As an offset to the success of the Board in dealing with swine fever, it has unfortunately to be recorded that there was an increase in the reported outbreaks of anthrax, and in the number of horses attacked with glanders.

The assistant secretary, Mr Anstruther, contributes a report in which the proceedings of the Board in dealing with the contagious diseases are discussed from an administrative point of view. From this we learn that the continuance of the present ineffective measures against glanders is due to the refusal of the Treasury to contribute from the exchequer towards the expenditure which would be involved by the slaughter with compensation of all horses reacting to the mallein test. It is to be hoped that the pressure of public opinion, exercised through the local authorities, will ere long induce the Treasury to reconsider this decision. The report contains the usual array of interesting details set forth in statistical form.

Veterinary Diagrams, with Keys and Exp'anatory Notes, By Henry C. Wilkie, F.R.C.V.S., Veterinary Division, Department of Agriculture, New Zealand. Wellington: John Mackay, 1904.

THESE diagrams include the skeleton and foot of the horse, and the udder of the cow, mare, and ewe. Although apparently designed mainly for the instruction of agriculturists, horse owners, and shoeing smiths, they are calculated to be of service to veterinary students, as their large size gives them an advantage over the illustrations of the same subjects which are to be found in the text-books of anatomy. The diagrams may be obtained from Messrs Gay & Bird, Bedford Street, Strand, London.

Report.

THE ETIOLOGY OF HOG CHOLERA.¹

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AFTER the fundamental work of Salmon and Smith relative to hog cholera and the corroboration of their observations by so many other investigators, it was regarded as settled beyond dispute that a certain micro-organism, *B. cholerae suis*, is the cause of hog cholera. Although certain results of Salmon and Smith, as well as the experience of others, show that it is difficult to reconcile all the facts as found with the acceptance of this bacterium as the exclusive factor in the production of the disease, more or less plausible explanations of these discrepancies have been offered; but these explanations did not seem entirely satisfactory, as will be shown later. Since this is the case, since the etiology is incompletely understood, it is difficult to attack properly the important practical questions of prevention and cure. In the present investigations we believe that, although we have by no means exhausted the subject, we have been able to make some very significant observations which throw light upon the perplexing question, and which we hope may eventually lead to a rational method of combating the disease.

In spite of the fact that the disease hog cholera is so well known, and that it has been so often defined and described in many excellent treatises, it will

[The complete report of this investigation has been published as Bulletin No. 72 of the "Bureau of Animal Industry," U.S.A. Department of Agriculture. In what is here reprinted the details of many important experiments are omitted. The researches are of the greatest possible interest to British veterinary surgeons, since it is generally admitted that American hog cholera and English swine fever are identical diseases.—Eds.]

nevertheless hardly be deemed superfluous to state at the outset just what our experience has taught us to regard as the essential features of the disease, on the one hand, and the variable and inconstant features, on the other. A definite statement in this regard is perhaps particularly called for, since all authorities do not agree upon the relative importance of the different manifestations seen in the disease. This discrepancy doubtless arises from the fact that in hog cholera, as in other diseases, in different outbreaks and at different times, one or the other set of symptoms and lesions predominate, and it is only by a study of various outbreaks in different parts of the country and over a long period of time that it is possible to arrive at an accurate knowledge of the variations to which the disease is subject.

Hog cholera is an acute and highly contagious disease which, so far as known, affects only hogs. It is characterised by fever, loss of appetite, weakness, and, where the affected animal does not quickly succumb, by rapid emaciation. Diarrhœa is usually present, though the affected animal may be constipated. Epistaxis frequently occurs, and there is often a gluing together of the eyelids. In the most virulent forms of the disease especially there may be ecchymoses in the skin over the ventral surface of the body. These are usually in the form of discrete splotches, but at times the entire ventral surface is diffusely reddened.

