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Bacteriological Studies on Eggs.

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The Bacteriology of Eggs and Egg Products with Special Reference to *B. coli.*

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INTRODUCTION.

The high cost of living has caused widespread efforts toward increased efficiency in our methods of production and distribution of food products. The egg industry is an industry in which enormous losses are sustained through decomposition, breakage, and incubation. He who has seen the wagon loads of rotten eggs hauled from the establishments of our big egg dealers fully appreciates the tremendous waste connected with this industry. In an effort to diminish this waste, the Department of Bacteriology of the Kansas State Agricultural Experiment Station, in cooperation with the Poultry Department of the same institution, has undertaken a detailed investigation of the factors influencing the spoilage of eggs and of egg products. The present paper contains the results of a part of these investigations.

ECONOMIC ASPECTS OF THE EGG INDUSTRY.

Kansas, like most of the other middle-western states, has a very extensive poultry industry. Remoteness from the great centers of consumption makes this territory a favorable field for the activity of firms which devote themselves to the freezing or desiccating of eggs in bulk. The daily capacity of the firms producing desiccated eggs, in Kansas alone, is about one thousand cases of thirty dozen each. The economic importance of this industry can scarcely be overestimated.

Cold storage does not inhibit deterioration of the eggs, but only retards it, as the well-known storage flavor develops early. With the modern methods of desiccating eggs, it is possible to obtain a product which retains for a long period the qualities of fresh eggs. Freezing, and especially desiccating, greatly reduces the weight and bulk of the eggs, one pound of the dried product representing about three and a half pounds of the raw egg meat obtained from thirty eggs. The dried or frozen products do not suffer from breakage or from freezing. On

account of its fat contents, desiccated egg resembles butter in its keeping qualities. It does not undergo deterioration at low temperatures; and when kept at room temperature, it changes slowly, acquiring finally a fishy odor which is accompanied by a decrease in solubility. In view of its keeping qualities, therefore, desiccated egg can be kept on hand easily and made ready for use by simply dissolving it in water. All this means an enormous saving in the cost of transportation and in the storage of eggs, as well as the avoidance of much waste due to breakage, decomposition and incubation. Moreover, the general use of this product for baking purposes would greatly decrease the demand for eggs in the shell, so that during all seasons the large cities could be supplied to a great extent by the surrounding country. This would make the egg market; infinitely more stable than it is now. The benefits which both the consumer and the farmer would derive therefrom, are evident.

In spite of the beneficial influences which this industry exercises, popular sentiment is often against it. This is largely because the public has exaggerated notions of the profits which the manufacturers derive from it; because the manufacturers are accused of monopolistic control of the egg market; and last but by no means least, because a few unscrupulous manufacturers have put on the market frozen and desiccated rotten eggs. It is to the interest of the consumer as well as to that of the honest manufacturer, and is necessary to the best development of this industry itself, that such unlawful practices should be strictly suppressed.

METHODS FOR JUDGING FROZEN AND DESICCATED EGGS.

In an effort to suppress the unlawful practices alluded to, methods for judging egg preparations have been recently devised. In the absence of physical signs of decomposition, these methods attach much significance to the total number of bacteria, and to the relative numbers of colon bacilli present. It is claimed by some investigators that colon bacilli are never found in any quantity in fresh eggs, and that when present in large numbers in egg products they have their origin in fecal matter or in old, bad eggs. This statement, however, is vigorously contested by the experts of the manufacturing interests. On account of the great practical importance of this

question, it was thought desirable to carry on extensive studies on the relation of *B. coli* to eggs.

BACTERIAL INFECTION OF EGGS DURING THEIR FORMATION.

It has been shown by various observers that newly laid eggs may contain bacteria. As regards the place of infection, Poppe says: "An infection of the yolk in the ovary must be excluded according to the present state of our knowledge of the absence of bacteria from the normal organs." Observations to the contrary, as made by Pernot, would therefore have to be attributed to a pathological condition of the ovary. The doctrine of the sterility of normal organs has recently received a serious shock, however, by the researches of Conradi. He examined the normal organs of healthy animals by a method which strictly excludes secondary infection, with the result that out of 162 organs examined, seventy-two were found to contain bacteria. It seems, therefore, that an infection of the yolks even in the normal ovary is possible, and that we have to consider this source of infection in investigating the infection of eggs during their formation.

From the ovary the yolk enters the anterior opening of the oviduct, the funnel-shaped *ostium infundibulum.* The oviduct is lined successively with albuminous, membrane-forming, and lime-secreting glands (uterus) which, in the upper part of the oviduct, are arranged in spiral ridges. In its spiral journey down the oviduct the yolk is successively covered with the secretions of these different glands. The egg finally leaves the oviduct and is set free through the cloaca. We see that in the female fowl there exists an open connection, by means of the cloaca, between the oviduct and the intestines. This explains the fact that the organisms found in the oviduct and in fresh eggs are those which generally inhabit the intestines. The oviduct is by no means always sterile, as Horowitz claims. Zimmerman, Abel and Draeer, Cao, McClintock, and Poppe found that micro-organisms occur in the oviduct of hens which are frequently mated, while this is not always the case with hens that are kept apart from males.

Copulation is not the only factor, however, which brings about a transportation of micro-organisms from the cloaca into the oviduct. Micro-organisms are frequently found in non-fertilized eggs, though, as was pointed out above, not so often as

in fertilized eggs. The occurrence of bacteria in such eggs is not due to a migration of motile organisms from the cloaca into the oviduct. Poppe found M. aureus, *M.* albus, and *M. sulfureus non* liquefaciens in the oviduct of a hen which was kept apart from a male bird; no copulation having taken place and these organisms being nonmotile, there must be some other factor which caused their occurrence in the oviduct. Antiperistalsis of the oviduct might result in the transportation of traces of feces and thereby micro-organisms from the cloaca into the oviduct. That antiperistalsis of the oviduct does occur is shown by the occasional presence of trematodes, feathers and stones within eggs, and also by the occurrence of eggs within eggs, etc.

THE RELATION OF B. COLI TO FRESH EGGS.

Since the organisms present in fresh eggs are the same as those found in the intestines of the hen, it would be natural to expect in the infected eggs an organism which is ever present in the intestines, *B. coli.* Though Kern, Rahner, Poppe, and Joest have never failed to find B. coli in the intestines of the chicken, not one of the investigators (Artault, Wiley, Pennington, Poppe) who made a study of the bacterial flora of fresh eggs seems to have found this organism. Most of the above investigators used the direct plate method for isolating and studying the organisms present. This necessitates the use of comparatively small amounts of the material under examination, so that B. coli, when present in small numbers, will probably escape detection. The number and variety in character of the eggs used by the above investigators were rather limited.

ORIGINAL INVESTIGATIONS.

It was thought desirable, therefore, to make a search for B. coli in and on a large number of eggs of varying character.

TECHNIQUE.

To detect B. coli *on the shell.*—The eggs were taken from the container with sterile forceps. With the egg held lengthwise between the fingers, the shell was rubbed off with a sterile, cotton swab, soaked in sterile physiological salt solution. The cotton was removed from its glass-rod carrier, and transferred with sterile forceps to a fermentation tube of lactose bouillon.

To detect B. coli within the egg.—The egg was now washed with soap and brush, after which it was immersed for five minutes in a 1:1000 solution of corrosive sublimate. From this it was removed with sterile crucible forceps, was transferred to a small conical graduate with the broad pole uppermost, and was thoroughly rinsed with sterile water. The outermost portion of the broad end was now briskly and briefly heated with the central cone of a bunsen flame. The expansion of the air in the air space bursts the shell very neatly, and without any chance for contamination. At the same time the heat applied is sufficient to dry the upper portion of the egg without in the least coagulating the albumin, Sometimes the shell did not burst, in which case a hole was made with sterile forceps.

From different portions of the albumin, one-tenth cubic centimeter, one cubic centimeter, and three cubic centimeter samples—two samples of the last size being taken—were transferred to lactose bouillon fermentation tubes by means of pipettes, the points of which had been broken off. The egg was mixed with the bouillon by repeatedly drawing it back and forth with the pipette. This done, the egg was removed from the graduate, care being taken not to touch the upper portions of the egg. If the lower part was still wet, it was dried with a clean towel. The egg was then inverted until all the albumin had run out, then the remainder was returned to the graduate, and the same inoculations were made from the yolk that had been made from the white. The fermentation tubes were incubated at 38° C., and when gas was formed the usual routine tests for *B. coli* were made.

RESULTS OF THE INVESTIGATIONS.

In the above manner, sixty eggs from the College poultry plant from thirty different hens were examined. These eggs were collected from trap nests twice a day, and were kept over night in a cool cellar. In the morning they were put into pasteboard carriers, each egg occupying a separate compartment, and were brought to the laboratory, where they were examined in the afternoon. Owing to the good care which the College poultry receives, and to the cleanliness prevailing in the pens and the trap nests, most of the eggs were clean, only a few of them being even slightly soiled with feces. No special

care, however, was taken with these eggs, the man who collected them not knowing anything of the experiments. The trapping system enabled us to trace each egg to the hen that had laid it, and made it possible to investigate the cause of abnormalities, should any be found in the eggs.

B. coli was not found in these eggs. It was found on the shells of twenty-seven per cent of them, however.

The conditions in the College poultry plant being about the best possible as regards the health of the fowls, food, housing, etc., it was thought desirable to examine eggs which were produced under less favorable conditions. For this purpose, two eggs were obtained from each of twenty-five different farms in the vicinity of Manhattan, Kan. The eggs thus obtained represented a very great variety of conditions as regards breeds, health, housing, feeding, etc.

B. coli was absent from the contents of all of these eggs, but was found on the shells of eighteen per cent of the eggs examined.

Numerous investigators have shown that bacteria contained in a bouillon culture are able to penetrate the egg shell and to infect the contents. Lange and Menini attribute this faculty only to motile bacteria. Sachs-Mucke, as a result of his experiments with dysentery bacilli, concludes that these bacteria reach the egg contents from bouillon cultures only when the shell has very fine cracks (blind checks). Poppe, in his recent investigations, comes to the conclusion that motile bacteria may reach the egg contents even if they are merely smeared on the shell. Considering the results obtained by these various investigators, we might expect to find *B. coli* in the contents of eggs which are soiled with feces, the more so since Wilm and Lange attribute to *B. coli* the power to penetrate the egg shell from bouillon cultures. It is obvious that if *B. coli* is thus found in eggs, its presence in egg preparations does not necessarily indicate the presence of any fecal matter. The eggs may be washed before they leave the farm and therefore pass as clean eggs.

To determine whether eggs soiled with fecal matter contain colon bacilli, twenty-five dirty eggs were obtained from a packing-house in Manhattan. These eggs were at least three weeks old, and were fairly covered with droppings. Upon candling, all but five of them proved perfectly normal. These five ab-

normal eggs showed a week, watery white in which the yolk would not stand up. *B. coli* was not found in any of these eggs, but was found on the shells of all of them.

It frequently happens that eggs are soiled with the contents of broken eggs. The shell of the eggs thus soiled may have been soiled also by feces at an earlier date. The colon bacilli and other putrefactive organisms present in the feces may rapidly multiply in the egg meat upon the shell. To see whether the colon bacilli thus developing in a favorable medium are able to penetrate the shell and to infect the contents, fifteen eggs which were heavily covered with feces and egg meat were secured on the market and subjected to an examination for *B. coli.* *B. coli* was never found in the contents of these eggs, while it was invariably present on the shell.

The season of the year in which these soiled eggs were laid (winter) would seem favorable to their infection from the contaminating feces. The places where the eggs are deposited are naturally pretty cold in winter; therefore, as soon as the egg has been deposited, it cools considerably. The contraction of the contents brought about by this cooling is likely to draw moisture from the surface of the shell through the pores into the interior. Together with the moisture, bacteria from droppings, etc., might also reach the egg contents. To see whether this is actually the case, several eggs, after being kept for three hours at 38° C., were smeared with a suspension of *B. prodiyiosus,* and were immediately afterward placed in an ice chest, where they remained for three hours at a temperature of about 12° C. They were then kept at about 18° C. so that the organisms which had penetrated the pores might develop. To distinguish an invasion caused by changes in temperature from an infection due to the motility of *B. prodigiosus,* which would not take place before several days, the eggs were examined at an early date, that is, after two days. Culture tests did not reveal the presence of *B. prodigiosus* within the eggs. Neither could a growth of *B. prodigiosus* be detected on the inside of the shell, except in one egg, the shell of which had a very fine crack. Here the red growth of *B. prodigiosus* was found all along the crack, between the membrane and the shell, which indicates that *B. prodigiosus,* under the above conditions, was not able to penetrate the intact shell or membrane. The

infection of eggs due to temperature changes is of great practical importance and will receive further investigation.

It may be objected that the results obtained with the soiled eggs are not representative for the same grade of eggs produced during summer. It may be argued that the low temperature prevalent at the time when these investigations were made (February and March), reduced the motility and vitality of the colon bacilli present in the feces, so that the bacilli were not able to penetrate the shell. Indeed, this argument seems justified, for Poppe has found that B. *paratyphosus* B is able to penetrate the egg shell at room and incubator temperature, while it is not able to do so at ice-chest temperature. It appeared necessary, therefore, to repeat the search for B. *coli* in soiled eggs which were kept at a temperature near that prevailing in summer.

For this purpose sixteen fresh eggs were heavily soiled with fresh chicken feces, and the eggs kept at room temperature. On the next day eight of these were cracked *without injuring the shell-membrane.* At intervals of two days, one egg of each, series was examined for *B. coli.* The samples for analysis from the unbroken eggs were obtained in the usual manner. The cracked eggs were quickly and carefully washed with, brush and soap, immersed for ten minutes in a 1:1000 bichloride ,solution,thoroughly rinsed in sterile water, and placed on a sterile graduate. With sterile instruments a small hole was made in the broad pole to allow for the escape of air, and after the pole had been scorched with a bunsen flame, the hole, was enlarged and portions of the albumin and yolk were removed in the usual manner.

