important, because in apples past their prime, the pectin undergoes deterioration and produces not only a low yield but a poor quality jelly as well. Seasonal differences in varieties (Table 13) do not appear to be particularly significant.

By consulting Table 14, it is possible to estimate the yield of apple jelly that may be obtained from a unit weight of apples providing the same manufacturing procedure is followed. Since approximately 67.5 per cent of the

weight of a finished apple jelly is sugar, and the weight of concentrated juice and the percentage of soluble solids in it are known, the finishing point of the jelly is readily determined by the simple expedient of weighing the sugar-juice mixture from time to time until the calculated yield of jelly is reached. For example in Table 14, it is shown that an average of 20 ounces of jelly per pound of apples may be expected where the ratio of fruit to extraction water is 3 to 3 by weight and the combined first and second 15-minute extractions are utilized. Then if 3 pounds of fruit are taken, the yield of sugar in 90 ounces of unconcentrated juice containing 5 per cent soluble solids (Table 2) is approximately 90×.05 or 4.5 ounces of sugar in the juice. This figure is not absolutely correct because only about 76.5 per cent of the solids in extracted apple juice is sugar (Table 5), but this correction is unnecessary for ordinary work. Since 3 pounds of apples are used, 3×20 or 60 ounces of jelly should result. This jelly must contain 60×0.675 or 40.5 ounces of sugar. Therefore 40.5—4.5 or 36 ounces of sugar must be added to the juice which is then concentrated until the weight of jelly in the pan is exactly 60 ounces. This is the finishing point of the jelly. The writer has used this method of preparing samples of experimental jellies and jams in numerous cases. In general, the results obtained by the procedure just outlined, usually approximate those obtained by the sheeting test as made by an experienced jelly maker.

The temperature test was useful but could not be relied upon in all cases if used by itself. The refractometer test was found to be of particular value in following the evaporation of water in a jelly mixture. The results checked those obtained by occasional weighings.

For a discussion of the role of sugar, acids, and salts in pectin jellies reference should be made to the series of bulletins issued by Tarr, Myers and Baker of the Delaware Agricultural Experiment Station. In many cases their results are applicabie as well, to fruit jellies. These fundamental contributions on the chemical and physical factors influencing jelly formation are very important and, when properly utilized by jelly manufacturers, should enable them to produce economically standardized products of high quality.

ACKNOWLEDGMENT

The writer is deeply indebted to Professor W. W. Chenoweth, who originally proposed this investigation, for his sustained interest in the project and for many timely suggestions freely given during its entire progress, and to Mr. Francis P. Griffiths who assisted in the final phases of the laboratory work.

SUMMARY AND CONCLUSIONS

Baldwin apples showed but slight yearly variation in composition of fruit, juice or yield of jelly. Red Siberian Crab, King David, Red Astrachan, Winesap and McIntosh were the most suitable varieties for jelly manufacture.

A study of juice extraction by heat showed:

1. Two short (15 minute) successive extractions were usually desirable to obtain an optimum yield of juice containing sufficient pectin and acid to give satisfactory yields of high quality jelly.

2. When only one extraction of the fruit was made, there was a serious loss in jelly yield. This was found to be due largely to the difficulty of extracting pectin.

3. Long extraction periods were unsatisfactory because of destruction of the jellifying power of the pectin. Jelly yields and quality were injured materially. For example, two 15-minute extractions removed from 50 to 80 per cent more soluble solids, pectin and acid than a single 30-minute extraction.

4. Although fair yields of solids, pectin and acid were obtained at an extraction temperature of 88°C. (190° F.), the optimum was found to be 100°C. (212°F.). Retorts or pressure cookers at 109°C. (228°F.) gave only slightly higher yields of solids and pectin in the juice than were obtained at the boiling point, and their use is not recommended for juice extraction.

5. The best ratio of fruit to water was 3:2 in the case of sliced apples, or 3:3 where chopped or grated apples were used. The yield of jelly per pound of fruit was greater where the ratio was 3:4 and least when it was 3:2.

6. Tartaric acid added to the apple-water mixture in concentrations of 0.2 to 0.4 per cent slightly increased the yield of solids and pectin in the extract as well as the total jelly yield. Added acid always improved the color and often the flavor and consistency of apple jellies.