The changes seen in the internal organs vary greatly even in different animals in one and the same outbreak in a given herd. In general these lesions may be said to be either those of a hæmorrhagic septicæmia or of an ulcerative enteritis, the latter particularly pronounced in the cæcum and colon. The hæmorrhagic lesions are characteristic of the rapidly fatal form of the disease known as "acute hog cholera," the ulcerative intestinal lesions being especially prominent in those outbreaks where the animals do not succumb so rapidly; both the ulcerative and hæmorrhagic lesions may, however, be seen in the same animal. These two forms are not supposed to represent distinct diseases, but merely different types of the same disease, as already stated, the chronic form apparently resulting from either a decreased virulence of the infecting organism or a heightened resistance on the part of the animals which are attacked.

Hog cholera may be conveyed from sick to healthy animals almost if not quite without exception by contact, by feeding the viscera of diseased animals, and by the subcutaneous injection of the blood of sick animals. The disease may also be produced, according to Salmon and Smith, by feeding cultures of *B. cholerae suis* and by subcutaneous or intravenous inoculation of that organism. Welch and Clement claim that the disease may be produced by the intratracheal inoculation of healthy animals, as well as by intravenous inoculation with *B. cholerae suis*. Salmon and Smith found it very difficult to produce hog cholera by feeding or by subcutaneous injection of cultures of *B. cholerae suis*, but they consider that in certain instances they succeeded in doing so by both methods.

The mortality among hogs attacked by the disease varies from 30 to 100 per cent. of the affected animals, the death rate from the acute type of disease usually exceeding 80 per cent. of the affected herd.

The Infectiousness of the Blood of Animals Affected with Hog Cholera.—It will be noticed in the brief description that we have just given of hog cholera that the statement is made that this disease is communicated readily to healthy animals by inoculating them subcutaneously with the blood of hogs suffering with spontaneous hog cholera. The infectious nature of the blood has long been recognised, Law¹ having recorded experimental evidence of that fact in 1878. Other experiments showing the infectiousness of the blood of sick hogs are contained in the *Bureau of Animal Industry* report issued in 1889.² On p. 110 of that report Salmon and Smith describe the inoculation

¹ "Ann. Rep. U.S. Com'r. Agr.," 1878, p. 377.

² Hog cholera: Its history, nature, and treatment. Wash., D.C., 1889.

of four hogs subcutaneously with the blood of hogs sick of hog cholera, three of the four inoculated animals dying as a result. We would call attention here to the striking difference between the readiness in producing the disease by the inoculation of blood from animals sick from the spontaneous disease, on the one hand, and the great difficulty experienced in producing the disease by subcutaneous injections of pure cultures of *B. cholerae suis*, on the other. This and other important differences will be fully discussed later.

It is suggested by Salmon and Smith that these successful results may have been due to the greater virulence of the organism in the blood, but they also suggest the possibility that the injected blood coagulating in the tissues protected *B. cholerae suis* from the injurious effects of the body fluids and from the attacks of phagocytes, and at the same time furnished the injected organisms with an abundant supply of suitable food. The bacteria being thus protected for a time might gradually acquire sufficient powers of resistance to enable them to enter the circulation and produce the disease.

At the outset of our present investigations it was deemed wise to repeat Salmon and Smith's experiments with the blood of diseased animals, and to ascertain to what the pathogenic power of the blood was due. Accordingly, healthy hogs were inoculated subcutaneously with the blood of sick animals, the infection being derived from a number of separate and distinct outbreaks of hog cholera. We succeeded with the greatest regularity in communicating the disease to healthy animals by the subcutaneous injection of diseased blood obtained during the earlier stages of an outbreak. For convenience the blood was defibrinated previous to injection. It was also found that the clear serum which separated from the red cells in the bottles after the diseased blood was drawn under aseptic conditions possessed the power of inducing the disease in the same manner as the defibrinated blood. In the rare exceptions where the disease was not communicated in this way, it was found that the animal that was injected possessed immunity, as proven by subsequent exposure to infection in a pen with animals sick of hog cholera; there was no exception to this. The subcutaneous injection of blood from diseased animals proved indeed to be such a convenient and reliable method of transferring hog cholera from one animal to another that we have used it almost exclusively for propagating the several strains of disease with which our experiments have been conducted. But while it is true we have experienced no difficulty in thus propagating the disease with inoculations of blood, it should be explained that the different outbreaks which furnished the virus with which we have worked were selected because they appeared to possess a high degree of virulence. It is quite possible that in mild forms of the disease the same difficulty might be experienced in transferring the disease by blood inoculations as is found in transferring it by contact or by any other method.