After fermentation tubes had been inoculated from the above eggs, the rest of the albumen and yolk was transferred separately, under aseptic precautions, to flasks containing about 200 c.c. of sterile bouillon. From these flasks subcultures were made, after forty-eight hours at 38° C., into lactose-bouillon fermentation tubes.

B. coli was absent from the contents of all the eggs examined. It was present on the shells of all these eggs.

The above experiment was repeated, egg meat heavily infected with a pure culture of *B. coli* being used for soiling the eggs. The eggs rested on their broad poles, and the infected egg meat, white and yolk mixed, was poured over them, the

excess running off. Twenty-four eggs in all were treated in this manner. Eight of them were cracked before they were soiled; eight were used in their original state; and eight were washed and scrubbed with a brush to remove the membrane covering the outside of the shell. It was thought that the removal of this membrane might favor the infection of the contents. These eggs were kept at room temperature, and one of each series was examined every other day. *B. coli* was never found in these eggs, though it was always found on the shell. Probably the season of the year and the moisture of the air have something to do with the infection of eggs from the outside. In the above experiment, the feces and egg meat smeared upon the shell of the eggs dried very rapidly. These experiments will therefore be repeated with summer eggs.

DO THE EGG CONTENTS HAVE BACTERICIDAL PROPERTIES FOR B. COLI?

The absence of *B. coli* from all these eggs, which offered such favorable conditions for infection by this organism, might lead us to the conclusion that the egg contents possess bactericidal properties towards *B. coli.* It has been shown by Wurtz, Horowitz, Turro, Laschtschenko, Poppe, and Scholl that the egg exerts a marked bactericidal action towards several organisms, *e. g., B. anthracis, B. subtilis, Proteus Zenkeri,* etc. The process of disintegration to which the bacteria are subject closely resembles that observed in Pfeiffer's phenomenon. Most investigators attribute this bacteriolysis to the action of the proteolytic enzymes which are present in the egg. According to Laschtschenko, osmotic influences play no role in the process. To determine whether the egg contents exert a bactericidal action upon *B. coli,* the following experiments were made:

To five cubic centimeters of albumin, taken under aseptic precautions from a fresh egg, were added three drops of a 1:1000 dilution of a 24-hour bouillon culture of *B. coli gallimarum.* The mixture was incubated at 38° C., and comparative counts were made at various intervals. For this purpose agar plates were poured which contained, respectively, one cubic centimeter and one-tenth cubic centimeter of a solution of one drop of the infected albumin in ten cubic centimeters

physiological salt solution. The following results were obtained:

Dilutions.	Immediately.	5 hrs.	24 hrs.	48 hrs.	72 hrs.
1. c.c.	60	10,000	Innumerable.	Innum.	Innum.
1/10 c.c.	....	1,200	6,000	50,000	Innum.

These results confirm the observation of Laschtschenko, that concentrated egg-albumin does not exert any bactericidal action upon *B. coli.* Indeed, the presence of large numbers of colon bacilli in frozen and desiccated eggs would be impossible if the egg possessed bactericidal properties for this organism,

It seems, therefore, that the only explanation we can give for the absence of *B. coli* from fresh eggs and from the oviduct is the lymphoid structure of the mucosa of the oviduct. This probably causes the removal, by leucocytic activity, of colon bacilli which have reached the oviduct, together with other intestinal organisms. It is interesting, in this connection, to note that Schattenfroh and Däubler have demonstrated that leucocytes possess bactericidal properties toward *B. coli.*

SOURCE OF *B. COLI* IN EGG PREPARATIONS.

The absence of *B. coli* from the contents of all the eggs, and from the shell of about seventy-seven per cent of the clean eggs examined, leads us to the conclusion that fecal matter is the source of the large numbers of colon bacilli often present in egg preparations.

Indeed this is what we should expect, considering the manner in which the albumin is separated from the yolk by some firms. Shrunk eggs, soiled and checked eggs (with no extravasation of contents), and weak-bodied eggs are largely used in the preparation of the frozen and desiccated products. No eggs are put up for human consumption that are not freely admitted to the market in the shell. After candling, the eggs are generally put into cold storage for some hours. The chilled eggs are broken in the usual housewife fashion, care being taken to select a clean spot in breaking and in pouring out the contents. In some factories the egg contents are poured out into the hand of the worker, who lets the albumin run through between her fingers into a cup and then puts the yolk into another cup. Of course, even with the greatest care, the hand of the worker will become contaminated with traces of fecal material from dirty eggs. In this manner fecal bacteria find their

way into frozen and dried products. Moreover, through the eggs are candled before they are broken, it is impossible to grade so closely as to prevent an occasional bad egg from finding its way into the breaking room. In the process of breaking, such an egg usually betrays itself by its odor, but often not until its contents, which teem with bacteria, have been poured out into the hands of the worker. In this manner bad eggs are a source of contamination for the hands of the workers, and through these for the frozen or dried egg product.

No eggs should be broken in this manner. Most firms use much better methods. They separate the white from the yolk by repeatedly pouring the egg contents from one half of the shell into the other, letting the white run out but retaining the yolk in the shell. This method reduces contamination of the hands of the worker by bad eggs. It is impossible to prevent some of the egg contents, however, from coming into contact with the outside of the shell, where it becomes infected with fecal material if the egg is dirty.

CONCLUSIONS.

How to reduce the bacterial content of frozen and desiccated egg:

1. Fecal contamination is the source of the large number of colon bacilli frequently found in egg products.

2. The bacterial content of canned eggs may be greatly reduced by separating only clean eggs. All soiled eggs which either directly or indirectly might give rise to fecal contamination of the egg meat should be utilized without separating the white from the yolk.

3. The girls who break the eggs should wash their hands whenever they come in contact with the contents of bad eggs or with other contaminating material. The wash water should be used only once; indeed, a pail with water in which the girls frequently wash their hands makes matters worse. The cups into which the eggs are broken, and all other utensils that come in contact with the egg meat, should be washed in clean water, whenever touched by contaminating material. These should not have any crevices, corners, etc., where material may accumulate, but should be as smooth as possible. Glass cups are best. Live steam should be applied liberally to sterilize utensils, etc.

4. The room in which the eggs are broken should be as cool as it can possibly be kept without making the inmates uncomfortable. The cleaner the room and everything in it, the fewer bacteria the product prepared in it will contain. The egg meat should be transferred to the freezing room as soon as possible.

A Discussion of the Healthfulness of Frozen and Desiccated Eggs

And of the Need and Value of Bacteriological Methods of Judging Them.

CLASSES OF FOOD POISONINGS.

We distinguish two great groups of food poisonings which are due to bacteria:

1. Food poisonings caused by nonspecific bacteria (putrefactive bacteria).

2. Food poisonings caused by specific bacteria (*B. enteritidis Gaertner* group, *paratyphoid B.* group, *B botulinus*).

We shall consider the danger of these two forms of poisoning from frozen and desiccated eggs and then discuss the need and value of bacteriological methods for determining them.

IS THERE ANY DANGER, FROM FROZEN AND DESICCATED EGGS, OF POISONINGS CAUSED BY NONSPECIFIC BACTERIA?

The gastro-intestinal disturbances sometimes observed after the consumption of decomposed foods, especially spoiled meat, are generally ascribed to the action of putrefactive alkaloids or ptomaines. These often arise as intermediary products in the breaking down of proteids by putrefactive bacteria. Such poisonings are due, therefore, to harmful substances which are preformed in indifferent media, and not due to an infection of the body with micro-organisms.

Numerous putrefactive bacteria have been found in the contents of good unbroken eggs. Artault, e. g., found (cited from Poppe) :

R. proteus in 60 per cent of the fresh and 100 per cent of the spoiled eggs

B. subtilis in 5 per cent of the fresh and 1 per cent of the spoiled eggs.

M. aureus in 2 per cent of the fresh and oneshalf of 1 per cent of the older spoiled eggs.

B. pyocyaneus in 1 per cent of the spoiled eggs.

B. prodigiosus in 4 per cent of the dirty eggs.

B. violaceus in 2 per cent of the dirty eggs.

Poppe gives the following for the frequency with which different species of bacteria were found in infected eggs:

	Yolk, per cent.	*Albumin, per cent.*
M. albus	26.4	50.0
M. aureus	6.7	14.5
M. luteus (flavus)	6.7	7
M. sulfureus nonliquefaciens	6.7	....
M. flavus tardigradus	6.7	....
M. candicans	6.7	....
B. putidus nonliquefaciens	6.7	7
B. fæcalis alcaligenes	6.7	....
B. proteus	6.7	....
B. mesentericus	....	7
Streptococci	20	14.5

In view of the large percentage of eggs that spoil, putrefactive organisms must necessarily be present in many eggs at some period in their history. Of course, in the earlier stages of decomposition, we must expect to find them in considerable numbers in eggs which do not yet show any physical signs of decomposition. If these putrefactive bacteria decompose egg meat with the formation of ptomaines before any physical signs of decomposition are apparent, they may do so in shell eggs as well as in bulk eggs.

The logical conclusion is: If the absence of physical signs of decomposition does not insure the absence of putrefactive alkaloids from frozen and desiccated eggs, neither does it insure their absence from shell eggs. If, therefore, we subject physically sound egg preparations to bacteriological or chemical tests for putrefactive alkaloids, we also have to do so with the shell eggs, if we do not want to commit a very gross injustice to the dealer in bulk egg meats. The consequences would be appalling. Fortunately such measures are not necessary. The fact that cases of poisonings by eggs are so extremely rare, in fact almost unknown, shows that poisonous putrefactive alkaloids are not formed in the decomposition of egg meat before physical signs of putrefaction are apparent. It seems, therefore, that we do not need bacteriological or chemical methods for judging the healthfulness of eggs with regard to nonspecific bacterial poisonings; our senses are sufficient for this purpose.

It might be objected that the bacterial flora of shell eggs is different from that of frozen and desiccated eggs because dur-

ing breaking the latter are contaminated with fecal bacteria. The bacteria which, according to Gage, are most frequently found in chicken feces are: *B. coli, B. mycoides, B. subtilis, staphylococci* and *sarcinz.* Most of these organisms are referred to earlier in this bulletin, in the list of bacteria isolated from good shell eggs. *B. subtilis* and *B. mycoides* were also isolated from fresh eggs by Pernot. The arguments given previously apply, therefore, to these bacteria also; that is, if the absence of physical signs of decomposition does not, insure the absence from the frozen and desiccated eggs of poisonous decomposition products due to these bacteria, neither does it insure their absence from shell eggs, etc. These arguments do not apply to B. coli however, because, so far as we know, this organism is never present in sweet, sound shell eggs. The occasional poisonous character of spoiled food, especially putrid meat, etc., has often been attributed to *B. coli.* Whatever the role that *B. coli* plays in such cases, the presence of this organism, even in large numbers, in egg preparations certainly cannot be considered as a sign of danger from poisonous bacterial products. The large numbers of colon bacilli found are not due to multiplication in the egg-meat of an originally small number of bacteria, but to fecal contamination during breaking and separating. Since, therefore, these organisms did not grow and multiply in the egg meat they could not possibly have given rise to poisonous decomposition products. This applies also to the large numbers of other bacteria that infect the egg meat during breaking. In other words, the occurrence of large numbers of bacteria, especially colon bacilli, in frozen and desiccated eggs, is not a sign of decomposition, and therefore does not indicate any danger from poisonous decomposition products.

There is hardly any chance for the multiplication of these organisms in egg preparations properly handled. The eggs are chilled before being broken; and, after they have been broken, generally not more than an hour elapses before the product is taken to the freezing room, where it is generally kept at a temperature of 0° F. A few firms use power churns for mixing the egg meat. In this case the churning lasts about two hours, and the churns are, therefore, supplied with a cold brine jacket to keep the temperature of the contents so low as to prevent bacterial development. The best firms also keep down

the temperature of the breaking room by refrigerating it or by providing it with a system of ventilation which supplies filtered chilled air. During desiccation, too, all possible precautions are taken to prevent bacterial development. The can from which the egg meat is automatically applied to the drying belt is insulated to prevent a rise in temperature of the contents, which were chilled before they were put in the can. Of course, the temperature during drying is favorable to bacterial growth. While the temperature of the air used in desiccation is about 140° F., the temperature of the egg itself is much lower on account of the rapid evaporation of water from the egg meat. The desiccation of each layer of egg meat on the belt requires fifteen minutes. At the beginning of this period, the temperature of the egg meat is considerably lower than that of the air used in drying, because evaporation proceeds very rapidly. At the end of this period, however, the temperature probably rises considerably because little or no evaporation takes place. This rise probably never exceeds 110° F., because this is the temperature of the air with which the layer of egg meat comes in contact as it approaches the dry state. We see that not more than fifteen minutes elapse before one layer of egg meat is dry. Since it takes about one-half hour for bacterial cells to undergo fission, it is apparent that practically no multiplication can take place during desiccation of the egg meat. The large number of bacteria in desiccated eggs, as compared with the number of bacteria in the same grade of fresh egg meat, is almost exclusively due to the reduction in volume which the egg undergoes during desiccation. One part of desiccated egg being obtained from three parts of egg meat, it is evident that the desiccated products will contain three times as many bacteria to the gram as the material, from which it was prepared.

