FURTHER STUDIES ON FATAL SEPTICEMIA IN YOUNG CHICKENS, OR "WHITE DIARRHEA." *

LEO F. RETTGER.

(*From the Sheffield Laboratory of Bacteriology and Hygiene, Yale University.*)

In a recent paper on "Fatal Septicemia in Young Chickens, or White Diarrhea," [1] by Rettger and Harvey, the writers gave a summary of the bacteriological work which had up to that time been done on this subject. As the title indicates, it was assumed that the fatal septicemia and the so-called white diarrhea are identical. Further investigation has strongly supported this assumption.

The term "white diarrhea" is often used without any real significance. The trained poultryman and scientist, however, associate with the term a definite malady whose importance as a poultry scourge is making itself felt throughout this country and Canada. What is known as white diarrhea is of a strictly infectious character, and occurs in epidemic form, like fowl or hog cholera. It is nothing unusual for poultry raisers to lose from fifty to seventy per cent of the chicks under four weeks old which have been exposed to this disease. In fact the malady has been so general and usually proves so destructive that poultrymen claim something must be done to check its ravages or poultry raising will be an unprofitable business.

There is an abundance of evidence that fatal septicemia and simple or uncomplicated white diarrhea are identical. During the past year numerous chicks have been received from various sources. These chicks were all described as having died of white diarrhea. While in a few of them the post-mortem examination revealed some irregularities, the findings were such as to leave very little doubt as to the close resemblance of the organs and tissues to those described in previous papers.

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Little is to be observed in chicks that have died which to the unaided eye appears to be of a decidedly pathological nature. The liver is usually quite pale, with occasional red streaks and patches. The intestines are pale and for the greater part empty. In fact, the chicks often have the appearance of being decidedly anemic. The ceca may be practically empty or be partly filled with a semi-solid or rather firm cheesy matter. The crop is empty or filled with either a slimy liquid or with food. The stomach usually seems normal. The chicks are always very much emaciated. During the past year special attention has been given to the presence of unabsorbed yolk. In every case where the specific organism was obtained from the internal organs of the chick there was found also more or less unabsorbed yolk. In chicks of the same ages which died from other causes, and in the control chicks, the yolks were usually found to be completely absorbed. The yolk sacs of the septicemia chicks varied in size from a small pea to an Italian chestnut.

The disease proves fatal during the first three weeks after the chick emerges from the shell, and more often during the early part of this period than towards the end. Birds are sometimes found dead within a day or two after they are hatched, and bacteriological examination discloses the organism in question. The symptoms may vary to a certain extent in different epidemics. This is probably due to a difference in the virulence of the bacilli or to the varied susceptibilities of the chicks. Two things stand out most prominently, however, in each case, namely, the rapid emaciation of the chicks and the whitish diarrheal discharges from the intestines.

The alleged cases of white diarrhea that are sometimes reported, in which pronounced lesions of one sort or another have been reported and in which the symptoms and general pathological picture are quite different from those described in connection with fatal septicemia, must be regarded as something entirely different or as white diarrhea in a complicated form. I have frequently tried to obtain chicks which had succumbed to this form of epidemic, but have always failed. It is quite possible that in these cases the

disease is caused primarily by the same organism as fatal septicemia, but that a secondary invasion by some other organism, as for example ameba or coccidium, takes place, and that the unusual symptoms and post-mortem appearances are in reality due to this double infection.

Since the publication of the last paper additional data have been obtained. Some of them may be of value to those who are especially interested in the subject, and it is for this reason that they are published at this time.

The malady which for a long time I have termed fatal septicemia, and for which I would suggest the name simple or uncomplicated white diarrhea, or better still, bacillary white diarrhea, has a definite etiology. In my later investigations, as in the former, I have always succeeded in finding the organism heretofore described, in connection with single cases of the disease or in widespread epidemics. Chicks which had all the appearances of having died of the malady, and which were designated as white diarrhea chicks, have been sent to me from various sources, including State agricultural experiment stations. While in some instances it was difficult to find the specific bacterium, a thorough search for it invariably revealed its presence in one or another of the organs or tissues. Normal or apparently unaffected chicks of corresponding ages never were found to harbor the organism.

A complete description of the bacillus may be found in previous papers.[2] It will suffice here to recall that the organism, which after long deliberation I have called *Bacterium pullorum*, has every mark of belonging to the colon-typhoid-dysentery group. That it differs from all the well-known members of this group is beyond all doubt.

The most important points to be observed in the isolation and identification of B. pullorum are its characteristic growths on tubes of slant agar which have been streaked with infected blood or other tissues, and its colonies in plates of one and one-half or two per cent agar. In fact I have found these two cultural tests to be of so much significance that I have constantly made use of them in my later diagnosis work.