What has been said above in regard to the result of inoculation with blood applies to blood obtained from animals at the beginning of an outbreak or during its height. Our own experience in propagating the disease by the successive inoculations of a number of hogs during a period of time covering several months has been that after a while the blood inoculations fail to cause the death of the animals. The virus seems to lose in potency after a number of passages through hogs.

The amount of diseased blood required to produce hog cholera when injected subcutaneously has not been definitely determined, but probably varies considerably, depending upon the virulence of the disease and the natural resisting power of the injected hog. We have produced the disease quite uniformly with from $\frac{1}{2}$ to 2 cc. of blood, and in some instances with much smaller doses. For keeping the disease in existence, larger doses (5 to 10 cc.) have usually been employed. This was done in order to avoid the loss of the disease through a possible lowering in virulence, the dose in most instances being no doubt much in excess of the amount required.

Although we can not speak definitely, owing to lack of experimental data, we would not expect the chronic form of hog cholera to be so readily communicated in this way. The lesions in the chronic form indicate a tendency toward a localisation of the infectious centres in the intestines, whereas the acute form possesses the characters of a septicæmia.

In addition to proving the ease with which hog cholera may be transferred from one animal to another by the subcutaneous inoculation of blood, the inoculation experiments indicate that this infectious property is not the result of the mechanical protection of *B. cholerae suis* by the clotted blood, the possibility of which was suggested by Salmon and Smith, as previously stated. All of our blood inoculations were made after the fibrin had been removed, the defibrinated blood containing corpuscles and serum, or the serum alone being used. The serum was diluted with ten volumes of sterile bouillon before injection, and could not have afforded any mechanical protection from the phagocytes or other injurious agents. We must therefore look for some other explanation of the fact first observed by Salmon and Smith, namely, that hog cholera may be easily induced by subcutaneous injections of diseased blood, whereas the injections of pure cultures of *B. cholerae suis* in the same manner fail, in the great majority of cases, to produce disease.

The Contagiousness of the Natural Disease Hog Cholera.—In connection with the infectiousness of the blood of hogs affected with hog cholera, the readiness with which transmission of the disease takes place by simple contact with diseased animals, or by exposure in infected pens, must be taken into consideration. This high degree of contagiousness of hog cholera is universally recognised by the farmer as well as by those who have made experiments to test the matter; for, aside from the abundant evidence of contagiousness to be derived from the records of natural outbreaks of hog cholera, such as the accidental introduction of a sick pig into a healthy herd, we would call especial attention to certain records contained in the *Bureau of Animal Industry* report.¹ On pp. 145 to 153 inclusive, of that report will be found the records of a number of hogs that had been subjected to various methods of treatment, the object of the treatment being to render them immune from hog cholera. An examination of those records will show that the treated animals and controls were subsequently placed in infected pens to determine the value of the immunising agent, and that after such exposure to the natural disease, thirty out of a total of thirty-three that were exposed died of hog cholera. These figures include the check animals which were exposed with the treated ones.

We are in possession of the records of a large number of recent experiments which confirm in every way the evidence cited above, and we would emphasise the importance of this contagiousness as a necessary feature of the disease.

Immunity in Hogs which have Recovered from Hog Cholera.—Hogs that have recovered from natural attacks of hog cholera are immune from subsequent attacks. This fact is quite generally known among hog raisers, and causes the average farmer to place a higher value upon these so-called "cholera proof" hogs than upon non-immunes. During the course of experimental work in the past few years we have had the opportunity of observing the course of a large number of separate outbreaks of hog cholera, and have tested the immunity of a number of animals that recovered from these natural attacks by means of field exposure and by injections of diseased blood, but have never seen one of them succumb to a second attack. Immunity acquired in this way seems to be remarkably high in degree and very permanent. The same thing has been found to be true of hogs which have recovered from the disease produced by subcutaneous injections of hog cholera blood, such animals being found immune from hog cholera when exposed to the disease in a natural way and also when injected subcutaneously with blood from diseased animals.

¹ Loc. cit.