In judging egg products, much significance has been attached by some investigators to the effect of injecting egg preparations into animals used for experimental purposes. No conclusions as to the healthfulness of the product under investigation can be obtained in this manner. Death or disease of an animal, upon the injection of egg products, may be due to a variety of causes. Several of the organisms regularly present in the product, notably *B. coli, B. proteus, staphylo–*

cocci, etc., may set up a fatal septicemia. The pathogenicity of the product upon injection may be due to the presence of specific pathogenic organisms such as *B. enteritidis* or *B. paratpphosus B*. It may also be due to the products of albuminous decomposition. Finally, it may be due to the normal constituents of the egg meat itself. Richet found that sterile egg yolk, when kept for some time under strictly aseptic conditions, produces more or less marked disturbances upon interavenous injection into guinea pigs. Richet attributes this toxicity to an autolysis of the egg contents. (Proteolytic enzymes are present in eggs.) According to this author, the injection of fresh yolk is not accompanied by any such toxic effects. Linossier et Lemoine, on the other hand, found that fresh meat, fresh eggs, and fresh milk normally possess toxic properties which may or may not increase upon keeping them under septic or aseptic conditions, In view of this great variety of factors which may bring about the death of an animal injected with egg meat, no information as to the healthfulness of egg products can be expected from such a procedure. Moreover, it is hardly necessary to reiterate the trite fact that the pathogenicity of a substance or an organism upon injection into an animal is absolutely no index to its pathogenicity upon ingestion.

IS THERE ANY DANGER OF SPECIFIC PATHOGENIC BACTERIA FROM EGG PRODUCTS?

By far the largest number of food poisonings are due, not to poisonous decomposition products, but to specific bacteria and their intracellular or extracellular toxins. Among these specific bacteria which give rise to food poisonings we distinguish two groups:

1. The pathogenic saprophytes.
2. The pathogenic parasites.

Van Ermengem defines pathogenic saprophytes as those micro-organisms which, though not able to multiply in the living organism, cause disease by the toxins preformed in their protoplasm or in their indifferent substrata. The pathogenic, or better toxicogenic, saprophyte *par excellence* is *B. botulinus.* The optimum temperature for this organism is 20° C.; therefore, it cannot lead a parasitic existence in the body of warm-blooded animals. At blood temperature, outside the body, it grows only very slowly and without the formation of toxin.

This prevents its development during desiccation of the egg meat. *B. botulinus,* being a strict anaerobe, will not develop during the churning of the egg meat, as this is accompanied with a thorough aeration of the product. The temperature during churning, as generally carried out, would favor development of *B. botulinus.*

Botulinus toxin that might be present in frozen or desiccated egg would be destroyed during cooking because it is inactived by temperatures as low as 60° to 70° C. Moreover, botulism, though quite frequent in Europe, is entirely unknown in America. The danger of intoxications of this sort from eggs is therefore negligible.

The pathogenic parasites that are most likely to occur in frozen and desiccated eggs and that might possibly endanger the health of the consumer are: colon and paracolon bacilli, bacteria of the *paratyphoid E* group, and bacteria of the *B. enteritidis Gaertner* group.

As regards *E. coli communis,* its pathogenicity upon injection is, of course, no indication that it will produce disease upon ingestion. Since the egg products in question are used for food, we shall limit our attention to investigations dealing with the disease-producing power of *B. coli* when ingested. It seems absurd, at first, to attribute pathogenic action to the ingestion of an organism which is a normal inhabitant of the intestines of man and animal. However, this seems less absurd when we consider that there are strains of *B. coli* which are much more virulent than others. This is what Kruse refers to when he says in Flügge's *Die Mikroorganismem:* "Emmerich and Korkunoff, in their feeding experiments with cultures of *B. coli,* did not succeed in bringing about an infection from the intestinal canal. Neither could Kartulis produce an infection by injection into the rectum. However, it is desirable that these experiments be repeated with highly virulent cultures and with a wide variation in the conditions of multiplication." One of these requirements proposed by Kruse, the employment of highly virulent cultures, seems to have been met by Jensen, who succeeded in killing calves within a few days by introducing, *per os* or per *rectum,* cultures of *B. coli* which he had isolated from calves suffering from severe diarrhea. Coppola is another investigator who produced disease by the introduction of *B. coli* into the alimentary tract. He succeeded in kill-

ing twenty per cent of the guinea pigs experimented upon. The source of his cultures and the other experimental conditions are not known to me since I do not have access to Coppola's original paper.

In connection with the question whether *B. coli* is able to set up infections from the intestinal tract, the following passage by Kruse is of interest:

"We cannot deny the possibility that the colon bacillus may become dangerous to the intestinal mucosa, just as it is sometimes found in other affections. According to a widespread opinion, this is supposed to be due to a rise in the virulence of the normal intestinal inhabitant, brought about in one manner or another. In other cases one has reason to accept the introduction of especially virulent bacteria with the food. This seems to have been the case in a small epidemic reported by Gaffky. Here the milk of a cow suffering from enteritis seems to have been contaminated with its excreta, thus serving as a carrier for the virus."

In the following words Escherich and Pfaundler suggest the probability of exogenous *coli* infections: "Jedenfalls liegt es naher, überall da, wo hochvirulente oder sonst in ihren Eigenschaften veränderte Colibazillen im Beginn einer Erkrankung gefunden und als Erreger derselben angeschuldigt werden, zunächst an die Möglichkeit eines ektogenen Coliinfektes zu denken, da ausserhalb des menschlichen Korpers die besonderen, zu einer Virulenzsteigerung führenden Bedingungen viel eher gegeben sein dürften."

The problem of the pathogenicity of *E. coli* is confused by the fact that *B. coli* does not represent a uniform type, but numerous varieties of a species, which shows considerable difference in its morphological, cultural and biological characters. Also, many organisms that have no relation to *B. coli communis* have been classified as such because they were isolated from the intestines and because they had certain properties in common with *B. coli communis.* The same is true of *B. paracoli,* which is often used as a collective name for many different species, intermediary between *B. coli* and *E. typhosus.* While the colon bacilli isolated in various diseases very frequently show deviations from the normal *B. coli communis,* these deviations are too irregular and manifold to allow us to speak of a pathogenic *B. coli* in the same manner in which we speak of a

normal typical *B. coli.* We therefore have no way of distinguishing the harmful from the harmless colon or paracolon bacilli; neither cultural nor biological characters nor the virulence in animal inoculations gives us any reliable information.

The chances that dirty eggs are contaminated with highly virulent bacilli of the colon group, if these be present in the excreta of any inhabitant of the farm, are pretty large. The poultry generally are the scavengers of the farm, and any infectious material is likely to be carried in their excreta, or on their feet, to the places where the eggs are deposited. From this place the infectious material may find its way to the shells of the eggs, especially if these become soiled, and ultimately to the frozen and desiccated product. In the egg meat itself, if kept at favorable temperatures, colon bacilli might experience an increase in virulence. The egg contains about 13 per cent of its nitrogen as nitrogen uncoagulable by heat; and, according to Lenti, the presence of easily soluble nitrogenous compounds, such as peptones and albumoses, in the culture-medium exerts a favorable influence upon the virulence of *B. coli.* The symbiosis with the *streptococci* and *staphylococci* present in egg preparations might also increase the virulence of *B. coli.*

We have to remember, however, that these factors for the increase in virulence of *B. coli* cannot enter into action because the products are kept under conditions that prevent bacterial development. Practically the only time when they would enter into play is the short period required to dissolve the desiccated product previous to its use. They can easily be controlled during this time by keeping the mixture at a low temperature. This can readily be done because the temperature has little effect on the rapidity with which the egg goes into solution.

On the other hand, the egg products are kept under conditions which probably greatly decrease the virulence of the colon bacilli contained in them. In the frozen product the low temperature (generally around zero) might certainly be expected to exert a deleterious influence upon the virulence of an organism which has its natural habitat in the intestines. The lack of moisture in desiccated eggs (only about 5 per cent water being present) might also be expected to decrease the virulence of the bacteria present.

In spite of all this, let us assume that an egg preparation contains highly virulent colon bacilli in numbers sufficient to

cause disease upon ingestion. These bacteria will invariably be killed during baking or cooking, for which purposes these eggs are exclusively used. Escherich and Pfaundler give the following table for the resistance of *B. coli* to different temperatures; the cultures were found dead after exposure:

To 62°-63° C. for 1 minute (v. Geuns).
To 59° C. for 5 minutes (v. Geuns).
To 60° C. for 15 minutes (Kitasato).
To 60° C. for 10 minutes (Weisser).
To 60°-61° C. for 5 minutes (Chantemesse and Widal).
To 59° C. for 15-30 minutes (Chantemesse and Widal).
To 55°-60° C. for 120 minutes (Fränkel).

This table shows clearly that *B. coli* cannot possibly survive the temperature to which the product is subjected during cooking or baking—about 100 degrees C.

It can be safely said, therefore, that the colon bacilli contained in egg preparations do not endanger the health of the consumer.

The colon bacilli present in egg preparations have their source largely in contamination with fecal matter. With this fecal matter, pathogenic bacteria may find their way into eggs. The bacteria which we have to consider here belong mainly to the B. *enteritidis Gaertner* group and to the *paratyphoid B* group. The very wide distribution in nature which these bacteria were recently found to possess, and their high resistance, make their occurrence in frozen and desiccated eggs very probable. In fact, we should expect their numbers to be proportional to the amount of fecal contamination, and, therefore, to the number of colon bacilli present. It might be thought, therefore, that the number of colon bacilli present could be used as an index to the danger from *paratyphoid* or *B. enteritidis Gaertner* infections through frozen and desiccated eggs. Unfortunately, however, such a simple method is not at our disposal, as the following considerations will show.

Poppe has shown that fowls which were fed large numbers of paratyphoid bacilli excreted these organisms for a few days in their feces. The eggs obtained during this period contained paratyphoid bacilli on the shells, but not in the contents. In the excreta of four healthy, chickens this author also found bacteria which were closely related to *B. paratyphoid B.* ***e.g.,*** the nonagglutinating form of *B. paratyphosus B* (*B. para-*

typhi C Uhlenhuth) and an organism which was identical with *B. paratyphosus B* in all its cultural features except that it did not change the color of litmus-whey to a dark ultramarine blue; it was agglutinated by *paratyphoid B* serum in dilutions of 1:100. In contrast with these results, Gage, in a very extensive study of the bacterial flora of the domestic fowl, does not report the presence of paratyphoid bacilli and reports only one case where *B. enteritidis* was found.

Paratyphoid and Gaertner bacilli, when present in the excreta of some person or animal on the farm, may be carried directly or indirectly in the feces, or on the feet of the chickens, to the place where the eggs are deposited. From this place they may find their way to the frozen and dried product through soiled and contaminated eggs. The high resistance of these bacteria to drying, etc., insures their longevity on the egg shell and thereby favors their infecting the egg meat while the shell is being broken. Multiplication of these bacteria might take place in desiccated eggs during the time required for dissolving this product previous to its use. During cooking or baking these organisms would probably be killed, but they contain a powerful endotoxin which is not destroyed even by temperatures as high as 100 degrees C.

Poppe has shown that *paratyphoid B* bacilli, when present on the shell of eggs soiled with the excreta of chickens, invade the egg contents within ten days and proliferate there. The danger of paratyphoid infections through eggs is therefore as great from dirty shell eggs as from the frozen or desiccated product prepared from such eggs. If, therefore, we subject egg preparations to bacteriological examination with a view towards obtaining information as to the danger of paratyphoid infections, we must do the same with shell eggs, otherwise we commit a very gross injustice towards the industry of freezing and desiccating eggs. The extreme scarcity of paratyphoid infections from eggs shows that there is no such danger from this source. The same reasoning applies also to Gaertner bacilli. These bacteria can be differentiated from *paratyphoid B* bacilli only by serological methods; it is therefore highly probable that they penetrate the egg shell in the same manner as the paratyphoid bacilli,

The danger of infections of Gaertner and paratyphoid bacilli through dirty shell eggs seems almost greater than through

frozen or desiccated eggs. Though the number of these bacteria which invade the interior of the egg may be small, the eggs are generally kept under conditions of temperature which are favorable to bacterial growth. The Gaertner and paratyphoid bacilli, if any should be present, might therefore multiply rapidly. In the frozen and desiccated eggs, on the other hand, conditions are unfavorable for bacterial development, and the exposure to prolonged cold or desiccation is very likely to lower the virulence of any pathogenic bacteria that might be present. Also, the egg preparations are used exclusively for cooking and baking. The temperature to which they are subjected during these processes will kill all bacteria and leave only their preformed, heat-resistant endotoxin for action. This is not always the case with shell eggs. In the preparation of soft-boiled eggs, poached eggs, eggnog, etc., the bacteria are not killed. In this case, therefore, we have to fear infection by the living pathogenic bacteria as well as intoxication by their endotoxin.

Even if we should isolate paratyphoid or Gaertner bacilli from egg preparations, this would not allow us to make any conclusions as to the healthfulness of the product. Hübener says with regard to these bacteria: "Unfortunately we are utterly unable, with our present methods, to distinguish between the pathogenic and nonpathogenic micro-organisms of the same group."

Komma in a bacteriological study of 102 samples of good, normal sausages found paratyphoid bacilli in thirty cases and B. *coli* in thirty-five cases. In twenty-two cases these two organisms were present simultaneously. Like other investigators (Ostertag, Uhlenhuth) Komma comes to the conclusion that the presence of paratyphoid bacilli does not entitle us to pronounce a food unfit for consumption, as long as we do not know a method for distinguishing the pathogenic members of this group from the nonpathogenic members. This author, however, thinks that the results of such bacteriological tests serve as an index for the amount of contamination to which the constituents of the sausage were exposed. Numerous bacteria of the partyphoid group, he thinks, always indicate some fault in the manufacture of the sausage.