7. Finely chopped apples gave a slightly more concentrated juice and greater jelly yield than sliced apples, but because of the difficulty of filtering and the cloudiness of the finished jellies, chopping is not recommended.

8. In short extraction periods the jelly strength of the jellies increased with the percentage of pectin present. In longer extractions where the jellifying power of the pectin had been injured by prolonged heat, this relation did not hold.

Apples suitable for jelly should yield approximately 20 ounces of jelly per pound of fruit.

Jelly yields depended primarily upon the amount of jellifying pectin present in the juice, and the amount of sugar used. Less sugar should be used where pectin is present only in limited amounts or where its jellifying power has been injured by hydrolysis.

In general those jellies containing the most pectin were very firm and tough in consistency unless additional sugar was used. The hydrogen ion concentration of the varieties tested was suitable for the formation of good yields of well flavored jellies.

There was a fair degree of correlation between the total titratable acidity and hydrogen ion concentration in apples, apple juice and jelly. That is, high total acidity and high hydrogen ion concentration were present in the same samples of apples, juices or jellies.

The sugar content of apple jellies ranged from 65 to 70 per cent with an average of 67.5 per cent. Jellies containing less than 65 per cent sugar were often tough while those with over 70 per cent were uniformly soft or syrupy. In making jellies from fruit, it was found much more desirable to proportion the sugar to the original weight of fruit than to an uncertain yield of juice of questionable composition.

The determination of jelly strength by means of the Bloom gelometer gave very concordant results.

Brix hydrometer readings on extracted apple juice gave an average of 0.18 per cent higher than by the Abbé refractometer and 0.15 per cent higher than by the gravimetric determination of solids in solution. The mean sugar content of the soluble solids in heat extracted apple juice was 76.5 per cent.

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APPENDIX

TABLE 1—Chemical Composition of the Varieties of Apples Used.

Variety	Year	No. of samples	Total solids	Soluble solids	Total sugar	Reducing sugar	Sucrose	Starch	Pectin as pectic acid	pH	Acidity as malic	Ash
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		*per cent*	*per cent*
Baldwin	1925	3	18.06	15.50	11.72	7.12	4.60	0.08	0.29	3.55	0.45	0.27
Baldwin	1926	2	16.79	11.12	9.93	6.22	3.71	tr	0.32	3.42	0.61	0.29
Baldwin	1927	2	15.90	13.60	11.01	7.58	3.43	0.11	0.37	3.47	0.53	0.33
Red Astrachan	1926	1	15.31	12.61	9.20	5.99	3.21	0.18	0.28	3.18	1.11	0.35
R. I. Greening	1926	2	15.70	12.91	10.00	7.80	3.20	0.10	0.31	3.37	0.51	0.31
Winesap	1926	2	18.61	15.79	13.52	10.03	3.49	tr	0.31	3.45	0.47	0.32
McIntosh	1926	3	15.70	13.28	12.09	9.13	2.96	0	0.27	3.60	0.38	0.28
Wealthy	1926	1	13.83	11.76	10.60	8.90	1.70	0	0.30	3.40	0.47	0.26
King David	1926	1	14.98	12.28	10.60	8.68	1.92	tr	0.47	3.25	0.91	0.28
Red Siberian Crab	1926	2	16.17	12.76	10.25	8.47	1.78	0.09	0.62	3.20	0.80	0.36

TABLE 2—Yield and Composition of Juice per Unit Weight of Apples. Combined First and Second Extracts at 100° C., 8 varieties.

