

OVARIAN INFECTION IN THE DOMESTIC FOWL AND  
DIRECT TRANSMISSION OF DISEASE  
TO THE OFFSPRING.\*

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In an extended discussion of the subject of inheritance and disease Adami<sup>1</sup> concludes that "specific infections are not inherited, but are of post-conceptual acquirement." Von Wassermann<sup>2</sup> holds the same opinion; in fact, this appears to be the generally accepted view. In the scientific interpretation of the term there can be no inheritance of disease. Parental properties are conveyed by the nuclear material of the spermatozoön and ovum; in other words, the inheritance of physiological and other properties is a function of the chromatin of the sperm and the egg. If germinal infection takes place and affects the offspring, the acquirement of the disease is by transmission only. Even this mode of handing transmission is disputed, however.

Instances of prenatal infection are too numerous to permit of doubt as to its occurrence, but it has at all times been difficult to exclude the possibility of intra-uterine infection, either as the result of a diseased condition of the placenta, or the passage of pathogenic organisms by way of the vagina through the amnion into the amniotic fluid. Syphilis is a common type of congenital disease, whereas tuberculosis appears to be transmitted rarely.

Calmette<sup>3</sup> claims that both placental and germinal transmission of tuberculosis are of uncommon occurrence in man. This view is strongly opposed by Baumgarten. In reviewing the literature Kerscher<sup>4</sup> found records of 181 alleged

\* Received for publication, March 23, 1914.

<sup>1</sup> Adami, J. G., in Osler, W., and McCrae, T., *A System of Medicine*, London, 1907, i, 17.

<sup>2</sup> von Wassermann, A., and Keysser, Fr., in Kolle-Wassermann, *Handbuch der pathogenen Mikroorganismen*, 2d edition, Jena, 1912, i, 659.

<sup>3</sup> Calmette, A., *Ann. de l'Inst. Pasteur*, 1910, xxiv, 771.

<sup>4</sup> Kerscher, abstracted in *Jahresber. ü. d. Fortschr. in d. Lehre v. d. path. Mikroorgan.*, 1910, xxvi, 507.

cases of congenital tuberculosis in man. Of this number 55 appeared to him to be well authenticated. The instances of placental infection were estimated at 35. Landouzy<sup>5</sup> believes that the danger of congenital transmission from the mother is greater than from the father. Gaertner<sup>6</sup> cites a number of cases of congenital tuberculosis which are apparently of placental origin. Katholický<sup>7</sup> states that inherited tuberculosis is not rare.

Numerous instances of placental transmission of disease, besides syphilis, tuberculosis, and leprosy, are recorded;<sup>8</sup> for example, anthrax (Paltauf), pneumonia (Levy, Netter, Viti), typhoid fever (Eberth, Ernst, Freund, Levy), pyogenic coccus infection (Auche, Lebedeff, Fraenkel), recurring fever (Spitz), and variola (Champ). Scarlet fever, measles, malaria, articular rheumatism, glanders, and Asiatic cholera are claimed to have been transmitted in this way, also.

As to germinal infection, particularly in man, there has been less conclusive evidence. Baumgarten has for many years held the view that germinal transmission plays an important part in congenital tuberculosis. His views have been accepted by other investigators, but have not been substantiated by scientific observation. The instances of apparent ovarian infection in tuberculous women, and in guinea pigs that were experimentally infected with tubercle bacilli, have been too few and too uncertain to allow positive conclusions to be drawn from them.

Rosinski<sup>9</sup> strongly adheres to the theory of germinal transmission of syphilis, and claims to have demonstrated the certainty of paternal transmission through the spermatozoa. In as far as ovular infection is concerned, the egg may become infected, at the time of its development and great physiological activity, by organisms which may have been latent for some time. Neumann<sup>10</sup> also accepts the idea of ovarian transmission of syphilis to the offspring, and believes it is more common than the placental. The unprotected ovum appears to him to be more liable to infection than the fetus which is enclosed in the placenta. He furthermore demonstrates paternal transmission of disease through the sperm cells.

Sibley<sup>11</sup> reported an incident which has considerable bearing on the question of germinal infection. Eggs for hatching purposes were exchanged between two farmers. One of the farms had apparently always been free from avian tuberculosis. Soon after the introduction of the new stock (exchanged eggs) tuberculosis appeared in acute form.

Maffucci<sup>12</sup> inoculated eighteen eggs with a broth culture of the avian tubercle bacillus. After natural incubation of these eggs eight chicks were obtained.