As regards egg products, it seems tempting to utilize the total and relative numbers of colon bacilli present as an index

for the sanitary precautions taken in the manufacture of the product. A large number of colon bacilli indicates a large amount of fecal contamination and, therefore, we might think, careless, insanitary methods of preparing and handling the product. This matter of judging, by means of bacteriological methods, the sanitary conditions under which egg products were prepared is, however, very difficult and complex. The qualitative and quantitative bacterial content of egg preparations is dependent largely upon climatic conditions. Every rainy day greatly increases the number of dirty eggs arriving at the packing houses. These eggs are most economically used in the manufacture of the frozen and desiccated products because their decreased keeping qualities and the low price they bring do not warrant their shipment to the great centers of consumption. We see, therefore, that the contamination of egg products is subject to great seasonal and local variations. It is at once evident that egg preparations produced during a rainy season, under the most painstaking and sanitary precautions, may show a higher bacterial content than eggs produced in a dry season under careless methods that do not endeavor to avoid contamination. Also, for this reason it would be unfair to apply the same standard to eggs produced in territories differing greatly in their rainfall.

The following argument also will show the inapplicability of such a method for judging the hygienic conditions under which the frozen or desiccated eggs were produced. There are two processes in use for desiccating eggs. In the one, the temperature of the egg probably never exceeds 110° F., while in the other, the egg is cooked and coagulated during desiccation. Therefore, the product prepared. by the first process contains all the bacteria present before desiccation, while the second product contains only the highly resistant spores, the vegetative forms being killed during desiccation. Bacteriological analysis of this latter product will therefore give us information neither as to the grade of eggs used, nor as to the hygienic conditions under which it was produced.

A simple numerical standard, such as we have for milk, cannot be applied to eggs. It is unnecessary, because frozen, and desiccated eggs do not play any role in infant mortality or in the spread of epidemics, and because egg products are always subjected, before consumption, to temperatures which

kill the bacteria. If a numerical standard were established, the manufacturers who use the first process described, could easily reduce the number of bacteria present, thereby depriving this standard of any meaning or value. This could be done in the following manner: After the product is desiccated in the usual manner, it is heated for some time to a temperature of from 60° to 70 ° C. This heating does not materially affect the quality of the product, because this contains only about five per cent water.

To show the effect of such a procedure, the following experiments were made. Some desiccated egg of different grades, ready for the market, was collected in stertile containers and mixed thoroughly. Care was taken to use a small-grained product, so as to obtain representative samples. The bottoms of several sterile petri dishes were covered with a thin layer

Grade of egg.	Time of exposure.	No. of bact. to the gr. after heating to 65° C.	No. of bact. to the gr. after heating to 70° C.
W Y Y Whole egg plus ⅓ of its volume of yolk.	Before exposure,	7,000,000	9,500,000
	One-half hour...	11,000,000	8,200,000
	One hour.....	6,000,000	4,500,000
	Two hours.....	900,000	450,000
Y Yolk.	Before exposure,	16,000,000	13,000,000
	One-half hour...	14,000,000	15,000,000
	One hour.....	7,800,000	1,100,000
	Two hours.....	1,200,000	600,000
X X X Tanners' eggs, made from questionable eggs, blood rings, etc., ready for market.	Before exposure,	110,000,000	85,000,000
	One-half hour...	95,000,000	58,000,000
	One hour.....	61,000,000	27,000,000
	Two hours.....	8,000,000	2,600,000
Tanners' eggs, before desiccation is complete (after removal from belt).	Before exposure,	.....	125,000,000
	One-half hour...	.....	10,000,000
	One hour.....	.....	63,000,000
	Two hours.....	.....	4,500,000
The above product ready for the market.	Before exposure,	.....	108,000,000
	One-half hour...	.....	92,000,000
	One hour.....	.....	40,000,000
	Two hours.	.....	1,500,000

of egg. The petri dishes were then exposed for various periods of time to 65°, 70°, and 75° C., in a water-jacketed oven. Bacterial analyses were made before and after heating. For this purpose one gram of the product was dissolved in ten cubic centimeters of sterile physiological salt solution by thoroughly shaking the mixture with broken glass in a high, strong-walled test tube. From this solution, agar plates were poured in different dilutions and incubated for two days at 38° C.

The results obtained are shown in the table, p. 357.

From these results it may be seen that heating to 65° C. to 70° C. greatly reduces the number of bacteria in the product. Samples were also heated to 75°C. It was found, however, that exposure to this temperature decreases the solubility of the product very rapidly. Immediately after heating, the egg has a slightly cooked flavor, which, however, disappears rapidly upon storage. The keeping qualities of the product seem to be exactly the same before and after heating. When the product was kept in an ice chest, no deterioration of the flavor and odor could be observed. At room temperature it changed slowly, whether the egg was heated or not. No sweating out of the fat occurs during heating up to 70° C. One sample of egg was heated before desiccation had progressed to the final stage. This sample, after being heated, was rather insoluble and had a cooked flavor, probably on account of the high water content. It will be seen that in two cases the bacterial counts after heating for one-half hour to 65° to 75° C. gave higher numbers than before heating. This, of course, is due, not to multiplication of the bacteria during heating, but to experimental error. (With a product like desiccated eggs it is very hard to obtain correct bacterial counts.) It is interesting to note that, after keeping desiccated eggs at 70°C. this product contains about the same number of bacteria as first-grade desiccated eggs. In the manufacture of tanners' eggs, the sound portion of spot-eggs, eggs showing blood rings, and slightly off-flavored eggs are used. After desiccation, this product can hardly be distinguished, by its physical appearance, from first-grade eggs. When one sees and tastes this product, it seems a needless waste that it should be excluded from human consumption. Perhaps future researches will show us that a good many eggs which are now prepared for tanners are perfectly healthful and fit for human consumption. This would avoid an enormous

waste and would furnish a nutrient and cheap egg product for the poor in our large cities. Probably this product would be cheaper and better than the grade of shell eggs which they can afford to buy.

All eggs that are really unfit for food, however, should be denaturized, to prevent unscrupulous people from selling them for food purposes. It has been found in a few large eastern cities that eggs which were supposedly sold to tanners were bought by unscrupulous dealers, deodorized by the addition of a small amount of formaldehyde, and sold to restaurants, etc., with great profits. After such eggs are sold to the tanners, it is very hard to keep track of them, and they may in a roundabout way make their appearance in cheap restaurants, etc. Denaturization of such preparations is the only sure way to prevent their use as food products. It would easily be possible to find a substance which, when added to tanners' eggs, would serve as a telltale without exerting a deleterious effect upon the leather.

CONCLUSIONS.

1. In the absence of physical signs of decomposition, there is no danger of poisonings or bacterial infections through egg products,

2. Bacteriological methods for judging the healthfulness of egg preparations are unnecessary and inapplicable.

3. The sanitary conditions under which frozen and desiccated eggs are produced cannot be judged by our present, bacteriological methods, because the bacterial content of egg preparations is subject to great seasonal and local variations.

4. Factory inspection should enforce sanitary methods of production and should exclude from the frozen and desiccated products all eggs that are not admitted to the market in the shell.

5. All tanners' eggs should be denaturized to prevent their use as food products.

6. The bacterial content of desiccated eggs can, by keeping them for from one to two hours at 65° to 70° C., be greatly reduced without decreasing their solubility very much. Practical tests will have to decide whether the advantages derived from the lower bacterial content would counterbalance the disadvantages arising from the somewhat lower solubility.

Bacteriological Studies of Newly Laid Eggs.

INTRODUCTION.

The enormous losses connected with the egg industry are to a great extent due to development of the embryo and to decomposition of the egg by micro-organisms. The large losses due to development of the embryo can easily be prevented by keeping the male birds from the females. We have to educate the farmer, first, to do his hatching as early as possible; then, to keep the males from the females. The difficulties which arise, when we try to reduce the loss of eggs due to decomposition, are numerous and complex. We are somewhat ignorant as to whether the destructive infection of eggs takes place during the formation of the egg, or after the egg is laid. Of course, we know that newly laid eggs may contain bacteria, and we also know that certain bacteria may penetrate the pores of the shell and thus invade the egg. But the vital question is: Which of these two methods of infection is most important economically? It is not until we have answered this question that we know where to improve conditions with the best returns. The following pages embody the results of part of a series of experiments on this problem which are being carried on at the Kansas Experiment Station. Though the results given here are only preliminary and of little direct practical importance, it was considered advisable to publish them because the methods used and the results obtained may be of help to investigators of this subject and may stimulate further research.

OBJECT OF EXPERIMENTS.

The object of the experiments described here was to obtain an insight into the infection of eggs during their formation; to determine the amount of such infection; to determine the influence of the age of the fowl upon the bacterial content of the eggs; and to find the relations, if there are any, between the bacterial content of eggs and their hatching quality. In this manner it was thought data would be obtained which might serve as a basis for comparing the bacterial content of fresh eggs with that of market eggs of various ages and under

various methods of handling, Such a comparison will show us the relative importance of the infection before and after the eggs are laid, and it will indicate where improvement is needed most and will result in the greatest benefit.

GENERAL CONSIDERATIONS.

In investigating the influence of various conditions of feeding, health, etc., upon the bacterial infection of eggs, the following considerations should be taken into account:

It is a well known fact that early spring eggs keep much better than summer eggs. This is probably due to the lower bacterial content, of spring eggs. Lamson from Storr's Experiment Station has shown that a much larger percentage of summer eggs are infected than of spring eggs. Therefore, in comparing the influence of two conditions of feeding, *e. g.*, it is not sufficient to compare simply the bacterial content of eggs obtained from the same hens, which are successively kept under these different conditions. The change in bacterial content might be due, not to the change in conditions under investigation, but to seasonal variation. We, therefore, have to work simultaneously with eggs from two different pens. The first pen is kept under one, and the second pen under the other condition. In this manner we avoid the error due to seasonal variation, because the seasonal variation is about the same in both pens. We introduce, however, a new error which is due to individual variation in the bacterial content of eggs from different hens. This error can be reduced by using a large number of hens to obtain a more nearly correct average. In addition to this, the two pens should be kept under absolutely identical conditions for a few weeks previous to the actual experiment. If an analysis of the eggs shows approximately the same amount of infection in both pens, the experiment proper may begin.

PLAN OF EXPERIMENTS.

With these considerations in mind, our experiments were arranged as follows:

Two pens were stocked with thirty-five birds each, of different age. The pens were supplied with trap nests, which enabled us to keep individual egg records, and to investigate the cause of abnormalities, should any be found, Alternate eggs from each hen were subjected to bacteriological analysis and to incubation. The incubation work was part of the

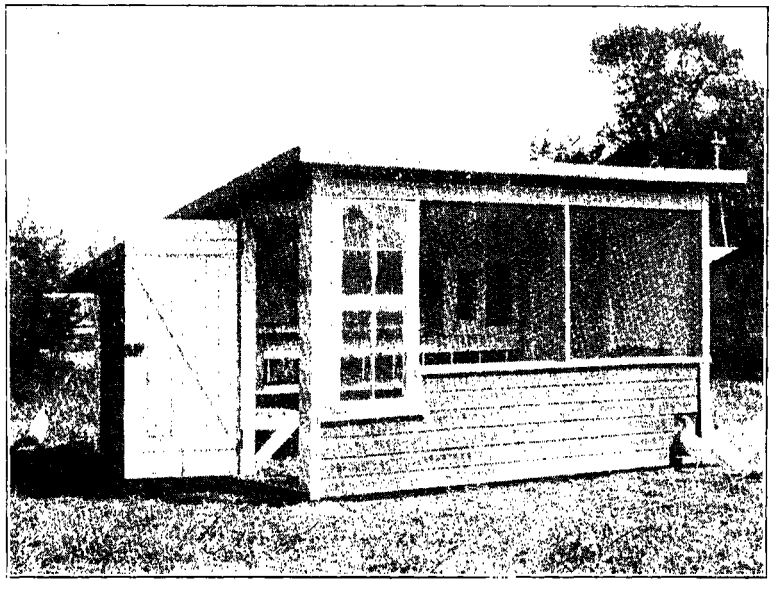
regular work of the poultry department and was under the direction of Mr. F. S. Jacoby, in charge of the Division of Poultry Husbandry. In this manner we attempted to obtain data on the relation between the bacterial content and the hatching qualities of eggs. The hens were kept for two weeks under absolutely identical conditions and supplied with the normal, well-balanced ration used at the College poultry plant. The birds had also been kept under identical conditions previous to their use for these experiments. During the two weeks preceding the actual experiment, a bacteriological analysis showed that about the same percentage of eggs was infected in both pens. The birds in one pen were then fed a highly carbonaceous ration, while those in the other pen were continued under the same conditions as before.

The bacteriological and incubation experiments were for one month continued as before. Our idea for investigating the influence of a highly carbonaceous ration upon the infection of eggs during their formation was as follows:

Many farmers feed their hens almost exclusively on corn. If the hens do not have sufficient range and access to insect food, as is often the case, corn furnishes them almost the only means of subsistence. As a consequence, the birds become excessively fat. The deposition of fat is especially marked in the viscera. The passage of the egg down the fatty oviduct causes much irritation. This irritation may cause antiperistalsis of the oviduct, thereby introducing traces of feces and numerous micro-organisms into the oviduct, where they may infect the egg.

DESCRIPTION OF FOWLS.

The birds in each pen consisted of nine S. C. White Leghorn hens, aged three years; nine Leghorn hens, aged two years; seventeen Leghorn pullets, aged one year. Each pen was mated with two S. C. White Leghorn cockerels, which were alternately admitted to the pens, every bird being with the hens one day at a time. This was done to insure fertility of the eggs. All birds in these pens were of strong, pure-bred stock, and had good records as egg producers. Death of a few birds and the obstinate refusal of some of them to lay in the trap nests explains the discrepancy between the number of birds stated heretofore and the number of individual egg records stated later.