Extraction period	Number of tests	YIELD OF JUICE		COMPOSITION OF JUICE			
		Per lb. of fruit	Per kg. of fruit	Soluble solids	Pectin as pectic acid	Pectin per kg. of fruit	Acidity as malic
Ratio of fruit to water 3 : 2							
min.		*oz.*	*grams*	*per cent*	*per cent*	*grams*	*per cent*
15	20	21.6	1353	7.43	0.187	2.53	0.290
15 + 10*	5	21.1	1322	7.71	0.193	2.55	0.345
30	12	19.1	1196	8.26	0.220	2.63	0.368
60	5	18.0	1127	8.60	0.227	2.55	0.421
Ratio of fruit to water 3 : 3							
15	20	30.7	1922	5.70	0.144	2.77	0.212
15 + 10*	5	31.8	1992	5.67	0.143	2.85	0.235
30	12	27.2	1704	6.08	0.175	2.98	0.250
60	5	28.1	1760	6.55	0.177	3.12	0.300
Ratio of fruit to water 3 : 4							
15	20	41.7	2611	4.49	0.129	3.37	0.155
15 + 10*	5	40.2	2519	4.67	0.131	3.30	0.187
30	12	35.4	2218	5.05	0.152	3.37	0.175
60	5	35.5	2550	5.27	0.163	4.15	0.220

* 10 minute standing period.

TABLE 3—Composition of Successive Extracts of Apple Juice. Extraction Period, 15 minutes at 100° C., 8 varieties.

Extract	Number of tests	3 : 2*		3 : 3*		3 : 4*	
		Soluble solids	Pectin as pectic acid	Soluble solids	Pectin as pectic acid	Soluble solids	Pectin as pectic acid
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
First extract	27	8.2	0.226	6.7	0.161	5.7	0.150
Second extract	27	5.3	0.170	3.7	0.122	2.9	0.111
Third extract	27	3.2	0.109	2.0	0.084	1.5	0.065
Residual pulp	24	3.1	0.240	1.9	0.195	1.7	0.170

* Ratio of fruit to water.

TABLE 4—Yield of Soluble Solids, Pectin and Acid per Unit Weight of Apples. Combined First, Second and Third Extracts plus the Residual Pulp.

Variety	Number of tests	SOLUBLE SOLIDS		PECTIN AS PECTIC ACID		ACID AS MALIC	
		Per lb. of fruit	Per kg. of fruit	Per lb. of fruit	Per kg. of fruit	Per lb. of fruit	Per kg. of fruit
		oz.	*grams*	*oz.*	*grams*	*oz.*	*grams*
Baldwin	91	2.15	134.7*	0.077	4.84*	0.064	4.01*
McIntosh	7	2.10	131.2	0.065	4.06	0.034	2.12
King David	7	2.36	148.0	0.064	4.06	0.106	6.64
Red Astrachan	7	1.41	87.9	0.065	4.08	0.115	7.20
Red Siberian Crab	8	2.42	151.6	0.070	4.38	0.143	8.94
Wealthy	7	1.47	92.0	0.057	3.59	0.078	4.87
Rhode Island Greening	7	1.91	119.7	0.070	4.38	0.087	5.42
Winesap	7	2.41	148.5	0.083	5.18	0.072	4.52

* Soluble solids 134.7 ± 9.55; pectin 4.84 ± 0.50; acid 4.01 ± 0.57.

TABLE 5—Relation of Soluble Solids and Sugar in Heat Extracted Apple Juice as Determined by Abbé Refractometer, Brix Hydrometer, and Gravimetrically.

Brix degrees	Computed from R. I.	Direct reading of sucrose — refractometer	Soluble solids — gravimetric determination	Total sugar as invert gravimetric method
6.2	5.67	6.30	5.71	4.10
3.7	3.48	4.10	3.39	2.49
1.8	1.44	2.00	1.51	0.98
6.7	6.45	6.90	6.65	4.96
3.3	3.40	3.75	3.42	2.01
1.8	1.38	2.00	1.41	0.97
5.1	5.05	5.20	5.09	4.05
7.9	7.90	8.00	8.10	6.76
5.0	5.00	4.61	4.71	3.81
10.8	10.65	10.90	10.71	8.93

Mean difference between Brix and refractometer determinations	+0.176
Mean difference between Brix and gravimetric determinations	+0.146
Mean difference between refractometer and gravimetric determinations	—0.030
Mean difference between sucrose scale reading on refractometer and total sugars (chemical)	+1.470
Mean sugar content of total solids in heat extracted apple juice	76.5%

TABLE 6—Titratable Acidity and Hydrogen Ion Concentration of Extracted Apple Juice and Jelly. Extraction Period 15 minutes at 100° C., with Ratio of Apple to Water 3 : 3.