A point of especial interest which is fairly well brought out is that a severe attack of hog cholera is not necessary for the production of immunity from subsequent attacks. In fact, the protection derived from a mild attack appears to be quite as complete and lasting as that afforded by a severer form of the disease. That this immunity is not transitory is shown by the fact that we have been unable to induce the disease in some of these immunes by injections of diseased blood made nearly a year after recovery from the original attacks.

Recapitulation of the Characteristics of Hog Cholera.—Before proceeding to a description of the various methods we have adopted in our efforts to reproduce hog cholera artificially, we wish to bring together in concise form those characteristics which appear to be essential before any disease may be classified as hog cholera, without, however, offering them as a means of differential diagnosis.

(1) The lesions found at autopsy should be those commonly met with in either acute or chronic hog cholera, and which have been already fully described.

(2) The disease should be contagious, as proven by intentional exposure of healthy animals placed in the pen with sick ones or by the history of an outbreak.

(3) The blood of diseased animals should be capable of producing an attack of hog cholera when injected subcutaneously into healthy hogs.

(4) Animals which have recovered from an attack of hog cholera should possess immunity when exposed to natural infection or when injected subcutaneously with the blood of hogs suffering with hog cholera.

The lesions found at autopsy are variable. This variability of the pathological lesions is seen during the course of practically all outbreaks of hog cholera. In some animals there is almost complete absence of lesions; in others there may be few or many of the lesions so generally regarded as pathognomonic of that disease. The hog may be desperately sick and yet when killed the autopsy may reveal only a few reddened lymphatic glands and possibly a few hepatised areas in the lungs; or, on the contrary, every organ may show pathological changes. Without evidence of contagiousness and without the *post-mortem* examination of other hogs from the same herd, some of the animals could not be said to have died of hog cholera. On the contrary, the contagiousness, the infectiousness of the blood, and the acquired immunity, after recovery, are very constant features.

Experiments with Pure Cultures.—Having become impressed with the constancy of the three characters which were found in all natural outbreaks of acute hog cholera studied by us, and finding after an examination of the literature upon the subject that the questions of the contagiousness of the disease and the infectiousness of the blood in animals injected with pure cultures of *B. cholerae suis* had apparently not been made the subject of experiments in earlier investigations, we determined to make a certain number of experiments which would be in a way supplementary to those already recorded by Salmon and Smith and by Welch and Clement, and which would at the same time enable us to compare the results obtained with the blood and tissues of hogs suffering from hog cholera, on the one hand, with those obtained with cultures, on the other; in short, to see whether hog cholera with the three essential characteristics of contagiousness of sick animals, infectiousness of the blood of sick animals, and immunity when exposed to spontaneous infection after recovery, is found in the disease produced by cultures.

Experiments were undertaken with a view of obtaining light on these points. In making these experiments some hogs were injected subcutaneously, others intravenously, and others were fed.

The results of the subcutaneous inoculation of hogs with cultures of *B. cholerae suis* show that such inoculations produce serious disease in a relatively small number of cases.

Of the nineteen hogs injected subcutaneously, only three died. Salmon and Smith also encountered this difficulty in producing disease by subcutaneous inoculation, but seemed to regard this as being due to lack of virulence of the organism. This explanation is hardly satisfactory in our cases, for the organisms were very virulent for rabbits and guinea-pigs, and produced death in hogs with great uniformity when injected intravenously even in minute amounts, besides producing disease and death when fed to hogs in very small doses. So there can be no doubt as to the virulence of the cultures employed. The fact already brought out, that the injection of blood serum of hog-cholera hogs produces disease promptly and uniformly, suggested the trial of additions of normal defibrinated blood to cultures for subcutaneous inoculation; but the injection of cultures mixed with defibrinated blood proved to be as little efficacious in producing disease as the injection of cultures alone.

The intravenous injection of even very small amounts of culture produced fatal disease with great regularity, as already stated, and the feeding of cultures frequently caused sickness and death.

In these experiments, then, *B. cholerae suis* was constantly fatal to hogs when administered intravenously, and usually so when administered by mouth, but not as a rule when injected subcutaneously.