<sup>5</sup> Landouzy, abstracted in *Jahresber. ü. d. Fortschr. in d. Lehre v. d. path. Mikroorgan.*, 1910, xxvi, 508.

<sup>6</sup> Gaertner, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1893, xiii, 131.

<sup>7</sup> Katholický, abstracted in *Jahresber. ü. d. Fortschr. in d. Lehre v. d. path. Mikroorgan.*, 1903, xix, 476.

<sup>8</sup> Cited by von Wassermann, A., and Keysser, Fr., *loc. cit.*

<sup>9</sup> Rosinski, abstracted in *Jahresber. ü. d. Fortschr. in d. Lehre v. d. path. Mikroorgan.*, 1904, xx, 690.

<sup>10</sup> Neumann, *Wien. klin. Wchnschr.*, 1904, xvii, 551.

<sup>11</sup> Sibley, cited by Gaertner, *loc. cit.*

<sup>12</sup> Maffucci, A., *Centralbl. f. Bakteriol.*, 1889, v, 237.

All but one of the chicks were active, but small and delicate; one died thirty-six hours after hatching, without indications of tubercular infection. The others died after periods varying from twenty days to four and a half months. At least six out of the eight chicks showed marks of tubercular infection, and in several of these the presence of tubercle bacilli was demonstrated. Baumgarten<sup>13</sup> inoculated twelve eggs with tubercle bacilli. Only two chicks were obtained from these eggs, but both gave evidence of tuberculosis.

Gaertner<sup>14</sup> injected tubercle bacilli of human origin into the peritoneal cavity of twelve female canary birds. All succumbed to tuberculosis. The contents of two of the nine eggs laid by these birds produced tuberculosis in guinea pigs, when injected into the peritoneal cavity. The remaining seven eggs gave apparently negative results.

These experiments strongly indicate that germinal infection and transmission can, and undoubtedly do, take place, at least in fowls. They are fully supported by the observations upon which this paper is based.

The investigations of Pasteur and others on pébrine have shown conclusively that infected silkworm eggs are a grave source of danger to the propagation of the silkworm, and that the selection of sound and uninfected eggs is necessary for the success of this industry. Little, if anything, seems to be known as to the manner in which the eggs become infected; whether in the process of formation, during the act of expulsion, or as the result of contact with infected matter after the eggs are laid.

There is also abundant evidence that the organism of Texas or Southern cattle fever, the piroplasma of Smith, is carried in the eggs of the tick (*Boöphilus bovis*). Here again the mode of infection of the eggs has not been determined, but that eggs from ticks which harbor the specific piroplasma are important agents in the transmission of this cattle disease has been satisfactorily shown.<sup>15</sup>

#### EXPERIMENTAL.

The following report is based entirely on a series of extensive observations made by the writer and his associates upon ovarian infection in the domestic fowl. This study was part of a general investigation of the economically important disease known in all parts of this country as bacillary white diarrhea of young chicks.<sup>16</sup>

Bacillary white diarrhea of chicks is a poultry scourge which was practically unknown prior to 1900. In its common manifestations it affects only chicks which are less than five or six weeks old. The greatest mortality occurs during the first two weeks. The symp-

<sup>13</sup> Baumgarten, cited by von Wassermann, A., and Keysser, Fr., *loc. cit.*

<sup>14</sup> Gaertner, *loc. cit.*

<sup>15</sup> Smith, T., and Kilborne, F. L., *Annual Reports of the U. S. Bureau of Animal Industry*, 1891-92, Nos. 8 and 9, 45, 177.

<sup>16</sup> Rettger, L. F., and Stoneburn, F. H., *Bull. Storrs Agricultural Experiment Station*, December, 1909, No. 60, 33; April, 1911, No. 68, 279. Rettger, L. F., Kirkpatrick, W. F., and Stoneburn, F. H., *idem*, December, 1912, No. 74, 153.

toms are those of acute bowel trouble; namely, diarrhea, listlessness, loss of appetite, subnormal temperature, and extreme weakness, especially in the later stages.

One of the earliest known epidemics came to the writer's notice in the summer of 1899. During an investigation of this epidemic an organism was isolated from the liver, heart, and lungs of chicks which died from the disease. Inoculation of normal chicks with this organism resulted in the death of the chicks, the symptoms and post-mortem appearances being the same as those for typical bacillary white diarrhea. In the numerous epidemics which have been studied since that time the same organism has invariably been found. Its definite relation to the disease has been established repeatedly by inoculation and artificial feeding experiments. A brief description of this microorganism, now generally known as *Bacterium pullorum*, follows.