Variety	Season	First extract		Second extract		Third extract		Jelly from combined first and second extract	
		pH	Acidity as malic	pH	Acidity as malic	pH	Acidity as malic	pH	Acidity as malic
Baldwin	1925	3.55	0.198	3.65	0.100	3.75	0.051	3.62	0.307
Baldwin	1926	3.47	0.201	3.60	0.110	3.67	0.047	3.57	0.310
Baldwin	1927	3.49	0.201	3.57	0.111	3.63	0.045	3.53	0.321
McIntosh	1926	3.64	0.117	3.71	0.068	3.82	0.039	3.55	0.220
Winesap	1926	3.54	0.265	3.57	0.134	3.59	0.560	3.51	0.295
King David	1926	3.33	0.436	3.36	0.238	3.45	0.105	3.40	0.542
R. I. Greening	1926	3.38	0.312	3.40	0.183	3.55	0.077	3.39	0.383
Red Astrachan	1926	3.15	0.430	3.30	0.240	3.47	0.139	3.20	0.640
Wealthy	1926	3.47	0.181	3.52	0.093	3.62	0.400	3.50	0.280
Red Siberian Crab	1926	3.21	0.450	3.25	0.247	3.40	0.810	3.26	0.660

TABLE 7—Distribution of Soluble Solids in Juice Extracted from Sliced and Chopped Apples. Extraction Period 15 minutes.

Variety	Ratio of fruit to water	Temperature	Number of tests	3 successive combined extracts		First extract		Second extract	
				Sliced	Chopped	Sliced	Chopped	Sliced	Chopped
		degrees C.		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Baldwin	3 : 3	88*	7	85.0	89.7	46.8	53.9	25.7	23.3
Baldwin	3 : 3	100*	14	89.4	92.9	53.0	57.1	23.9	24.7
Baldwin	3 : 3	109*	11	92.6	85.9	60.6	56.2	22.8	26.4
Baldwin	3 : 4	88	6	89.0	91.8	50.3	54.4	27.9	24.8
Baldwin	3 : 4	100	14	91.2	95.4	59.6	58.8	22.9	26.6
Baldwin	3 : 4	109	6	91.4	94.6	58.5	59.1	24.8	27.2
Baldwin	3 : 2	88	6	80.0	87.0	37.2	40.0	26.0	26.8
Baldwin	3 : 2	100	14	87.1	87.6	50.5	45.6	24.8	27.7
Baldwin	3 : 2	109	6	90.2	86.1	58.3	43.2	20.9	28.8
Red Astrachan	3 : 3	100	2	91.8	95.3	66.7	60.5	20.2	27.8
Red Siberian Crab	3 : 3	100	2	96.0	97.1	60.8	65.7	25.7	22.9
Wealthy	3 : 3	100	2	97.8	99.0	69.9	71.1	23.2	21.8
McIntosh	3 : 3	100	2	85.7	90.1	43.6	51.2	30.0	26.9
King David	3 : 3	100	2	91.4	95.2	62.4	63.0	19.7	18.0
Winesap	3 : 3	100	2	91.2	88.8	52.8	51.9	25.8	21.2
Rhode Island Greening	3 : 3	100	2	93.3	89.9	47.4	47.2	30.5	27.4

* Respective Fahrenheit equivalents are 190°, 212° and 228°.

TABLE 8 —Percentage of Pectin in Juice Recovered from Sliced and Chopped Apples. Extraction Period 15 minutes.