The symptoms produced by the organism when fed are those of intestinal disturbance mainly; the symptoms after intravenous injection are profound depression, loss of appetite, with death in a few days. The autopsy in both cases frequently shows hæmorrhagic lesions in the kidneys and other organs, and greater or less necrosis and even ulceration of the mucosa of the intestines, particularly of the cæcum in the region of the ileocaecal valve, the intestinal lesions being more marked where cultures are fed. So far, then, as symptoms and lesions are concerned, the disease in hogs produced by *B. cholerae suis* may very closely resemble those found in natural outbreaks of hog cholera. At least we are unable to make out any essential differences between the two diseases in these respects. But in certain other very important respects there exists a most striking difference; for, while the disease contracted by natural infection is highly contagious and the blood of animals sick from natural infection is almost always infectious for other hogs by subcutaneous inoculation, and, moreover, while hogs recovered from disease contracted in a spontaneous outbreak possess a high degree of immunity from subsequent infection, all these features are wanting in the disease produced by cultures of *B. cholerae suis*.

The lack of contagiousness in the disease produced by *B. cholerae suis* is rather surprising, for it would be natural to suppose that hogs made sick with the cultures would readily communicate the disease, particularly in those cases accompanied by the profuse diarrhoea which is often seen, notably in those where the hogs have been made sick by feeding cultures; yet in only one case—and that not free from objection—is there any indication that healthy hogs contract disease by associating intimately with hogs sick from cultures of *B. cholerae suis*. In this case the symptoms, which developed in seventeen days, were those of a passing sickness, shown by diminution of appetite and a gluing together of the eyelids, but all symptoms subsided in three or four days. This animal afterwards survived exposure in a pen with hogs sick of the natural disease. So, while it can not be asserted positively that this animal was not made sick by contact with the culture hog 207, there are several considerations which make this at least improbable, for a hog, 217, inoculated with blood drawn from the tail of the culture hog 207 remained well after the inoculation and did not acquire immunity, as was shown by the death of the hog in the exposure pen. Again, a hog, 221, fed with viscera of hog 207 became somewhat sick from eating the viscera, but also failed to acquire immunity. So, if of the two hogs, 217 and 221, the one inoculated with blood and the other fed with the viscera of hog 207, neither became immune,

then it is not probable that hog 214 should have acquired immunity from simple association.

There were twenty-one hogs in all exposed to hogs which were given cultures of *B. cholerae suis* by different methods of administration, and hog 214 was the only one of the twenty-one to show any signs of sickness. Of these twenty-one hogs exposed to culture hogs, eighteen were subsequently placed in a pen with hogs suffering from natural hog cholera, and sixteen of the eighteen contracted the disease, showing that all except two of the eighteen hogs were susceptible and would have contracted the disease from the culture animals if these had been suffering from infectious hog cholera, as we understand the disease. Of the two hogs remaining well in the exposure pen, one, hog 214, has been discussed above, where it is shown that the animal probably did not derive immunity from the sickness contracted while in the pen with the hog made sick by inoculation with a culture, but in all likelihood possessed natural immunity. The most reasonable explanation of the failure of the other one of these hogs, 241, to become sick in the exposure pen is also that the animal had natural immunity, since it showed no evidences of sickness in the pen with the hogs inoculated with cultures, and one of these, 239, became sick and died in the exposure pen after recovering from the sickness produced by the culture. Hog 240, the companion of hog 239, died from the effects of the inoculation with the culture.

The injection of blood from a hog suffering from the natural infection is uniformly followed by the disease. This is the experience of all who have tested it, and we have abundant experimental proof of this fact. Indeed, we use this means of propagating the disease for experimental purposes in preference to any other on account of its certainty, as already stated. The injection of blood from animals sick from the administration of cultures, on the contrary, did not produce disease in any of the eleven hogs inoculated in this way. Of these eleven hogs, ten were exposed to natural infection, nine became sick, and seven died. This, again, is in marked contrast with the natural disease, for, in the rare instances of recovery after inoculation with the blood from a hog suffering from hog cholera, there is almost, if not always, without exception, the production of immunity.