*Morphology, Cultural Characteristics, etc., of Bacterium pullorum.*—The organism is a long, slender bacillus (0.4 to 0.5  $\times$  2 to 4 micra) with slightly rounded ends. It usually occurs single, chains of more than two bacilli being rarely found. It is a non-motile, non-liquefying, non-chromogenic, facultative anaerobe. It is stained readily by the ordinary basic anilin dyes, but does not retain its stain by the Gram method. It does not produce spores.

*Agar Plates.*—Small colorless colonies make their appearance in twenty-four hours at 37° C. They increase in size slowly, and seldom attain more than one millimeter in diameter. They may be round, oval, or spindle-shaped. The surface is marked with one or two rosette figures. The border is comparatively smooth.

*Slant Agar.*—When streaked in the usual manner, a visible growth is obtained in twenty-four hours which spreads little, and remains delicate, even after three or four days of incubation. When, however, the entire surface of the agar is streaked with a platinum loop the characteristic cultural appearance of the pyogenic streptococcus is obtained, the growth being made up of minute discrete colonies which may be so small as to require a magnifying lens for their detection. This cultural characteristic is of extreme importance in identification work.

*Bouillon.*—The growth is like that of *B. typhi*, though as a rule not so luxuriant.

*Gas Production in Sugar Bouillon.*—Some of the strains produce gas in dextrose and mannite broth, whereas others lack this property. No reactions of any importance are obtained in any of the other laboratory sugar media.

*Indol and Nitrite Production.*—Neither indol nor nitrite could be detected in Dunham's peptone solution at the end of one week's incubation.

In its morphological, staining, and in certain of its cultural characteristics *B. pullorum* resembles *B. typhi*, and must, therefore, be classed as a member of the coli-typhi-enteritidis group of bacilli.<sup>17</sup>

Numerous feeding experiments in which the food was artificially infected with *Bacterium pullorum* demonstrated without any doubt that bacillary white diarrhea is highly infectious, and that it is transmitted from chick to chick through infected droppings. This fact furnishes an explanation of the rapid spread of the disease and its frequent occurrence in epidemic form. It does not, however, establish the immediate source of infection in those chicks which are the first to become affected.

#### THE OVARIES OF THE BREEDING STOCK AS THE IMMEDIATE SOURCE OF INFECTION.

One of the peculiar conditions that had frequently been observed in diseased chicks was the failure to absorb the yolk. In fact, this is one of the most striking symptoms of the disease, and makes itself manifest in the protrusion of the abdomen below the vent. Bacteriological examinations of the contents of the yolk sac invariably revealed the presence of *Bacterium pullorum* in the yolk.

It was but natural to assume that the yolk played an important part in bringing about a diseased condition of the chick while still in the egg. This assumption was further supported by the successful search for the bacterium in question in the yolks of infected chicks at the time of hatching, and of chicks at various stages of their development within the egg. Furthermore, when eggs which came from breeds of hens whose progeny had been known to be subject to white diarrhea were examined, *Bacterium pullorum* was frequently found. Not only eggs which were in various stages of incubation harbored the organism in the yolk, but perfectly fresh eggs as well.

It required but one more link in the chain of evidence to trace white diarrhea infection to the laying hen; namely, the demonstration of the presence of the organism in the ovary. This was ac-

<sup>17</sup> For a more detailed account of *B. pullorum*, see Rettger, L. F., and Harvey, S. C., *Jour. Med. Research*, 1908, xviii, 277; 1909, xxi, 115. Rettger, L. F., and Stoneburn, F. H., *Bull. Storrs Agricultural Experiment Station*, December, 1909, No. 60, 33.

complished without difficulty. Small flocks of hens, the eggs and chicks of which had revealed a history of white diarrhea infection, were killed and examined. Out of the first lot of 23 hens, 21 had abnormal ovaries which were found to contain *Bacterium pullorum* unaccompanied by any other organism.

A normal ovary which is undergoing natural development is made up of numerous ova of varying sizes. Some of the ova may be as large as an egg yolk; on the other hand, there are numerous minute ova many of which require magnification in order to be seen. The small ova are round and colorless, and may be likened to small tapioca grains that have been soaked in water. The color of the developing ova varies from a light yellow to a rich normal yolk color.