Variety	Ratio of fruit to water	Temperature	Number of tests	3 successive combined extracts		First extract		Second extract	
				Sliced	Chopped	Sliced	Chopped	Sliced	Chopped
		degrees C.		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Baldwin	3 : 3	88*	7	64.2	72.8	33.6	42.9	19.1	17.5
Baldwin	3 : 3	100*	14	68.5	70.9	37.7	40.2	24.6	26.7
Baldwin	3 : 3	109*	11	79.2	74.0	46.6	35.5	20.6	26.5
Baldwin	3 : 4	88	6	68.4	73.8	31.8	36.9	22.2	21.6
Baldwin	3 : 4	100	14	83.7	83.0	43.6	44.6	25.0	24.8
Baldwin	3 : 4	109	6	88.3	78.7	48.7	36.1	16.7	26.9
Baldwin	3 : 2	88	6	63.5	63.1	26.0	35.1	21.9	18.8
Baldwin	3 : 2	100	14	71.6	73.5	39.8	38.4	21.9	22.2
Baldwin	3 : 2	109	6	76.7	77.1	44.8	35.6	21.3	26.9
Red Astrachan	3 : 3	100	2	86.2	89.7	59.2	52.2	14.3	25.2
Red Siberian Crab	3 : 3	100	2	84.2	83.4	37.6	48.7	28.2	22.8
Wealthy	3 : 3	100	2	84.9	84.4	52.6	55.2	21.5	20.1
McIntosh	3 : 3	100	2	80.6	73.8	39.3	33.2	26.7	21.9
King David	3 : 3	100	2	74.6	74.6	31.9	48.9	22.9	11.2
Winesap	3 : 3	100	2	69.0	74.3	35.0	37.3	20.3	20.5
Rhode Island Greening	3 : 3	100	2	78.4	76.9	32.4	34.0	28.2	25.9

* Respective Fahrenheit equivalents are 190°, 212° and 228°.

TABLE 9—Influence of Period of Extraction and Temperature upon the Percentage Recovery of Soluble Solids and Pectin. Ratio of Baldwin Apple to Water 3 : 3.

Temperature	Number of tests	Extraction period	3 successive combined extracts		First extract		Second extract	
			Soluble solids	Pectin	Soluble solids	Pectin	Soluble solids	Pectin
degrees C.		*minutes*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
88	10	15	87.8	69.3	48.4	36.7	26.0	19.8
88	3	15 + 10*	87.7	60.6	44.3	24.2	26.7	18.6
88	3	30	85.4	62.1	42.6	28.7	27.2	19.8
88	3	60	87.5	79.0	50.7	36.8	24.8	25.9
100	24	15	90.3	77.3	54.0	39.6	24.6	23.5
100	6	15 + 10*	89.8	77.9	52.6	37.3	25.0	24.2
100	6	30	87.4	79.7	53.9	41.4	23.6	25.6
100	6	60	92.8	84.8	59.2	47.3	24.1	24.8
109	10	15	91.1	75.4	52.9	35.2	26.4	25.5
109	4	15 + 10*	93.0	77.7	58.7	43.2	24.1	21.6
109	5	30	93.9	83.0	61.5	48.2	22.9	25.2
109	4	60	93.1	87.5	63.8	56.4	19.4	20.0

* 10 minute standing period.

TABLE 10—Effect of Variety and Ratio of Apple to Water on the Recovery of Soluble Solids, Pectin and Malic Acid. Extraction Period 15 minutes at 100° C.