Finally, the administration of cultures of *B. cholerae suis* seems to have little, if any, effect upon the production of immunity from natural infection. Incidentally, this has been shown above, in connection with the discussion of the power to produce disease with cultures; but it is worthy of more than passing mention, since it constitutes such a marked contrast with the results following recovery from the spontaneous disease, and from inoculations with blood from hogs suffering from the spontaneous disease.

There were eighteen hogs which survived the administration of cultures; sixteen of these were injected subcutaneously and two fed. Of these eighteen hogs, sixteen died, one became sick but recovered, one remained well when exposed to natural infection. Of these two which survived, both had previously been made sick by subcutaneous injection of cultures. Of the sixteen that contracted hog cholera and died in the exposure pen, six had recovered after being sick from subcutaneous injection of cultures, two from feeding, and eight had shown no sickness from the administration of cultures.

The difference between the disease produced by *B. cholerae suis* and spontaneous hog cholera, in respect to the immunity of hogs which recover, is very marked; for it can not be admitted that even the hog which failed to become sick in the exposure pen derived its immunity from the administration of cultures, since it may be claimed with equal probability that this animal had natural immunity. Indeed, it seems more probable that the latter explanation is correct in view of the many instances in which illness after administration of cultures failed to produce immunity.

It is clear, therefore, that while the disease produced experimentally with cultures of *B. cholerae suis* corresponds in some respects with spontaneous hog

cholera, the two diseases show marked points of difference. They differ in contagiousness, infectiousness, and in the production of immunity from natural infection in animals that recover.

As an explanation of these differences between the natural disease and that produced by *B. cholerae suis*, the possibility that they could be accounted for by a sudden and profound alteration in the disease-producing properties of *B. cholerae suis* occurred to us. But this did not seem probable, for the reason that many cultures employed in the foregoing experiments were tested only a few days after isolation from the body of the sick hog; moreover, the same cultures proved to have retained full virulence for guinea-pigs and rabbits after cultivation for several months.

But if these conflicting results between the natural disease and that produced by *B. cholerae suis* are not due to a rapid change in virulence of the organism for hogs they must be due to the presence in the blood of some agent which is absent from the pure cultures, and which, acting either alone or in conjunction with *B. cholerae suis*, is able to bring on a typical attack of hog cholera.

In order to determine the presence or absence of this hypothetical virus in the blood of hogs sick of hog cholera, it became necessary to see whether we could obtain infectious blood from which *B. cholerae suis* was absent.

Two methods of obtaining such blood were finally adopted. The first was based upon the supposition that if two micro-organisms were concerned in the production of hog cholera they would probably not always invade the body simultaneously. Hence, by drawing blood from hogs at different stages of the disease we might be able to find a time during the illness when *B. cholerae suis* would be absent and the hypothetical virus would be present in the blood.

Experiments with Diseased Blood free from B. Cholerae Suis.—In order to obtain blood from the same hog on a number of consecutive days, we have used a very ingenious method which was devised by Dr W. B. Niles. In hogs the superficial blood vessels are either so small or are so situated that it is impracticable to use them for obtaining blood by the methods ordinarily employed. Dr Niles found that by cutting off the end of the tail an abundant supply of blood could be obtained, and, in addition, the blood drawing was comparatively simple and did not cause serious injury to the hog.

The general plan adopted for carrying out these so-called "tail-blood" experiments was as follows: A hog known to be healthy was injected subcutaneously with blood from a sick animal. Beginning forty-eight hours later, blood was drawn from the tail of the injected hog; a certain amount of the blood obtained in this way was injected subcutaneously into a healthy hog, and an equal quantity placed in neutral beef broth and incubated, the culture being made in order to determine whether or not *B. cholerae suis* was present. The object of these experiments was to determine whether the time at which the blood becomes infectious coincides with the appearance of *B. cholerae suis*, or whether this is independent of the appearance of that organism.

There was one contingency which it was feared might interfere with this experiment. In injecting the original hog with diseased blood the chances were in favour of *B. cholerae suis* being present in the injected blood. Under such circumstances it was feared that this organism would invade the body immediately and thus defeat our object. However, as no better method appeared available at the time, the experiments were conducted according to the plan described above, and it will be seen from the results that our fears were groundless, for *B. cholerae suis* did not appear in the general circulation until several days after the hogs were inoculated.