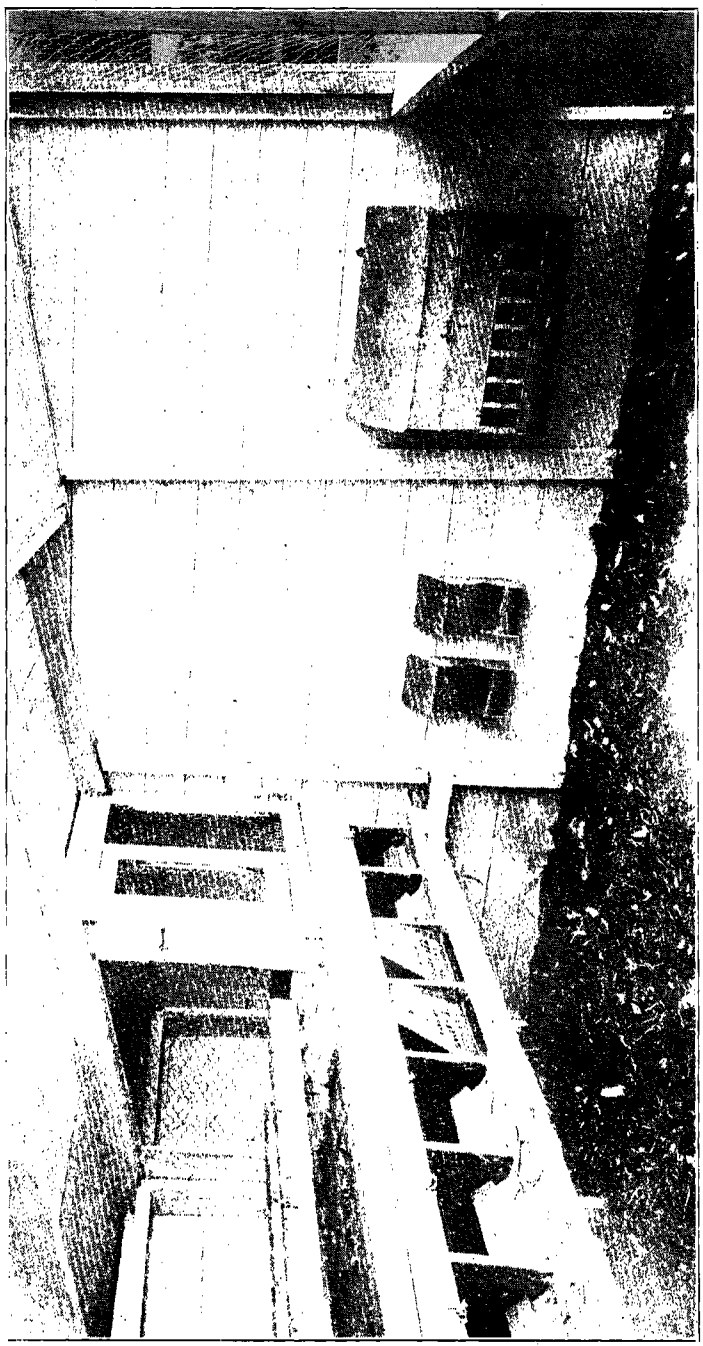


DESCRIPTION OF PENS.

The pens were of the shed-roof type with an open front facing south; the floors were ten by twelve feet, and the pens were eight feet high in front and five feet in the rear. For protection in cold weather, the pens were supplied with curtain fronts six feet four inches long and four feet wide. The window in front was six feet high. The floor was always covered with a thick litter of straw in which the birds had to scratch for their grain. The perches were constructed movable, with a dropboard which was easily cleaned. The pens were disinfected and whitewashed before they were stocked with birds. The size of the yards surrounding the pens was 100 feet by 150 feet. These yards were kept in grass throughout the year.

FEEDING.

Each pen was equipped with one grain can, one dry mash hopper, one grit hopper, and one hopper for oyster shell. The whole grain was scattered on the floor in the morning and in the evening, and the birds had free access to the dry mash during the entire day. The grain mixture fed from the be-



Interior arrangement of house shown on page 363.

ginning of the experiment until May 4 had the following composition (Henry's *Feeds and Feeding*, Madison, Wis., 1901):

	Prot.	*C. H.*	*Fat.*
10 pounds corn chop	.790	6.670	.430
10 pounds wheat	1.020	6.920	.170
5 pounds oats	.460	2.365	.210
Totals	2.270	15.955	.810

Nutritive ratio $\frac{2.270}{15.955 + .810 \times 2.25} = 1 : 7.83$

The ground feed fed from the dry mash hoppers was composed of:

	Prot.	*C. H.*	*Fat.*
4 pounds bran	.488	1.568	.108
6 pounds shorts	.732	3.000	.228
4 pounds corn meal	.316	2.668	.172
1 pound alfalfa meal	.177	.388	.107
1 pound oil meal	.293	.327	.078
4 pounds meat scrap	2.648	.012	.548
Totals	4.654	7.963	1.241

Nutritive ratio $\frac{4.654}{7.963 + 1.241 \times 2.25} = 1 : 2.3$

The desired nutritive ratio was 1:5. To obtain this, the fowls were fed twice as much whole grain as ground feed. The following table gives the complete analysis and nutritive ratio of the feed consumed. It was, however, not always possible to induce the fowls to eat enough dry mash to make the nutritive exactly 1 : 5.04.

	Prot.	*C. H.*	*F*
20 pounds corn chop	1.580	13.340	.0
10 pounds oats	.920	4.730	.20
20 pounds wheat	2.040	13.840	.340
4 pounds bran	.488	1.568	.108
6 pounds shorts	.732	3.000	.228
4 pounds corn meal	.316	2.668	.172
1 pound alfalfa	.177	.388	.107
1 pound oil meal	.293	.3?	.078
4 pounds meat scrap	2.648	.[illegible]	.548
Totals	9.194	3[illegible]	2.861

Nutritive ratio $\frac{9.194}{39.873 + 2.861 \times 2.25} = 1 : 5.0[illegible]$

The fowls were also fed green food [illegible]h as green alfalfa, rye, and forage obtained from the [illegible]. The hens were never shut up in the house the entire day during the winter months,

and therefore could take exercise in the yard the whole year round. Grit and oyster shell were fed from the hoppers, so the birds could help themselves according to their own desire. Fresh water was given them twice a day, except during the winter months, when it was supplied four times a day. Once in a while warm water was given on very cold days. On May 4, pen 21 was changed to the following ration:

Grain mixture.	*Prot.*	*C. H.*	*Fat.*
10 pounds corn chop	.790	6.670	.430
2 pounds wheat	.204	1.384	.034
5 pounds Kafir	3.900	2.855	.135
5 pounds rice	.240	3.610	.015
Totals	1.624	14.519	.614

Nutritive ratio 1: 9.8

Dry mash.	*Prot.*	*C. H.*	*Fat.*
15 pounds corn meal	1.185	10.005	.645
5 pounds shorts	.610	2.500	.190
3 pounds oats (rolled)	.426	1.990	.198
2 pounds alfalfa meal	.354	.775	.034
Totals	2.575	15.271	1.067

Nutritive ratio 1: 6.8

This ration was fed in the same manner as indicated above (twice as much whole grain as dry mash.) The composition of the total amount of food consumed is given below:

	Prot.	*C. H.*	*Fat.*
20 pounds corn chop	1.580	13.340	.860
4 pounds wheat (No. 2)	.408	2.768	.068
10 pounds Kafir	.780	5.710	.270
10 pounds rice	.480	7.220	.030
15 pounds corn meal	1.185	10.005	.645
5 pounds shorts	.610	2.500	.190
3 pounds oats (rolled)	.426	1.990	.198
2 pounds alfalfa meal	.354	.776	.034
Totals	5.751	44.309	2.295

Nutritive ratio 1: 8.9

After a few weeks of this feeding, the hens diminished very rapidly in laying, the ration being too wide. We therefore changed the ground feed to a mixture of twelve pounds of corn meal, five pounds of shorts, three pounds of oats, two pounds

of alfalfa meal, and two pounds of meat scrap. The total mixture of whole grain and dry mash was therefore:

	Prot.	*C. H.*	*Fat.*
20 pounds corn chop	1.580	13.340	.860
4 pounds wheat	.408	2.768	.068
10 pounds Kafir	.780	5.710	.270
10 pounds rice	.480	7.220	.030
12 pounds corn meal	.927	8.000	.516
5 pounds shorts	.610	2.500	.190
3 pounds oats (rolled)	.426	1.990	.198
2 pounds alfalfa meal	.354	.776	.034
2 pounds meat scrap	1.324	.....	.274
Totals	6.889	42.304	2.440

Nutritive ratio 1: 6.94

Except for this difference in feeding, the two pens were treated exactly alike.

INCUBATION.

The trap-nested eggs were distributed according to the hen's number, and a record was kept of the eggs from each hen, giving the day when the eggs were laid. The eggs from the two pens were kept in a dark, cool place, where they were turned daily until a sufficient number had accumulated to fill an incubator; the time elapsing between the date of laying and the date of incubation was never more than ten days. The eggs laid between April 24 and May 3 were hatched in a Queen incubator; all the other eggs were put in a Prairie State incubator No. 2. The Prairie State machines were run at 102° F. for the first week, thereafter the temperature being changed to 103° F. All eggs were turned in the morning and evening after the third morning. After the fifth day the eggs were cooled down to blood temperature. In the bottom of the machine a sand tray with lukewarm water was placed to supply moisture. The eggs were tested on the seventh and fourteenth days, and a strict record was kept of infertile, dead-germ, and broken eggs. The eggs that did not hatch were recorded as either pipped or as dead in shell.

The eggs in the Queen incubator were cared for in exactly the same manner as the eggs in the Prairie State machine, except that no sand tray was used in the Queen, because this is a hot-water machine.

BACTERIOLOGICAL TECHNIQUE.

We first tried to determine the percentage of eggs infected as well as the number of bacteria present. For this purpose the method described by Pennington, which consists essentially in the plating of dilute samples of egg, was used. With this method, however, it was absolutely impossible to obtain reliable results for newly laid eggs. This method may be perfectly satisfactory for market eggs, which generally contain numerous bacteria, In the case of our fresh eggs, however, about 90 per cent of which did not show any growth at blood temperature, the secondary infection of the plates, which we found impossible to prevent in our laboratories, gives rise to serious errors. After numerous preliminary experiments we came to the conclusion that we must give up the determination of the numbers of bacteria present, and that we must be satisfied with a determination of the number of eggs infected. For this purpose the following method was finally devised:

The egg is cleaned with brush and soap and immersed for ten minutes in a 1:500 solution of corrosive sublimate. It is transferred with sterile crucible forceps to a small conical graduate, acute pole uppermost. The corrosive sublimate is removed and the egg dried by washing it first with alcohol and then with ether. The acute pole is scorched to kill spores, etc., that might remain. The egg is then immediately removed from the graduate by the operator's holding it near the blunt pole, turning the acute pole down. With sharp, stout forceps which have been sterilized in the flame, a hole about one-half c.m. in diameter is made into the acute pole below. Holding the egg with the acute pole down, and making the stab from below, prevents contamination from above. The shell around the hole is flamed briskly and the egg is put with the acute pole upon the neck of a tall 300 cubic centimeter Erlenmeyer flask containing 100 c.c. of sterile bouillon. The blunt end of the egg is now heated with a bunsen flame while a close watch is kept on the hole. The heating expands the air in the air space and this expels the contents of the egg. As soon as about half of the albumin has run into the flask, the heating is interrupted. The cotton plug is quickly removed from a second sterile flask, the neck of the flask is flamed, and the egg is transferred from the first to the second flask. Sometimes it is necessary to invert

the egg, as soon as the heating is discontinued, to prevent all the albumin from running into the first flask. In this case it often happens that a little of the egg contents runs down the outside of the shell where it may become contaminated. To prevent such material from getting into the next flask, it is cemented to the shell by being heated with the flame. The expulsion of the albumin into the second flask should be done slowly and watched closely. As soon as the yolk appears in the hole, the heating is interrupted and the egg is tilted from one side to the other, to allow the rest of the albumin to run out. In the same manner the yolk is expelled in two portions. The success of this method depends largely upon the size of the hole. If this is too small, it is hard to separate the white from the yolk; if it is too large, it is difficult to expel the yolk in two separate portions. Sometimes the yolk obstructs the hole before all the albumin is obtained. If the yolk does not retract after cooling, the egg is inverted for a moment. Often the vitelline membrane will not rupture, and the yolk will come out in one piece. This can be prevented by puncturing the membrane with a sterile platinum needle. In this manner four flasks are obtained from each egg, two of them containing albumin and two of them containing yolk. The flasks are repeatedly shaken to mix the contents well. It is of advantage to have tall flasks because the contents can be mixed easier without wetting the cotton plug.

Two flasks, one with albumin and one with yolk, are incubated at 58° C. for forty-eight hours, and the other two flasks are incubated at room temperature for five days, After this period of development, subcultures are made to determine if growth has taken place. In our case agar plates were poured in two dilutions from the contents of the flasks. The plates were examined under the microscope because there often are numerous minute colonies which cannot be detected by the naked eye.

It might be objected that the heating of the egg, in expelling the contents, may kill some of the bacteria present in the latter. This, however, is not the case. The temperature of that part of the egg contents which is expelled, is never sufficiently high to cause the death of the bacteria present. That part of the albumin which is next to the shell membrane is coagulated at places where heat is applied. The coagulated albumin is firmly cemented to the shell membrane, the two forming one strong,

thick membrane which effectively checks conduction of heat to the interior of the egg.

In applying the method described above to the bacteriological analysis of such eggs as show a considerable percentage of infection, the addition of a little methylene blue to the bouillon saves much work. It is sufficient to add a trace of the pigment, just enough to color the bouillon green. After the incubation period has elapsed, the flasks are looked over before they are shaken. In many flasks growth is indicated by the reduction of the methylene blue. This is not, however, an absolute indicator of bacterial development. From all those flasks which do not show any reduction, subcultures have to be made to ascertain the absence of growth.