Variety	Ratio of fruit to water	Number of tests	First extract			Second extract			Third extract			Residual pulp			Jelly		
			Soluble solids	Pectin	Acidity as malic	Soluble solids	Pectin	Acidity as malic	Soluble solids	Pectin	Acidity as malic	Soluble solids	Pectin	Acidity as malic	Yield per pound of fruit	Grade *	Jelly strength
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*oz.*		*grams*
Baldwin	3 : 2	18	49.1	41.5	51.7	25.9	22.7	23.1	11.8	10.9	12.3	12.9	24.9	12.9	14.2	2	87
	3 : 3	20	52.9	43.3	55.8	23.7	24.9	21.4	11.7	11.1	10.8	11.7	20.7	12.0	20.0	2	80
	3 : 4	18	58.1	43.6	57.8	26.3	25.0	21.2	7.8	15.1	8.7	7.8	16.3	12.3	21.2	2	77
Red Astrachan	3 : 2	2	60.6	42.3	63.8	21.0	23.1	24.7	10.5	18.1	6.9	7.9	16.5	4.6	14.5	2	150
	3 : 3	2	66.7	59.2	73.7	20.2	14.3	13.8	7.9	12.7	7.5	5.2	13.8	5.0	22.8	1	121
	3 : 4	2	69.6	55.2	66.7	17.4	24.2	17.2	8.7	9.3	6.7	4.3	11.3	3.9	26.0	1	100
Red Siberian Crab	3 : 2	2	51.5	33.9	48.0	25.0	19.3	25.7	13.2	11.5	12.3	10.3	35.3	14.0	16.7	2	205
	3 : 3	3	60.8	37.6	66.3	25.7	28.2	22.3	9.5	18.4	6.8	4.0	15.8	4.6	26.1	2	182
	3 : 4	2	69.5	42.2	65.9	22.2	21.1	23.3	5.5	12.6	6.4	2.8	21.1	1.1	29.2	1	114
Wealthy	3 : 2	2	65.2	53.2	52.1	22.9	22.4	19.4	7.2	9.0	13.2	4.7	15.4	15.3	14.3	1	135
	3 : 3	2	69.9	52.6	62.0	23.2	21.5	22.0	4.7	10.8	10.1	2.2	15.1	5.9	20.2	1	172
	3 : 4	2	74.6	52.1	72.1	20.0	20.8	19.7	4.5	10.8	5.0	0.9	16.3	3.2	22.7	1	134
McIntosh	3 : 2	1	49.3	43.1	56.9	27.2	20.4	19.3	10.6	12.2	10.2	12.9	21.3	13.6	13.6	3	175
	3 : 3	2	43.6	39.3	49.0	30.0	26.7	29.0	12.1	14.6	13.0	14.3	19.4	9.0	19.7	2	160
	3 : 4	2	48.6	39.3	51.5	18.7	19.4	24.1	21.1	13.3	13.8	11.6	28.0	7.6	23.7	1	68
King David	3 : 2	2	51.7	41.1	56.1	24.8	22.8	20.8	12.1	11.4	11.6	11.1	24.7	11.5	15.2	2	120
	3 : 3	2	62.4	34.9	65.7	19.7	22.9	18.9	12.3	16.8	9.6	5.6	25.4	6.2	19.7	1	81
	3 : 4	2	61.8	46.8	67.3	21.2	22.7	21.6	11.5	11.1	7.3	5.5	19.4	3.8	24.7	3	47
Winesap	3 : 2	2	48.2	28.0	55.6	21.4	20.3	19.0	15.3	14.2	11.7	15.1	37.5	13.7	15.9	2	178
	3 : 3	3	52.8	35.0	59.0	25.8	20.3	24.9	12.6	13.7	8.4	8.8	31.0	7.0	19.9	2	128
	3 : 4	2	62.6	27.9	71.6	19.1	28.6	13.8	9.2	19.3	7.3	9.1	24.2	4.3	21.3	1	84
R. I. Greening	3 : 2	2	51.0	39.5	52.5	20.0	20.9	20.8	14.4	11.4	11.9	14.6	25.2	14.8	14.9	2	113
	3 : 3	2	47.1	32.4	57.4	30.5	28.2	25.9	15.1	17.8	9.1	6.7	21.6	7.6	20.2	1	118
	3 : 4	2	51.3	52.1	69.5	23.2	22.4	19.2	13.2	12.4	7.1	9.3	13.1	4.2	25.8	1	84

* Grade 1—good; grade 2—fair; grade 3—poor, unmarketable.

TABLE 11—Effect of Variety and Ratio of Fruit to Water on the Recovery of Soluble Solids, Pectin and Malic Acid. Extraction Period 30 minutes at 100° C.