In the first experiment the blood was infectious on the second day, whereas *B. Cholerae suis* did not appear till the third day. In the second experiment the blood was infectious on the second day and *B. cholerae suis* did not appear until the fifth day. Beyond question, then, the blood of hogs suffering from hog cholera may be infectious for other hogs in the absence of *B. cholerae*

suis, as is shown from the results given above. Even if we had only the results in these two cases this conclusion would not be unjustifiable, but when it is supported by the evidence of the filtration experiments below it becomes unavoidable.

We have laid the emphasis which the matter deserves upon the contagiousness of the natural disease, as contrasted with the lack of contagiousness in the disease produced by the use of cultures of *B. cholerae suis*, and it would complete these experiments with the tail blood taken from sick hogs *intra vitam* if it could be shown that hogs made sick by the injection of blood devoid of *B. Cholerae suis* could convey the disease to other hogs by association; but this matter was overlooked at the time the experiments were conducted. Still, since it will be shown in connection with the filtration experiments that those hogs inoculated with blood free from *B. cholerae suis* suffered from a highly contagious disease, it is but reasonable to suppose that the disease produced by the injection of tail blood, in all other respects resembling that produced by the filtered blood, should correspond with this in contagiousness as well.

Experiments with filtered Blood.—In addition to the experiments with the blood drawn from the tail during life, a second plan adopted was the filtration of diseased blood through Berkefeld and Chamberland cylinders. The filtration experiments were in part conducted before the tail-blood experiments; but for a better understanding of the results it has been deemed best to group all experiments of that character together.

It may be well, before entering upon a description of the filtration experiments, to explain briefly the conditions under which they were performed. First of all we would emphasise the fact that the most rigid precautions against accidental infection have been adopted in all cases. All pens had wooden floors. The disinfection of pens and feeding troughs was in every instance most thoroughly carried out; the attendants in feeding the experimental animals never entered the pen nor was any implement used for cleaning the pens without thorough disinfection, both prior to and after use. The large majority of all hogs in these experiments were held in quarantine several weeks, and, where such a quarantine was impracticable, the animals were obtained from farms which were personally known to be free from disease. As a result of a rigid enforcement of these precautions there have been in the course of our experiments only a very few instances of disease that could be attributed to accidental infection. Any doubtful cases of this character have been left out of our report or else the conditions have been thoroughly explained.

In the foregoing pages it will be noted that we have insisted upon contagiousness as a necessary feature of hog cholera, and yet it will be found that in some of our filtration experiments pen checks were not used, and therefore the contagiousness of the disease in such instances was not established. In explanation of this fact it should be remembered that our conception of the disease hog cholera has been more or less modified by the results of our experiments; hence in the earlier work some details were omitted which were later found to be desirable. In work of this character, even with all possible facilities at our command, we have not failed to encounter difficulty in making each experiment absolutely complete within itself; but when the work is considered as a whole, we believe the sources of error will be found to have been eliminated so far as possible, and the results show very satisfactory uniformity.

The experiments have been made with eight distinct strains of disease. A part of the experiments were conducted at Washington, D.C., and a part at a temporary station in south-western Iowa. In obtaining virus from natural outbreaks for experimental purposes we have selected those instances of disease which showed unusual virulence and which would be classed as "acute hog cholera." Preceding each series of experiments will be found a short description of the outbreak from which the virus was obtained originally. The filters

used were Berkefeld laboratory cylinders Nos. 5 and 7 (normal), and "Filtres Chamberland, Système Pasteur," marked "F" and "B," and all filtrations were, of course, conducted with sterile apparatus.

The serum was drawn off with a sterile pipette after the blood had stood for twenty-four hours or more in the refrigerator. The actual amount of serum used in most cases was 50 cc., though in some cases a smaller amount of serum was used, as it was not always possible to obtain 50 cc. free from red blood cells. The serum was then diluted with neutral beef broth or physiological salt solution in the proportion of 1 part of serum to 10 parts of the diluent, as stated in each case. The dilution was made for the reason that experience had taught us that the undiluted blood serum filtered very slowly, and in order to facilitate the passage of any virus that might be present. In most cases a fresh bouillon culture of about 4 or 5 cc. of *B. prodigiosus* was added to test the efficiency of the filtration. The vacuum employed varied from twenty-two to twenty-five inches, and the time of filtration was usually very short, about a half hour or less, though at times it was much longer on account of the presence of red blood cells. The apparatus was arranged in some cases so that the filtration took place from within the cylinder outward; in other cases from without inward.