The following is the simplest and most satisfactory method of isolating and identifying the organism:

After an incision has been made with a sterile knife a little blood or other material is removed from the organs on a straight wire or platinum loop, and streaked over the surfaces of slant agar tubes. The greater the amount of blood removed in this way the better the chances of obtaining the organism. The tubes are incubated at 35–37° C. In the course of twenty-four hours a careful examination, preferably with a hand magnifying lens, will reveal minute colonies which look like small droplets of fat. The colonies are separate or discrete, and usually remain so, even after several days of incubation. The colonies never grow to be large, although there is considerable increase in size after the first twenty-four hours. The growth on agar that has been streaked with the infected blood, etc., has all the appearances during the first twenty-four to thirty-six hours of the ordinary streptococcus.

After a microscopic examination of the individual bacteria, agar plates are poured from some of the small, well-isolated colonies. The plates are kept for twenty-four to forty-eight hours at 35–37° C. If the colonies are those of B. pullorum they will be small, oval, spindle-shaped or round, with one or more of the characteristic rosette markings on the surface, as previously shown. Frequently the colonies are less compact than I have heretofore described, appearing to have a tendency to break apart or disintegrate to a certain extent. They never get large, like the colonies of the hog cholera or the fowl cholera bacillus. From the colonies the usual subcultures are made, as well as fermentation tests.

The behavior of the organism on agar is quite constant. The same thing is true with reference to its development in gelatin (stab), milk, and potato. The different strains vary, however, in their gas-producing power in dextrose-bouillon. Some of the organisms which I have isolated have failed and still fail to produce any gas in this medium. On the other hand, two particular strains fermented the sugar, and produced five per cent and twenty-five per cent gas, respectively, in this medium. The more active fermenter lost this

property within a period of seven or eight months, so that for a while it was strictly anaërogenic. At the present time the fermenting power is gradually being restored again. Work on this peculiar deportment of the organism in sugar-bouillon is being carried on from the standpoint of variation, and some very interesting results have already been obtained. These will be reserved for a later publication.

All of the strains of organisms studied produce acid in dextrose-bouillon. Lactose is apparently not affected. Mannite is fermented by some strains, but not by others.

The bacillus may be present in all of the internal organs of the chick, but particularly in the liver, spleen, lungs, and heart. The most careful search may reveal only a few scattered bacilli in one or two of the organs, as indicated by the growths on slant agar, while the remaining organs appear sterile. It is often very difficult to find B. pullorum in the tissues of chicks that have undergone much putrefaction, as the bacillus is easily overrun or crowded out by other bacteria, especially the colon bacillus. It is very important, therefore, that the bacteriological examinations be made soon after the death of the chick, or in birds that have been kept cold enough to prevent marked post-mortem decomposition.

Reference has been made to the presence of unabsorbed yoke in chicks which have died of fatal septicemia or white diarrhea. During the past eighteen months I have given particular attention to this phase of the subject. That the birds which succumb to the disease invariably have some unabsorbed yolk is in itself of much importance, and has been the cause of a great deal of speculation. Poultrymen and pathologists have held to the view that the unused yolk is at least indirectly responsible for the malady.

Not only have I observed the presence of unabsorbed yolk in all of the white diarrhea chicks that have been examined within the last eighteen months, but in practically every instance have I been able to detect B. pullorum in the yolk. It was nearly always found pure and in large numbers. By opening the yolk sac with a sterile knife or scissors, and thrusting a platinum wire into its contents enough yolk is

removed to streak three or four tubes of slant agar and to obtain numerous minute colonies in each of the tubes. By this procedure the bacteriological diagnosis of the disease may be made quite simple. Even when the liver, spleen, heart, and lungs contain so few of the bacilli that the latter are found with some difficulty, they may easily be detected in the yolk.

What appears to be of considerable importance, also, is the frequent occurrence of the specific organism in the crop, stomach, and intestine. I have often found the bacillus in the crop in large numbers, and especially when the crop was filled with a more or less slimy liquid, or when little or no food had been taken for some time. In a few cases B. pullorum was found practically pure and in large numbers. As a rule, however, the organism was mixed with other forms, especially B. coli and members of the subtilis group. It was very difficult to detect the bacilli in question in the dirt of the floors or grounds on which the chickens were running.

The organism was also found in the stomach, but in smaller numbers. The results obtained by examinations of the intestines varied greatly, depending to a large extent on the age of the chick. In chicks that died from the disease when they were only two or three days old it was comparatively easy to obtain B. pullorum by the usual agar tube method. In one instance in particular the bacillus was found practically pure. In older chicks, however, in which the colon bacillus had gained a good foothold it was much more difficult to detect the organism in the intestinal contents, and in many cases attempts resulted in failure. For these examinations the contents of the small intestine and of the ceca were, as a rule, taken.