In this manner about six hundred eggs from seventy-four birds were examined. During the time which elapsed between the, removal of the eggs from the nests and the bacteriological examination (generally about twenty-four hours), the eggs were kept in a cool, dry place.

RESULTS.

The results are given in the tables on following pages. Explanation of tables: Each of the tables contains the results for one pen during one week. Four vertical columns are given under each day. Every egg used in the experiment is represented in the first vertical column, either by an O or by an X. O denotes that the egg was subjected to bacteriological analysis; X, that it was incubated. The second vertical column under each date contains the results of incubation and of the search for bacteria growing at 38° C. Y denotes infection in the yolk, W in the white. The results of incubation (for hatching) are expressed in the letters J, G, D, P; J means infertile egg, G dead germ, D dead chicken in shell, P pipped shell (low vitality). Eggs which we marked infertile need not necessarily have been infertile; development of the embryo may have stopped at so early a stage as not to have been recognized upon candling, so that the egg was considered infertile. Y or W in the third or fourth column indicates that the yolk or the white of the egg contained baceria growing at room temperature. Eggs which do not show any letters in the last three columns were free from bacteria or hatched satisfactorily.

April.	20	21	22	23	24	25	26	No. of eggs incubated	No. of eggs hatched	No. of eggs analyzed	No. showing growth at 38° C.	No. showing growth at room temp.	No. of eggs containing bacteria
PEN 20.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.						
7754	O	X P	O		X	O							
7758		O	X L	O	X		O						
7760	O		X D			O							
7762	X	O		X D	O	X D							
7763	X J			O	X	O							
7765		X	O		X J	O							
7766	X		O	X P		O	X						
7767			X G	O W		X		16	8	17	1	0	1
6936		X	O	X J		O	X G						
6937		X		O	X		O						
6941	O	X J	O		X J	O							
6943		X G		O	X D		O						
6944	O			X D	O	X							
6948	X J		O	X J		O	X D						
6949	O Y Y	X P	O		X J	O	X J	17	4	16	1	1	1
7782				O Y		X D							
7785		X J	O	X J									
7787	O		X	O		X G	O						
7788			O			X	O						
7789													
7791	X	O		X D									
7792		O		X		O	X						
7793					O		X P						
7794	X			O	X		O						
7795		X	O		X	O	X D						
7796		X	O Y Y		X D		O W						
7904	X	O	X	O Y	X J		O						
7907	X	O	X	O	X		O W						
7909	O			X G	O		X						
7910					O	X D		27	16	26	2	4	5
							Totals.....	60	28	59	4	5	7

April.	27	28	29	30	May 1	2	3	No. of eggs incubated	No. of eggs hatched	No. of eggs analyzed	No. showing growth at 38° C.	No. showing growth at room temp.	No. of eggs containing bacteria
PEN 20.	Bacteria at room temp	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.						
7754	X	0	X P		0	X D	0						
7758		X	0			X	0						
7760	X			0		X	0						
7762	0	X		0	X		0 L						
7763	0	X G			0 Y	X	0 Y						
7765	X	0		X	0 Y		X						
7766		0	X J										
7767		0 Y	X D		0			16	11	17	1	3	4
6936				0		X J							
6937		X	0		X	0 Y	X J						
6941	X D	0	X G		0	X L							
6943	X J		0		X D		0						
6944	0		X D		0		X						
6948	0 Y		X D	0		X G	0						
6949		0	X D		0 W Y		X G	14	3	14	1	3	3
7782													
7785				0		X J							
7787		X	0 Y			X D	0						
7788			X		0	X							
7789													
7791						0	X D						
7792	0	X	0 Y W	X G									
7793			0										
7794	X D	0				X							
7795		0	X	0		X	0 Y W						
7796	X D		0			X							
7904	X D	0	X	0			X						
7907	X		0 Y	X	0		X P						
7909	0												
7910	0 L	0						20	12	19	1	3	4
							Totals.....	50	26	50	3	9	11

May.	4	5	6	7	8	9	10	No. of eggs incubated	No. of eggs hatched	No. of eggs analyzed	No. showing growth at 38° C.	No. showing growth at room temp.	No. of eggs containing bacteria
PEN 20.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.						
7754		X	O		X	O Y							
7758		X G	O	X		O Y	X G						
7760		X J		O		X G							
7762	X J	O W		X D	O Y		X D						
7763		X G	O	X G		O Y	X G						
7765	O		X		O	X							
7766	O W	X D			O		X J						
7767								17	5	13	3	3	6
6936													
6937		O	X P		O								
6941													
6943	X G		O			X G							
6944		O	X G		O	X J							
6948		X			O		X L						
6949		O Y	X		O	X P		8	2	8	0	1	1
7782													
7785													
7787		X	O Y		X								
7788			O		X		O						
7789							O						
7791		O											
7792	O L			O L	O		X G						
7793	X J												
7794	O Y		X		O Y	X							
7795	X		O Y		X D	O Y	X						
7796													
7904	O	X J		O	X J		O Y						
7907	O		X L		O		X						
7909	X D			O			X G						
7910		X		O			X G	17	9	17	1	5	6
							Totals.....	42	16	38	4	9	13

May.	11	12	13	14	15	16	17	No. of eggs containing bacteria.....
PEN 20.	Bacteria at room temp.	Bacteria at room temp	Bacteria at room temp	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	
7754	X		O			X J		
7758		O			X	O	X J	
7760	O		X P		O Y		X J	
7762	O		X P	O Y Y		X G	O Y	
7763	O	X J		O	X	O	X G	
7765	O			X	O		X J	
7766	O L	X G	O	X D		O Y	X G	
7767								4
6936								
6937								
6941			O		X D	O		
6943	O		X D		O Y	X L		
6944	O	X J		O Y	X J			
6948		O	X P				O	
6949	O	X G		O	X	O		2
7782								
7785			O W					
7787	O Y	X J		O	X		O	
7788				X		O		
7789			X D					
7791			X P		O		X J	
7792	O W	X		O	X			
7793								
7794	O Y		X		O	X G		
7795		O	X	O		X J	O	
7796								
7904	X					O Y	X J	
7907	O Y Y		X	O			X G	
7909	O		X L	X		O W		
7910	O	X D	O	X D	O	X L		7
						Totals.....		18

	No. of eggs incubated.....	No. of eggs hatched.....	No. of eggs analyzed.....	No. showing growth at 38° C.....	No. showing growth at room temp.....	No. of eggs containing bacteria.....
7754						
7758						
7760						
7762						
7763						
7765						
7766						
7767	16	4	15	1	4	4
6936						
6937						
6941						
6943						
6944						
6948						
6949	7	1	11	0	2	2
7782						
7785						
7787						
7788						
7789						
7791						
7792						
7793						
7794						
7795						
7796						
7904						
7907						
7909						
7910	19	9	21	2	6	7
Totals.....	42	14	47	3	12	13

May.	18	19	20	21	22	23	24	No. of eggs incubated	No. of eggs hatched	No. of eggs analyzed	No. showing growth at 38° C.	No. showing growth at room temp.	No. of eggs containing bacteria
PEN 20.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.						
7754	0 Y	X			0								
7758		0 Y	X		0								
7760	0	-	X P										
7762		X	0	X P		0	X G						
7763	0 Y		X J	0	X								
7765			0 Y	X		0	X						
7766		0	X G		0	X J							
7767	X D							13	6	13	1	3	4
6936													
6937				X D	0								
6941													
6943	X G	0	X D		0	X G							
6944			0	X G		0							
6948		X G	0										
6949	X J		0 Y			X		8	1	7	0	1	1
7782													
7785													
7787	X			0	X P	0							
7788		X			0								
7789				0									
7791			0 Y										
7792	0	X D	0		X								
7793		0			X								
7794			0		X	0							
7795	X		0			X D							
7796													
7904		0 Y	X			0							
7907		0	X				0						
7909	X		0										
7910	0		X					13	10	17	1	1	2
							Totals.....	34	17	37	2	5	7

May.	25				26				27				28				29				30				31				No. of eggs incubated	No. of eggs hatched	No. of eggs analyzed	No. showing growth at 38° C.	No. showing growth at room temp.	No. of eggs containing bacteria
PEN 20.	Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria a roo t mp.									
7754													X								O				X	D								
7758	x	J											O				x	J							O		Y							
7760									O			W					x	P							O									
7762	O								x	D			O								x				O									
7763													O				x	J							O									
7765					O			W					x	G			O								x									
7766	O		Y		x	J															O				x	D								
7767																													12	3	14	0	4	4
6936																																		
6937	x				O		Y						x				O	Y			x													
6941																																		
6943	O				x	D							O				x	G			O													
6944																					x	J												
6948	x	J							O								x	J							O									
6949	O			W	x	J							O	Y							x	J							10	3	9	2	2	4
7782																																		
7785																																		
7787																																		
7788					x	J															O													
7789													x												O	L								
7791																	x	J																
7792	O	Y															x	D							O									
7793																																		
7794	x								O		Y										x													
7795																																		
7796									O																									
7904	x	D			O								x	J			O				x	J			O		Y							
7907					x	G							O	Y			x	D																
7909	x	D							O								x																	
7910																													13	4	10	2	2	4
																													36	10	33	4	8	12

Totals.....

June.	1	2	3	4	No. of eggs incubated	No. of eggs hatched	No. of eggs analyzed	No. showing growth at 38° C.	No. showing growth at room temp.	No. of eggs containing bacteria
PEN 20.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.						
7754										
7758	X		0 Y							
7760			X G	0						
7762		X P	0 Y	X						
7763	X J	0	X J							
7765	0 Y		X P							
7766		0	X							
7767	0		X	0	9	4	8	1	2	3
6936										
6937	0	X		0						
6941										
6943	X G	0		X D						
6944	0	X		0						
6948				X						
6949	0 Y	X D		0	6	3	7	0	1	1
7782										
7785										
7787				X						
7788	X J	0		X						
7789		X D	0							
7791	0	X		0						
7792	X D	0		X L						
7793	0		X							
7794		0								
7795	0		X D	0						
7796										
7904		X	0	X						
7907	0		X							
7909		0								
7910	0	X			12	8	13	0	0	0
				Totals.....	27	15	28	1	3	4

April.	20	21	22	23	24	25	26	No. of eggs incubated	No. of eggs hatched	No. of eggs analyzed	No. showing growth at room temp.	No. showing growth at 38° C.	No. of eggs containing bacteria
PEN 21.	Bacteria at room temp.	Bacteria at room temp	Bacteria at room temp.	Bacteria at room temp	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.						
7756		X J	O				X						
7757	O Y		X		O		X						
7759	X		O	X D	O		X D						
7761													
7764						X	O						
7768	O		X P	O									
7769		O	X	O	X D								
7799	O			X D	O	X J		18	6	12	1	0	1
6938			X	O		X	O Y						
6939			X										
6945	X				O Y	X							
6946	O Y			X	O	X D							
6947			O		X J		O						
6950			X		O								
7751	O		O	X G	O		X P						
7752			X	O		X	O Y W						
7753	O		X J			O	X	15	10	15	1	4	4
7771	X D	O	X		O								
7772		O		X	O	X							
7773			O		X	X							
7774			O		X								
7775	X	O	X D										
7776		O	X		O	X							
7778				O	X	O							
7779		O	X	O		X	O L						
7780					O	X							
7781		O	X	O		X	O						
7783	O Y			X		O							
7784			O		X J		O Y						
7902													
7905	O	X		O	X		O						
7906			O		X	C	X D						
7908	X G	O	X G	O	X	O	X						
72	X		O		X D								
73	X					O		30	23	31	1	1	2
								Totals.....	58	39	58	3	5	7

April.	27				28				29				30				May 1				2				3				No. of eggs incubated.	No. of eggs hatched.	No. of eggs analyzed.	No. showing growth at 38° C.	No. showing growth at room temp.	No. of eggs containing bacteria.
PEN 21.	Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.									
7756	0								X	D			0				X								0									
7757					0												X				0				X									
7759	0								X	D			0				X	D							0									
7761																																		
7764					X				0	W			X								0				X									
7768																					X	D			0									
7769	0				X				0								X				0		Y		X									
7799	0																												13	9	15	1	1	2
6938					X				0			W					X	P			0													
6939																	0	Y			X	D												
6945	0				X				0												X													
6946	0	Y	Y		X	G			0	Y							X				0				X									
6947					X	J			0												X													
6950																																		
7751	0				X				0				X	P			0				X	D												
7752	X	D															0								X	D								
7753					0				X	L							0				X	D			0				16	7	16	3	2	4
7771					X																0													
7772	0		Y						X	J											0	W	Y											
7773	0				X	D			0												X													
7774													0				X																	
7775					0								X	D			0								X									
7776	0				X								0				X	D							0									
7778	X				0				X												0			W										
7779	X	G			0																													
7780					0																													
7781	X	J							0				X								0				X									
7783	X	D			0				X				0								X				0									
7784													X				0	Y			X	D												
7902																									X									
7905	X	D							0				X				0		Y						X	D								
7906	0												X				0								X	D								
7908	0		Y						X	J											0													
72	0								X				0																					
73	X	D																																
																													29	16	29	2	5	6
Totals.....																													58	32	60	6	8	12