Variety	Ratio of fruit to water	Number of tests	First extract			Second extract			Third extract			Residual pulp			Jelly		
			Soluble solids	Pectin	Acidity as malic	Soluble solids	Pectin	Acidity as malic	Soluble solids	Pectin	Acidity as malic	Soluble solids	Pectin	Acidity as malic	Yield per pound of fruit	Grade *	Jelly strength
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*oz.*		*grams*
Baldwin	3 : 2	6	45.2	40.2	47.9	24.8	19.2	27.1	13.0	10.0	12.5	17.0	17.7	12.5	13.2	2	80
	3 : 3	6	53.9	41.4	57.2	23.6	25.6	25.8	9.9	12.2	8.2	12.6	20.3	8.8	20.0	1	72
	3 : 4	6	60.2	45.3	64.1	23.2	26.2	24.0	11.0	12.3	6.6	5.6	16.2	5.3	23.5	2	65
Red Astrachan	3 : 2	2	60.4	42.6	61.3	26.7	28.2	24.8	8.9	19.0	10.8	6.0	10.2	3.1	14.4	2	146
	3 : 3	2	62.0	55.5	61.8	23.8	20.6	22.2	7.1	12.9	10.7	7.1	11.0	5.3	21.0	1	111
	3 : 4	2	60.5	52.2	58.2	27.8	25.2	25.0	7.0	12.3	10.9	4.7	10.3	5.9	26.1	1	106
Red Siberian Crab	3 : 2	2	47.1	37.9	48.7	28.6	22.2	27.0	15.7	12.3	14.0	8.6	27.2	10.3	15.5	3	195
	3 : 3	3	51.3	40.0	60.6	31.6	25.9	22.3	11.8	11.2	10.5	5.3	22.9	6.6	24.2	2	170
	3 : 4	2	67.1	44.8	68.4	22.8	25.2	22.6	7.6	11.4	5.7	2.5	18.6	3.7	28.7	1	180
Wealthy	3 : 2	2	67.5	53.2	61.9	25.6	21.2	24.2	4.6	8.1	9.8	2.3	17.5	4.1	14.0	1	122
	3 : 3	2	72.5	54.8	69.0	19.8	22.9	23.2	5.5	9.1	4.7	2.2	13.2	3.1	19.8	2	133
	3 : 4	2	74.9	57.7	79.1	19.8	18.3	13.1	3.1	9.6	4.9	2.2	14.4	2.9	22.2	3	76
McIntosh	3 : 2	2	54.4	41.0	49.9	21.9	22.2	29.3	12.2	13.2	9.5	11.5	23.6	11.3	13.5	3	175
	3 : 3	2	56.1	42.4	65.9	26.4	25.2	25.4	8.2	9.4	4.8	9.3	23.0	3.9	19.8	2	114
	3 : 4	2	57.7	45.1	62.3	18.6	21.2	19.4	12.4	13.5	12.2	11.3	20.2	6.1	23.9	2	74
King David	3 : 2	2	43.3	36.5	14.8	23.6	24.2	20.5	19.4	13.4	17.6	13.7	25.9	17.1	14.7	2	144
	3 : 3	2	53.0	43.3	56.3	20.6	21.1	24.8	15.9	12.4	11.7	9.6	23.2	7.2	19.9	1	72
	3 : 4	2	65.1	45.3	67.0	17.4	17.2	18.3	12.6	12.7	10.2	4.9	24.8	4.5	24.3	3	35
Winesap	3 : 2	2	51.6	43.7	52.3	18.3	17.5	19.2	13.4	12.6	11.2	16.7	26.2	17.3	15.6	3	227
	3 : 3	2	57.5	46.6	63.6	20.0	21.5	19.7	12.1	4.6	8.9	10.4	27.3	7.8	19.3	2	143
	3 : 4	2	60.2	46.8	67.0	19.1	27.3	17.7	10.3	7.4	9.5	10.3	18.5	5.8	22.5	1	118
R. I. Greening	3 : 2	2	51.0	42.0	54.3	21.6	22.0	20.1	14.0	14.5	11.8	13.4	21.5	13.8	14.6	3	240
	3 : 3	2	55.7	43.1	61.4	19.9	19.0	20.0	12.4	16.4	9.3	12.0	21.5	9.3	19.4	3	138
	3 : 4	2	55.2	45.2	68.3	22.7	26.9	18.8	15.3	15.5	8.5	6.8	12.4	4.4	25.0	1	117

* Grade 1—good; grade 2—fair; grade 3—poor, unmarketable.

TABLE 12—Influence of Added Acid upon the Recovery of Soluble Solids and Pectin. Ratio of Baldwin Apple to Water, 3 : 3.