The question of the natural transmission of fatal septicemia or bacillary white diarrhea is still far from solved. We have sufficient evidence, however, to show that chicks may acquire the disease through food and water that have been infected with the specific organism. The complete results of this work will be reported in a subsequent paper. If the disease

is spread naturally through food and water which are constantly being infected by the droppings of the diseased chicks, and this would seem most probable, the stamping out of the epidemic must in a large measure at least be effected by essentially the same methods as those which are used in the suppression of fowl cholera; namely, isolation of the birds and disinfection of the incubators, brooders, poultry yards, etc.

I have carried on a number of experiments to determine the action of various disinfectants on B. pullorum. The susceptibility of the organism to corrosive sublimate, carbolic acid, and formaldehyde has already been commented on. The organism is readily injured or destroyed by very small quantities of mineral acids and of lactic acid. Neutral bouillon cultures of B. pullorum and of B. coli communis which were twenty-four hours old were treated with definite and varying amounts of an aqueous solution of pure lactic acid (Kahlbaum) and incubated at 37–38° C. At the end of two hours transplantations were made in sterile bouillon. Bacterium pullorum was found to be extremely sensitive to very small amounts of the lactic acid. Bouillon tubes were rendered sterile when the acid was present in the ratio of 1 : 400, or .25 per cent. The resistance of B. coli was fully twice as great as that of the chick organism. In other words, it required .5 per cent lactic acid to kill all of the colon bacilli during the two hours of exposure.

Since chicks which are exposed to the disease and those which already suffer with it harbor the specific organism in large numbers in the crop, in particular, I have had the conviction that certain feeding experiments might profitably be attempted. I would suggest, therefore, that those who have the facilities to do so undertake the feeding of sour milk, as Metchnikoff advises in the treatment of certain intestinal diseases in man. Ordinary well-soured milk, either alone or mixed with the regular feed given at least two or three times a day would probably render the crop, stomach, and upper portion of the intestine sufficiently acid to hold the organism in check, or even to kill it. I intend to carry out such

experiments, at least on a small scale, but have in the past been unable to do so, on account of the lack of the necessary facilities.

There is a possibility that the chicks may be infected before they leave the shell. Such infection may come originally from the hens which laid the eggs, or during the period of incubation, the bacilli getting into the eggs through the walls of the shell. We have examined at least five dozen eggs procured from various sources, and have not succeeded in finding B. pullorum in the yolks of any so far examined. This search is being continued and may still yield positive results.

It seems strange that the organism of simple white diarrhea should have been overlooked so long. Frequent attempts have been made to discover some specific agent which is responsible for the disease, but usually with negative results. Milks[3] of the Louisiana Agricultural Experiment Station at Baton Rouge has recently found a bacillus which he claims is the cause of an infectious disease in young chicks in which the symptoms and post-mortem appearances are undoubtedly those of white diarrhea. His description of the organism leaves little doubt that he was working with the same bacillus as that which I have so frequently found. Since his investigations were carried on independently the results are especially important.

Morse[4] announced in a preliminary publication that he had discovered the true cause of white diarrhea. In a large number of what he regarded as white diarrhea chicks which had been sent him for examination he claims to have observed a parasite which is identical with Coccidium tenellum. This parasite was found by him in the intestines, and particularly the ceca, of the infected chicks. The coccidium was observed in its encysted stage in the feces, on the walls of the intestine, and to a certain extent in the intestinal epithelial lining. Hadley[5] also believes that he has demonstrated that white diarrhea is caused by a coccidium, or at least that the coccidium plays an important part in the etiology of the disease. Hadley was unable, however, to find the cysts observed by Morse, but saw only what is known as the schizont stage of the organism, in a certain per

cent of the diseased chicks, and embedded in the epithelial lining of the intestine and ceca. Quite recently Hadley informed me that he had succeeded in finding my organism in some of the chicks which had died. In chicks which he sent to me I had no difficulty in establishing the identity of B. pullorum.

I have failed to find what without doubt is the encysted form of the coccidium in the feces, in the contents of the ceca or intestine, or on the intestinal walls of chicks which have been under my observation. Even should the coccidia be present real proof is lacking to show that they are the cause of the disease in question. Coccidia are known to be of very common occurrence in birds of the air and in chickens and other barnyard fowl. The data at hand do not warrant the supposition that coccidia play an important part in the etiology of white diarrhea, or at least in the more simple form of the disease.

In conclusion I wish to emphasize the following: Fatal septicemia and ordinary white diarrhea are one and the same disease. The malady is caused by a specific organism whose important characteristics I have described, and to which I have given the name of *Bacterium pullorum*. This organism occurs in the internal organs and in the unabsorbed yolk. The easiest and most satisfactory method of identifying the bacillus is by the agar streak and subsequent plating method given in this paper.

[I take this opportunity of expressing my obligation to Dr. Erwin F. Smith for his most valuable advice in the choice of a name for the new organism.]

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