May.	4	5	6	7	8	9	10	No. of eggs incubated.	No. of eggs hatched.	No. of eggs analyzed.	No. showing growth at 38° C.	No. showing growth at room temp.	No. of eggs containing bacteria.
PEN 21.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.						
7756	x G		O		x J	O Y							
7757	O	x		O		x D							
7759	x D		O	x D		O Y	x P						
7761						O Y							
7764		O Y	x J		O	x D							
7768	x L		O	x G	O	x D							
7769		O Y	x J		O	x L							
7799								12	1	13	2	3	5
6938			O Y										
6939	O W	x D		O			x D						
6945	O	x D		O	x		O						
6946		O	x J	O	x J		O						
6947	O												
6950			x										
7751	O	x D		O	x P	O Y							
7752	O	x J		O	x L		x D						
7753		x D	O Y		x J	O Y Y	x						
7771		x G	O		x P			15	3	17	3	3	5
7772	x J	O		x	O L		O						
7773	O	x		O		x P	O						
7774													
7775	O Y W			x J	O		x						
7776	x		O Y W	x G		O Y W							
7778	x	O					x						
7779													
7780				x		O Y							
7781		O	x		O L	x	O						
7783	x												
7784		O			x	O Y W							
7902													
7905	O Y	x P		O Y	x D		O						
7906	O	x G		O	x		O						
7908		x P	O		x	O	x						
72		x D	O		x D	O							
73	O Y	x G			O		x	27	15	28	2	7	8
							Totals.....	54	19	58	7	13	18

May.	11				12				13				14				15				16				17				No. of eggs incubated	No. of eggs hatched	No. of eggs analyzed	No. showing growth at 38° C.	No. showing growth at room temp.	No. of eggs containing bacteria
PEN 21.	Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.									
7756	X				O								X	G			O				X	J												
7757	O				X	D							O								X	J												
7759					O				X				O								X	G			O									
7761																																		
7764	O												X	G											O			W						
7768	O				X	D															O		Y		X	D								
7769	X	D			O				X	J			O			W					X	J												
7799																													13	2	13	0	3	3
6938	X	D			O								X	P			O							O										
6939	O												X								O			W										
6945	X	P																			O	Y	Y											
6946	X	J							O			W	X	D											O		Y							
6947	X	J											O	Y											X	J								
6950					O			W					X				O				X				O	Y								
7751	X				O		Y		X								O				X													
7752	O								X	J															O									
7753					O								X	D							O		Y	W	X	J			16	6	17	3	7	9
7771	O												X				O								X	G								
7772	X	P							O												X	J												
7773					X																O													
7774																																		
7775	O		Y		X	P							O				X								O		Y							
7776	X	L							X																									
7778					O								X								O		Y											
7779																	O								X	D								
7780					X	P			O								X	G			O		Y											
7781					X				O												X	J			O			W						
7783																									O		Y							
7784					X								O	Y			X								O		Y							
7902																																		
7905					X				O								X																	
7906	X								O				X								O													
7908					O				X				O								X	D			O	Y								
72	X				O								X												O		Y							
73	O												X				O	L																
																													25	16	26	2	8	10
Totals.....																													54	24	56	5	18	22

May.	18	19	20	21	22	23	24	No. of eggs incubated.....	No. of eggs hatched.....	No. of eggs analyzed.....	No. showing growth at 38° C.....	No. showing growth at room temp.....	No. of eggs containing bacteria.....
PEN 21.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.						
7756													
7757	0												
7759		X G	0										
7761													
7764	X		0		X								
7768			0										
7769	0 Y			X J									
7799								4	2	5	1	0	1
6938													
6939													
6945													
6946	X J		0		X G								
6947		0 Y	X		0	X							
6950			X	0		X							
7751	0	X		0									
7752													
7753		0	X		0	X							
								9	7	7	0	1	1
7771	0 W												
7772		0		X									
7773	X		0										
7774													
7775	X G												
7776	0	X		0									
7778			X G		0								
7779	0 W		X D										
7780													
7781		X											
7783		X	0 Y	X		0							
7784		X	0										
7902													
7905													
7906													
7908	X J												
72													
73								11	7	10	1	2	3
								24	16	22	2	3	5

Totals.....

May.	25				26				27				28				29				30				31				No. of eggs incubated	No. of eggs hatched	No. of eggs analyzed	No. showing growth at 38° C.	No. showing growth at room temp.	No. of eggs containing bacteria
PEN 21.	Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.				Bacteria at room temp.									
7756																																
7757																					X											
7759																																
7761																																
7764																																
7768																																
7769																																
7799																													1	1	0	0	0	0
6938																									X	J								
6939																	X	P																
6945					X																													
6946																	0								X	D								
6947																																		
6950																									0									
7751																									X	G								
7752													X	J			0								X	J								
7753	0	Y	Y	W					X	D							0	L											8	1	4	1	1	1
7771																																		
7772																									0									
7773																																		
7774																																		
7775																																		
7776																																		
7778																																		
7779																																		
7780																																		
7781																	0	Y			X	L												
7783																																		
7784																																		
7902																																		
7905																																		
7906	X	J							0				X				0																	
7908																																		
72																									X	D								
73									X								0												4	2	5	1	0	1
																													13	4	9	2	1	2

Totals .....

June.	1	2	3	4	No. of eggs incubated.	No. of eggs hatched.	No. of eggs analyzed.	No. showing growth at 38° C.	No. showing growth at room temp.	No. of eggs containing bacteria.
PEN 21.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.	Bacteria at room temp.						
7756										
7757										
7759	x J		O							
7761										
7764		O W		x						
7768										
7769				O						
7799					2	1	3	0	1	1
6938				O						
6939										
6945	x		O W							
6946			O							
6947		O								
6950	x D		O	x D						
7751	O	x J	O Y	x D						
7752	O		x D							
7753	x J		O Y Y	x G	8	1	9	2	2	3
7771				x J						
7772		x J	O							
7773										
7774	O L									
7775		O	x D							
7776				x G						
7778				x D						
7779				O						
7780	x									
7781	O		x G							
7783										
7784										
7902										
7905										
7906	x	O Y		x						
7908	O			x D						
72		O	x L							
73	x	O Y		x	12	5	8	0	2	2
				Totals.....	22	7	20	2	5	6

DISCUSSION OF RESULTS.

DISTRIBUTION OF BACTERIA IN EGGS.

Perhaps the most striking feature that impresses one in looking over these tables is the very small number of eggs infected in the white, as compared with the number of eggs infected in the yolk. Out of the total number of eggs that contained bacteria, only about 25.9 per cent contained them in the white. The reason for this may be that the infection of the eggs takes place mostly in the ovary and not in the oviduct. It might be thought that if infection took place in the oviduct, the albumin of the eggs would show more infection than the yolk. This argument is not free from objections, however. We have to take into account the bactericidal properties of the albumin. Infection might take place in the oviduct and some bacteria might rapidly penetrate into the yolk before being killed by the bactericidal substances in the albumin. Once in the yolk, they are comparatively safe, for the yolk possesses only very slight bactericidal properties. Therefore, while the bacteria in the white may be killed, those in the yolk may survive. This might give rise to such results as were obtained in our experiments.

It is interesting to compare the following data, containing the percentage of infected eggs showing bacteria in white:

Pen 20, from April 20 to May 3, 33 per cent; from May 4 to June 4, 14 per cent.

Pen 21, from April 20 to May 3, 26 per cent; from May 4 to June 4, 32 per cent.

Before feeding the fattening ration, the number of whites infected was somewhat lower in Pen 21 than in Pen 20. After this feeding, the number of whites infected in Pen 21 was more than twice the number of whites infected in Pen 20. These results might suggest an increased infection in the oviduct due to the effects of the fattening ration. However, the excess of infection in the whites from Pen 21 over the infection in those from Pen 20 is due, not to an increase of this infection for Pen 21, but to its decrease for Pen 20. We cannot definitely say, therefore, that the effects of the fattening ration caused an increased infection in the oviduct.

SUMMARY

	Three-year-old hens.						Two-year-old hens.					
	No. incubated.	No. hatched.	No. analyzed.	No. growth at 38° C.	No. growth at room temp.	No. infected.	No. incubated.	No. hatched.	No. analyzed.	No. growth at 38° C.	No. growth at room temp.	No. infected.
Pen 20.												
First week	16	8	17	1	0	1	17	4	16	1	1	1
Second week	16	11	17	1	3	4	14	3	14	1	3	3
Third week	17	5	13	3	3	6	8	2	8	0	1	1
Fourth week	16	4	15	1	4	4	7	1	11	0	2	2
Fifth week	13	6	13	1	3	4	8	1	7	0	1	1
Sixth week	12	3	14	0	4	4	10	3	9	2	2	4
Seventh week	9	4	8	1	2	3	6	3	7	0	1	1
Totals	99	41	97	8	19	26	70	17	72	4	11	13
Per cent	....	41.4	....	8.2	19.6	26.8	....	24.3	....	5.5	15.2	18.0
Pen 21.												
First week	13	6	12	1	0	1	15	10	15	1	4	4
Second week	13	9	15	1	1	2	16	7	16	3	2	4
Third week	12	1	13	2	3	5	15	3	17	3	3	5
Fourth week	13	2	13	0	3	3	16	6	17	3	7	9
Fifth week	4	2	5	1	0	1	9	7	7	0	1	1
Sixth week	1	1	0	0	0	0	8	1	4	1	1	1
Seventh week	2	1	3	0	1	1	8	1	9	2	2	3
Totals	58	22	61	5	8	13	87	35	85	13	20	27
Per cent	....	37.9	....	8.2	13.1	19.7	....	40.2	..	15.3	23.5	31.9

It seems that variations in the infection of eggs in the oviduct may be brought about in two ways: First, by a variation of the factors which bring about a mechanical introduction of bacteria into the oviduct—copulation, antiperistalsis of the oviduct, and perhaps constipation or other intestinal troubles; second, by a variation in the protective agencies of the oviduct against bacterial invasions—phagocytosis, bacteriolysis. In general, lower vitality of the fowl might be expected to weaken these protective agencies, thereby increasing the bacterial content of the oviduct and, therefore, of the eggs.

BACTERIA FROM EGGS GROWING AT DIFFERENT TEMPERATURES

From the summary of our results it may be seen that the number of eggs showing bacteria at room temperature is very much larger than that showing growth at blood temperature. In Pen 20, only 7.2 per cent of the eggs show growth at blood temperature, against 17.5 per cent at room temperature. For Pen 21, these numbers are 9.5 per cent and 18.7 per cent. This difference in the growth obtained at different temperatures is interesting in connection with the hatching qualities of eggs. The bacteria in most of the infected eggs do not grow at blood

OF RESULTS.

Pullets.						Whole pen.								
No. incubated	No. hatched	No. analyzed	No. growth at 38° C.	No. growth at room temp.	No. infected	No. incubated	No. hatched	Per cent hatched	No. analyzed	No. growth at 38° C.	Per cent growth at 38° C.	No. growth at room temp	Per cent growth at room temp.	No. infected	Per cent infected
27	16	26	2	4	5	60	28	46.7	59	4	6.8	5	8.5	7	11.9
20	12	19	1	3	4	50	26	52.0	50	3	6.0	9	18.0	11	22.0
17	9	17	1	5	6	42	16	38.1	38	4	10.5	9	23.7	13	34.2
19	9	21	2	6	7	42	14	33.3	47	3	6.4	12	25.5	13	27.6
13	10	17	1	1	2	34	17	50.0	37	2	5.4	5	13.5	7	18.9
13	4	10	2	2	4	35	10	28.6	33	4	12.1	8	24.2	12	36.4
12	8	13	0	0	0	27	15	55.5	28	1	3.6	3	10.7	4	14.8
121	68	123	9	21	28	290	126	43.4	292	21	7.2	51	17.5	67	22.9
.....	56.2	.....	7.3	17.1	22.8										
30	23	31	1	1	2	58	39	67.2	58	3	5.2	5	8.6	7	12.1
29	16	29	2	5	6	58	32	55.2	60	6	10.0	8	13.3	12	20.0
27	15	28	2	7	8	54	19	35.2	58	7	12.1	13	22.4	18	31.0
25	16	26	2	8	10	54	24	44.4	56	5	8.9	18	32.1	22	39.3
11	7	10	1	2	3	24	16	66.7	22	2	9.1	3	13.6	5	22.7
4	2	5	1	0	1	13	4	30.8	9	2	22.2	1	11.1	2	22.2
12	5	8	0	2	2	22	7	31.8	20	2	10.0	5	25.0	6	30 0
138	84	137	9	25	32	283	141	49.2	283	27	9.5	53	18.7	72	25.4
.....	60.9	.....	6.6	18.2	13.4										

temperature. Therefore, since they cannot develop during the hatching of the eggs, they cannot interfere with the hatching qualities of the eggs. On the other hand, there is a possibility that these bacteria develop during the period intervening between the laying and the hatching of the eggs and, either directly or indirectly, affect the vitality of the delicate embryo.

The relative scarcity of eggs showing bacterial growth at blood temperature may be due to the bactericidal substances present in the egg. These bacteriolysins, being most active at 38 degrees C., might kill the bacteria in those egg cultures which are kept at blood heat, while they might not affect the development of the bacteria in those kept at room temperature, Experiments will be made to determine whether or not this. is actually the case.

RELATION BETWEEN AGE OF FOWLS AND BACTERIAL CONTENT OF EGGS.

From the summary of our data, we cite the following percentage of infected eggs:

Pen 20, three-year-old hens, 27; two-year-old hens, 18; pullets, 23.

Pen 21, three-year-old hens, 20; two-year-old hens, 32; pullets, 23.

Average of both pens, three-year-old hens, 24.6; two-year-old hens, 25.6; pullets, 23.

These data do not show any constant relation between the age of the fowls and the number of eggs infected.

RELATION BETWEEN AGE OF FOWLS AND HATCHING QUALITIES OF EGGS.

The following is a condensation of the data obtained on this subject:

Per cent of eggs hatched.

Pen 20, three-year-old hens, 41.4; two-year-old hens, 24.3; pullets, 56.2.

Pen 21, three-year-old hens, 37.9; two-year-old hens, 40.2; pullets, 60.9.

Average of both pens, three-year-old hens, 40.1; two-year-old hens, 33.0; pullets, 59.0.

These results seem to show that eggs from pullets hatch better than eggs from older hens. This is opposed to the usual point of view of poultrymen and will be further investigated,

INFLUENCE OF FATTENING RATION UPON NUMBER OF EGGS INFECTED.