Temperature	Tartaric acid added to apples	Number of tests	3 successive combined extracts				First extract				Second extract			
			Soluble solids		Pectin		Soluble solids		Pectin		Soluble solids		Pectin	
			Sliced	Chopped	Sliced	Chopped	Sliced	Chopped	Sliced	Chopped	Sliced	Chopped	Sliced	Chopped
degrees C.	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
100	0.	12	86.7	92.2	72.6	76.1	50.2	54.6	33.0	38.6	25.4	25.8	25.1	23.1
100	0.2	6	88.5	91.7	74.2	79.0	54.8	53.5	40.6	41.8	24.3	27.1	21.0	25.8
100	0.4	12	91.6	92.5	82.2	80.0	58.0	55.5	42.1	41.6	24.7	25.4	23.8	21.5
100	0.8	6	90.5	92.0	78.7	83.5	59.2	52.5	44.8	42.9	22.2	27.0	21.3	26.2
88	0.	12	85.1	88.2	66.0	67.0	42.8	50.7	26.8	34.1	25.5	23.7	19.3	17.9
88	0.4	6	88.3	91.2	71.3	77.1	45.6	52.8	33.4	44.2	28.7	26.0	21.1	21.1
109	0.	12	92.8	89.0	79.2	73.7	57.9	49.5	43.6	35.2	23.5	28.3	23.9	21.5
109	0.4	7	92.0	93.2	77.3	77.8	59.2	55.8	43.0	25.8	23.6	26.6	21.8	28.3

TABLE 13—Recovery of Soluble Solids, Pectin and Malic Acid in Baldwin Apples for Three Seasons. Extraction Period 15 minutes at 100° C.

Season	Ratio of apple to water	Number of samples	SOLUBLE SOLIDS		PECTIN		MALIC ACID		JELLY		
			First extract	Second extract	First extract	Second extract	First extract	Second extract	Yield per pound of fruit	Grade *	Jelly strength
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*ounces*		*grams*
1925	3 : 2	14	50.5	27.7	37.7	24.6	49.8	26.0	14.0	2	80
1925	3 : 3	14	53.0	24.7	43.6	25.0	54.1	21.0	19.2	1	73
1925	3 : 4	14	59.6	26.6	39.8	21.9	57.6	21.2	24.7	2	72
1926	3 : 2	2	49.7	23.2	47.1	19.1	57.5	19.5	13.3	2	128
1926	3 : 3	3	53.0	22.2	43.2	20.0	56.2	21.2	19.0	1	95
1926	3 : 4	2	57.7	28.7	41.2	28.0	58.8	22.6	25.1	1	106
1927	3 : 2	2	47.9	26.8	39.8	24.3	47.9	23.7	13.9	1	99
1927	3 : 3	3	52.6	24.2	43.2	29.8	57.2	21.9	18.7	1	94
1927	3 : 4	2	57.4	23.7	44.7	27.9	57.1	19.7	24.7	1	88

* Grade 1—good; grade 2—fair; grade 3—poor, unmarketable.

TABLE 14—Jelly Yields per Unit Weight of Apples.
Combined First and Second Extracts at 100° C., 8 varieties.

Extraction period	Number of tests	YIELD OF JELLY: Per pound of fruit	YIELD OF JELLY: Per kilogram of fruit	Jelly strength	Grade*	Pectin as pectic acid
			Ratio of fruit to water 3 : 2			
minutes		*ounces*	*grams*	*grams*		*per cent*
15	16	14.26	893	118	1-2	0.283
15 + 10**	5	14.95	937	123	1-2	0.272
30	12	13.20	827	143	2	0.318
60	5	12.90	808	78	3	0.317
			Ratio of fruit to water 3 : 3			
15	16	20.02	1251	88	1	0.221
15 + 10**	5	19.90	1239	90	1	0.229
30	12	19.90	1239	95	1-2	0.239
60	5	18.33	1141	57	2-3	0.271
			Ratio of fruit to water 3 : 1			
15	16	24.60	1132	67	1	0.219
15 + 10**	5	22.40	1395	70	1-2	0.235
30	12	23.55	1471	76	2	0.229
60	5	19.40	1196	52	3	0.298

* Grade 1—good; grade 2—fair; grade 3—poor, unmarketable.

** 10 minute standing period.

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