A typical infected ovary is composed of two distinct types of ova, the normal and the abnormal. The normal resemble those of perfect ovaries. The abnormal may vary as to size, and occasionally as many as twelve or more comparatively large ova, of practically uniform size, may be seen. The small cysts are usually less angular and irregular than the large ones. The color of the large cysts may vary greatly. Some are light, others dark; almost all shades of yellow and brown may be present. Occasionally the color suggests gangrene. The larger abnormal ova are usually quite angular in form, and of a firm consistency. At times they are so compressed as to appear flattened. The contents of the pathological ova are quite characteristic, consisting chiefly of a solid cheese-like matter which is permeated by a clear amber-colored fluid.

An infected ovary can be recognized readily, even by those who have had no special training. Hence, the direct examination of ovaries constitutes one of the best methods of determining the presence of white diarrhea infection in breeding stock. The bacteriological examination of the pathological ova is desirable, however, as a corroborative test.

#### CYCLE OF INFECTION.

Three possible explanations as to the manner in which ovarian infection with *Bacterium pullorum* is brought about naturally present themselves. First, chicks which have been victims of, but which have survived an attack of bacillary white diarrhea, may continue to harbor the organism of the disease for long periods of time, even to maturity, when the infection becomes localized in the ovary; second, fowls may become diseased by the ingestion of food and water which have been contaminated by the droppings of infected chicks, or carriers; and third, infection may be brought about by

association or contact with fowls which harbor the organism in question, as through the agency of body lice or of mites. There is also a possibility that the male may play some part in the transmission of the bacterium, although numerous attempts to demonstrate this have failed, and no evidence can be gathered that *Bacterium pullorum* is ever present in the testes.

It has been definitely shown that mature hens may acquire infection by feeding on food that has been artificially infected with broth cultures of *Bacterium pullorum*, and that the ovaries in particular become involved; but there is much doubt as to the probability that fowls which contain the organism in the ovum as the localized seat of injury eliminate the organism in the droppings, so that their excreta are a menace to other fowls. Although the question as to whether ovarian transmission from adult to adult hens takes place to an appreciable extent needs further elucidation, the carrying of infection to full maturity by chicks that survive the disease has been conclusively demonstrated in our investigations of the past year.

The experiments were conducted as follows.

A large number of chicks which were from twenty-four to forty-eight hours old were employed. They were divided into two lots, one of which was artificially infected with broth cultures of *B. pullorum*, while the other was not given this treatment, but served as a control for the first lot. In each experiment typical white diarrhea made itself felt throughout the artificially infected pens, with a high mortality, whereas the control pens remained unaffected. After the disease had fully run its course, or when the chicks were from seven to eight weeks old, the female chicks were transferred to permanent poultry houses where they were kept under daily observation until they were a year old, when the final examinations were made. The houses and the yards into which they opened had been thoroughly cleaned and disinfected, and had been left unoccupied for at least three or four weeks.

Of the 138 chicks which grew to maturity and lived until the termination of the experiments, 88 were known to have been infected with the organism in question, while the remaining 57 were survivors of the original controls. Of the 88 that were infected as chicks, 21 gave positive agglutination tests when they were about a year old, and showed unmistakable evidence of ovarian infection with *B. pullorum* at the time of autopsy. In other words, almost 25 per cent. of the chicks which were known to have been infected became bacillus carriers, the ovaries being apparently the only organs that were involved.

On the other hand, but one of the control lots showed any indications of ovarian infection, 56 of the 57 controls giving negative results in the agglutination tests and at the final examination. It is possible that the single exception may have suffered accidental infection as a chick or later in life.

Since the female chicks had been separated from the males at an early age (seven to eight weeks), and as no males were kept in the permanent houses, it may be concluded that the ovarian infection was brought about as the result of early infection of the chicks; hence our evidence as to a cycle of infection in bacillary white diarrhea is complete. The original source of infection is the ovary of the mother hen. The infected ovary produces eggs which carry the bacterium of white diarrhea in them; consequently chicks which are developed in these eggs are infected at the time of hatching. The infected chicks become an immediate source of danger to other or normal chicks, and epidemics of white diarrhea take place. Surviving female chicks become permanent bacillus carriers, and when mature they harbor the organism of the disease permanently in the ovary.