This can best be studied by comparing the curves for the weekly percentage of eggs infected in the two pens; these curves are printed on a subsequent page. The time in weeks is marked on the abscissa, the percentage on the ordinate. By comparing the two curves which represent the weekly variation in the number of eggs showing growth at 38 degrees C., we find that the curve for Pen 21 (this pen was fed the unbalanced ration) rises considerably above the curve for Pen 20. During the first four weeks these two curves run pretty close together, then they separate, until during the sixth week they are farthest apart. At this period the percentage of eggs showing growth at 38 degrees C. is 22 per cent for Pen 21 and only 12 per cent for Pen 20. Unfortunately the experiment could be continued only four days; during this period Pen 21 showed 10 per cent of eggs giving growth at 38 degrees C. while Pen 20 showed only 3.6 per cent. In comparing the curves which represent the variation in the total number of eggs infected (growth either at room or at blood temperature), we cannot recognize any definite influence of the wide ration upon the number of eggs infected. During the first two weeks the

curves run pretty close together, Pen 20 showing a somewhat larger number of infected eggs than Pen 21. This changes rapidly during the fourth week because of a sudden decrease in the number of infected eggs in Pen 20 and an increase in Pen 21. During the fifth week, the numbers of infected eggs from Pen 21 experience a sudden decrease, the numbers for Pen 20 continue to decrease. During the sixth week, the number of infected eggs is much smaller for Pen 21 than for Pen 20. During the four days following, the number of infected eggs increases suddenly for Pen 21 and decreases for Pen 20, so that their number is higher in Pen 21 than in Pen 20. Perhaps these violent changes in our curves are due to incorrect averages. This shows the necessity of using a larger number of hens and eggs, and, before all, extending such experiments over a long period of time.

Comparing the number of eggs from the two pens before and after feeding the unbalanced ration to Pen 21, we obtain the following results :

Percentage of eggs showing bacteria.

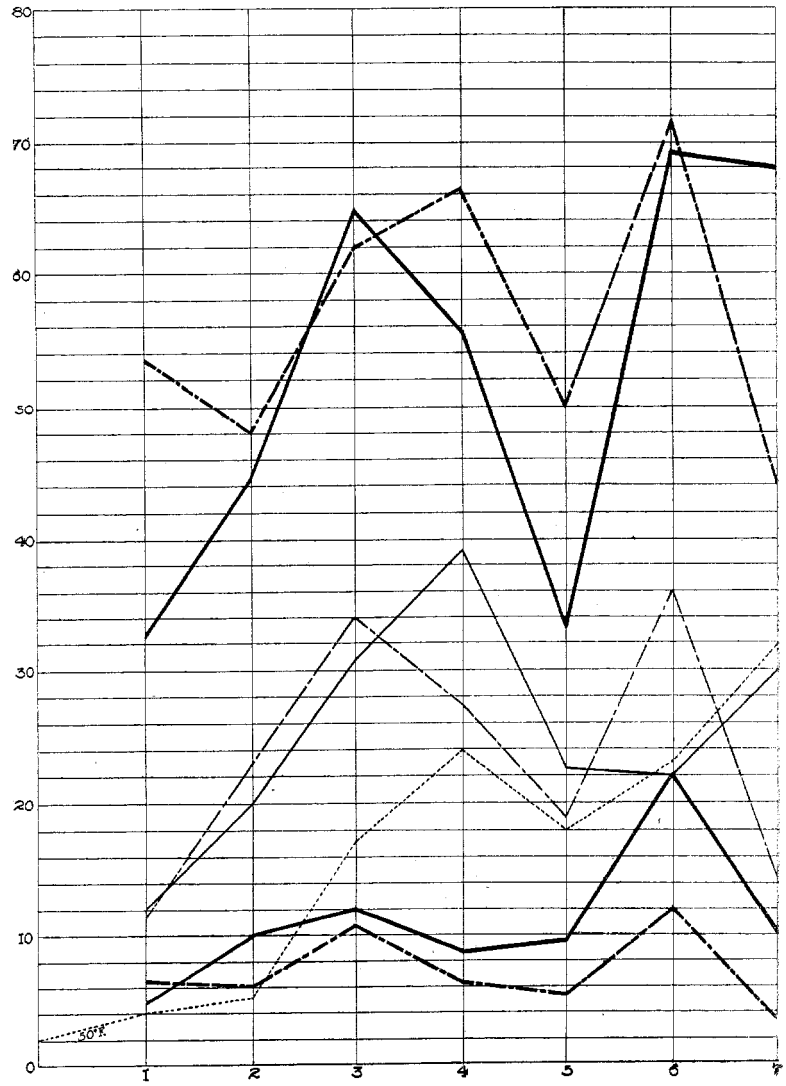
Pen 20, before May 4, 16.5; after May 4, 26.8.

Pen 21, before May 4, 16.1; after May 4, 32.1.

The number of infected eggs for Pe 21 shows an increase of about 6 per cent over that due to seasonal variations, as indicated by Pen 20. When one considers the great fluctuation in the number of infected eggs from Pen 20 (this pen was kept under the same conditions throughout the experiment), it seems doubtful if this excess is due to the effects of the fattening ration.

SEASONAL VARIATION IN THE BACTERIAL CONTENT OF EGGS.

For Pen 20, which was kept under the same conditions throughout the experiment, the number of infected eggs was 16.5 per cent from April 20 to May 3 and 26.8 per cent from May 4 to June 4. This shows a striking increase in the number of eggs infected. The average temperature was 53° F. from April 20 to May 3, and 70° F. from May 3 to June 3. The dotted line in the lower part of our curve chart shows the variation in the mean weekly temperature. Comparing this line with the line representing the variation in infection, we notice, on the whole, a tendency for the number of infected eggs to rise and fall with the rise and fall in temperature.



Heavy solid line. (Pen 21.)
Upper line, per cent of eggs unhatched.
Lower line, per cent of eggs infected.
Heavy broken line. (Pen 20.)
Upper line, per cent of eggs unhatched.
Lower line, per cent of eggs infected.
Light solid line. (Pen 21.)
Per cent of eggs showing bacteria at 38 deg. C.
Light broken line. (Pen 20.)
Per cent of eggs showing bacteria at room temperature.

SEASONAL VARIATION IN THE HATCHING QUALITIES OF EGGS.

Comparing with the mean temperature the curves which represent the weekly loss during hatching, we find, on the whole, a vague tendency for the loss to increase or decrease with the rise or fall in temperature. In other words, there is a slight seasonal variation, eggs obtained during the cool spring months hatching better than eggs produced during the late spring and summer. This is further corroborated by the following data:

Number of eggs hatched.

Pen 20, April 20 to May 3, 49 per cent; May 4 to June 4, 40 per cent.

Pen 21, April 20 to May 3, 61 per cent; May 4 to June 4, 48 per cent.

Pen 20 shows a decrease of 9 per cent in the number of eggs hatched.

INFLUENCE OF FATTENING RATION UPON HATCHING QUALITIES OF EGGS.

The above data and the comparison of our two incubation curves show that the fattening ration did not in the least diminish the hatching qualities of the eggs.

RELATION BETWEEN BACTERIAL CONTENT OF EGGS AND THEIR HATCHING QUALITIES.

A comparative study of the tabulated results of incubation and of bacteriological analysis does not reveal any definite relations between the hatching qualities and the bacterial content of eggs. Neither does a comparison of curves, representing the weekly variation in the hatching qualities and in bacterial content, allow us to draw any conclusions. The striking feature of these curves is their frequent and violent change of direction.

BACTERIAL CONTENT OF EGGS FROM INDIVIDUAL HENS.

The following table gives the number of eggs from each hen analyzed and found to contain bacteria :

PEN 20.

No. of hen.....	No. of eggs analyzed.....	No. infected.....	No. of hen.....	No. of eggs analyzed.....	No. infected.....	No. of hen.....	No. of eggs analyzed.....	No. infected.....	No. of hen.....	No. of eggs analyzed.....	No. infected.....
7754	12	2	6936	3	0	7782	1	1	7794	11	4
7758	14	4	6937	11	3	7785	3	1	7795	13	3
7760	11	2	6941	7	0	7787	11	3	7796	4	1
7762	15	5	6943	13	1	7788	9	0	7904	15	5
7763	15	4	6944	12	1	7789	3	0	7907	13	4
7765	13	4	6948	10	1	7791	7	1	7909	9	1
7766	12	2	6949	15	7	7792	12	3	7910	8	0
7767	5	2	.....	.....	...	7793	4	0	.....	.....	.....

PEN 21.

7756	8	1	6939	5	3	7772	10	2	7783	8	2
7757	9	1	6945	8	3	7773	8	0	7784	8	5
7759	12	1	6946	13	5	7774	2	0	7902	0	0
7761	1	1	6947	8	2	7775	9	3	7905	9	3
7764	9	4	6950	7	3	7776	9	2	7906	12	1
7768	8	1	7751	15	3	7778	8	2	7908	11	2
7769	11	4	7752	9	1	7779	6	1	72	8	1
7799	3	0	7753	13	5	7780	5	2	73	6	2
6938	8	3	7771	7	1	7781	11	2	.....	.....	.....

The above list, in connection with our weekly record sheets, offers interesting data for studying the infection of eggs from individual hens. To facilitate such a study, the most interesting egg records are given in the next table, in which analyzed eggs are represented by the dates when they were laid, heavy type indicating that the egg was infected.

PEN 20.

7758.	4-21	4-23	4-26	4-29	5-3	5-6	**5-9**	5-12	5-16	**5-19**	5-22
	5-28	**5-31**	**6-3**								
7762.	4-21	4-24	4-27	4-30	**5-5**	**5-8**	5-11	**5-14**	**5-17**	5-20	5-23
	5-25	5-28	5-31	**6-3**							
7763.	4-23	4-25	4-27	**5-1**	**5-3**	5-6	**5-9**	5-11	5-14	5-16	**5-18**
	5-21	5-28	5-31	6-2							
7765.	4-22	4-25	4-28	**5-1**	5-4	5-8	5-11	5-15	**5-20**	5-23	**5-26**
	5-29	**6-1**									
7767.	**4-23**	**4-28**	5-1	6-1	6-4						
6937.	4-23	4-26	4-29	**5-2**	5-5	5-8	5-22	**5-26**	**5-29**	6-1	6-4
6949.	**4-20**	4-22	4-25	4-28	**5-1**	**5-5**	5-8	5-11	5-14	5-16	**5-20**
	5-25	**5-28**	**6-1**	6-4							

PEN 20—concluded.

7787.	4-20	4-23	4-26	**4-29**	5-3	**5-6**	**5-11**	5-14	5-17	5-21	5-23
7794.	4-23	4-26	4-28	**5-4**	**5-8**	**5-11**	5-15	5-20	5-23	**5-27**	6-2
7904.	4-21	**4-23**	4-26	4-28	4-30	5-4	5-7	**5-10**	**5-16**	**5-19**	5-23
	5-26	5-29	**5-31**	6-3							
7907.	**4-21**	4-23	**4-26**	**4-29**	5-1	5-4	5-8	**5-11**	5-14	5-19	5-24
	5-28	6-1									

PEN 21.

7764.	4-26	**4-29**	5-2	**5-5**	5-8	5-11	**5-17**	5-20	**6-2**		
7769.	4-21	4-23	4-27	4-29	**5-2**	**5-5**	5-8	5-12	**5-14**	**5-18**	6-4
6938.	4-23	**4-26**	**4-29**	5-2	**5-6**	5-12	5-16	6-4			
6939.	**5-1**	**5-4**	5-7	5-11	**5-16**						
6946.	**4-20**	4-24	**4-27**	**4-29**	5-2	5-5	5-7	5-10	**5-13**	**5-17**	5-20
	5-29	6-8									
6947.	4-22	4-26	4-29	5-4	**5-14**	**5-19**	5-22	6-2			
7751.	4-20	4-22	4-24	4-27	4-29	5-1	**5-4**	5-7	**5-9**	**5-12**	5-15
	5-18	5-21	6-1	**6-3**							
7753.	4-20	4-25	4-28	5-1	5-3	**5-6**	**5-9**	5-12	**5-16**	5-19	5-22
	5-25	**6-3**									
7772.	4-21	**4-24**	**4-27**	**4-2**	5-5	5-10	5-13	5-19	5-31	6-3	
7776.	4-21	4-24	4-27	4-30	5-3	**5-6**	**5-9**	5-18	5-21		

From this table we may see that there is a tendency for the infected eggs to occur in small groups of two, three, or even four. The record for hen No. 6949 shows this most markedly. Of course, there are also numerous cases where this tendency does not appear. But on the whole, it seems that the infected eggs are not uniformly distributed over the whole number of eggs analyzed, but occur in small groups. No endeavor was made to express this tendency in a more concise mathematical form, because only alternate eggs had been subjected to bacteriological examination, and because the number of eggs being laid outside the trap nests was quite large. We are carrying on experiments at present in which these inaccuracies are to a large extent prevented. By examining all the eggs laid and by making careful observations, we endeavor to come to an understanding of the factors that influence the infection of eggs within the hens, and, ultimately, to control them.

CONCLUSIONS.

1. Eighteen and one-tenth per cent of the total number of eggs analyzed showed bacterial growth at room temperature, while only 8.3 per cent showed growth at blood temperature.

2. Of the infected eggs, 82 per cent were infected in the yolk, 25.9 per cent in the white, and only 7.9 per cent in both white and yolk.

3. The bacterial content of eggs undergoes great seasonal changes, generally increasing with the rise in temperature.

4. No definite relation could be traced between the bacterial content of eggs and their hatching qualities.

5. No relation could be found between the age of the fowls and the bacterial content of their eggs.

6. No definite influence of the fattening ration upon the number of eggs infected and upon their hatching qualities could be observed.

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