Numerous ovaries that have been examined by us have been so badly diseased in the manner described that the production of normal egg yolks had probably long ceased. On the other hand, there is abundant evidence that fowls which possess pathological ova continue laying through an entire laying season or longer. It is probable, too, that the most active layers are the most susceptible to ovarian infection, since the physiological activity of the ovary is such as to lessen its vitality and make it an easy prey to an organism which attacks the ovary more easily than any other organ. On the other hand, an ovary which is comparatively inactive or dormant is less apt to be disturbed.

As a rule, only a comparatively small number of the eggs that are laid by fowls having abnormal ovaries contain the organism of bacillary white diarrhea. It is often necessary to examine as many as eight or ten eggs from a single fowl before any positive evidence may be obtained by this method as to ovarian infection, although the ovaries are decidedly involved. This fact greatly lessens the value of routine egg-testing as a diagnostic measure, especially in as far as selection of individual fowls for breeding is concerned. As an aid in determining ovarian infection in flocks of fowl, however, it is of much importance.

The comparative infrequency with which *Bacterium pullorum* is, as a rule, transmitted to the egg, even when the ovary has been gen-



erally affected by the organism, is but another illustration of the interesting fact that nature does her utmost to protect the offspring in its period of embryonic development.

It is also of particular interest to note that eggs which carry the bacterium in question in their yolks harbor the organism in such small numbers that it is practically impossible to detect it unless the entire yolk is employed in the examination, or unless the eggs have been incubated at body temperature for at least three or four days, or until multiplication of the organism within the yolk has taken place. It is certain that while the yolk is held within the ovary or the oviduct of the fowl there is little or no reproduction of *Bacterium pullorum*; it is only after the time of laying that the apparently inhibitive influence of the yolk on the bacterium is reduced or completely lost.

There is no apparent difference between infected and uninfected eggs, aside from the presence of *Bacterium pullorum* in the former, and this can be detected by cultural methods only. In shape, size, color, odor, taste, and consistency the yolks of eggs that contain the bacterium of bacillary white diarrhea can not be distinguished from those of normal eggs.

The contents of eggs that are produced by normal fowls are, with rare exceptions, sterile at the time of laying and for several weeks after, if the shells remain whole, dry, and clean. This has been conclusively demonstrated by the writer.<sup>18</sup> Furthermore, numerous bacteriological examinations have shown that ovaries which are not infected with *Bacterium pullorum*, and which appear to be normal, are invariably sterile. The oviduct has also been found to be sterile to within a distance of three or four inches of the cloaca. It may be said, therefore, that in the fowl nature ordinarily makes the necessary provisions for the safeguarding of the young against early invasion by microorganisms.

The claim is not made here that the demonstration of ovarian infection with *Bacterium pullorum* in fowls is proof that germinal transmission of disease in mammals, and especially in man, can or does take place; but it makes the possibility of such transmission in man all the more apparent. The mere fact that the ova of fowls

<sup>18</sup> Rettger, L. F., *Centralbl. f. Bakteriol., 2te Abt.*, 1914, xxxix, 611.

and mammals are different as to their structure and development is not sufficient evidence that bacterial invasion of the mammalian ovule is impossible or even improbable. The yolk or food substance in the egg of fowls is not necessary for the successful invasion and establishment of *Bacterium pullorum*, as all the evidence at hand indicates that this organism does not multiply, at least to any noticeable extent, in the ovum or yolk until long after the egg leaves the body, but that it lies dormant during the entire period of ovular development, and even for a time after the egg is completed. These statements apply only to ova that develop normally and are expelled into the oviducts, and not to the pathological cysts that are retained in the ovary.

These observations are in accord with Baumgarten's view regarding germinal transmission as well as with the opinions of other investigators to whom reference has already been made.

#### SUMMARY.

Ovarian infection and germinal transmission of disease have been conclusively demonstrated in our investigations of bacillary white diarrhea in the common domestic fowl. The disease, which has caused so much loss to the poultry industry in recent years, primarily affects young chicks that are but a few weeks old.

Chicks which survive frequently become permanent bacillus carriers, the ovary being the important seat of infection. The eggs from such carriers often harbor the organism of the disease in the yolk. Chicks that develop in infected eggs become in turn infected, and have the disease at the time of hatching. The disease is transmitted to normal chicks through the infected droppings; thus an epidemic is produced, and the cycle of infection is completed.

There is no evidence to indicate that germinal transmission through the male takes place. In view of the frequent negative results bearing on this question it seems probable that it does not.