The hogs used in the experiments were young animals weighing from 20 to 40 pounds, and the injections were made inside the thighs, using 22 cc.—11 cc. on each side—equivalent to 2 cc. of undiluted serum, except where otherwise stated.

Experiments with Blood from Herd I.—About the middle of February, 1903, the animals in this herd, which was located in south-western Iowa, began showing symptoms of illness. The farm was visited by Dr Charles M. Day, who reported that the *ante-mortem* symptoms were those of hog cholera. *Post-mortem* examinations of two of the sickest hogs revealed the lesions of acute hæmorrhagic hog cholera. As a result of this outbreak twenty-two out of twenty-three hogs died.

Blood from a sick animal in this herd was injected subcutaneously into hog 650, the latter animal being at once shipped to Washington. This hog, 650, served as the starting point for a large number of experiments. By successive subcutaneous inoculations of blood the disease was kept in existence for many months, no difficulty being experienced in transferring the disease to healthy animals in this manner, and the autopsies upon hogs which died from these injections were quite typical of hog cholera.

Filtration Experiment No. I.—A healthy hog, 704, was inoculated subcutaneously inside the left thigh with 4 cc. of defibrinated blood from hog 689, and this hog was sick at the time the blood was drawn, as a result of an injection of 5 cc. of diseased blood from a hog belonging to herd I.

Hog 704 remained well until six days after inoculation, when the first symptoms of illness were observed, and three days later, when the hog was very sick and weak, it was etherised and blood drawn from the heart. The autopsy showed a few punctate hæmorrhages in the kidneys and a few areas of congestion in the colon. The intestinal contents were bile-stained. The blood was defibrinated and kept on ice in a sterilized flask for one day, when the clear serum which had separated from the corpuscles was drawn off with a sterile pipette and diluted with physiological salt solution. A portion of the mixture was now filtered through a Chamberland cylinder "F."

The portion of the diluted serum which was not filtered was preserved for two days on ice, when it was filtered through a No. 7 Berkefeld laboratory cylinder, the conditions of filtration being the same as on the previous day when the Chamberland cylinder was used. In both of these filtrations, as well as in all others, every precaution was, of course, taken to prevent outside contamination, all flasks and filters being thoroughly sterilized before they were used. The culture in bouillon from the unfiltered serum of hog 704 was densely clouded twenty-four hours after being placed in the incubator. Upon plating

from this flask a pure culture of a large organism resembling *B. subtilis* was found. *B. cholerae suis* could not be detected.

The cultures made from the Chamberland and Berkefeld filtrates remained in the thermostat for three weeks without showing any signs whatever of bacterial growth.

Rabbit 739, which was inoculated with 10 cc. of the Berkefeld filtrate, died thirty-three days after inoculation. The autopsy disclosed no lesions whatever with the exception of echinococcus cysts in the liver. The echinococcus infection was no doubt the cause of death.

Rabbit 740, inoculated with the unfiltered serum, and rabbit 737 and guinea pig 3620, which were both inoculated with the Chamberland "F" filtrate, were all alive and well six months after inoculation.

Hog 749, inoculated with diluted, unfiltered serum, died thirty days after inoculation. There was no local inflammation at the point of inoculation. The inguinal glands were red and somewhat enlarged; spleen engorged with blood, slightly enlarged; kidneys congested, a few punctate ecchymoses in the cortical portion. In the small intestine and in the cæcum and colon a number of small erosions were found. The surface of these erosions was covered with a yellowish necrotic material which was easily removed. *B. cholerae suis* was obtained from the liver and spleen.

Hog 748, inoculated with Berkefeld filtrate, died nineteen days after inoculation. There was no inflammation at the point of inoculation. Inguinal glands enlarged and dark red; lungs showed two small areas of red hepatisation and a few small ecchymoses on the surface. This animal was otherwise normal with the exception of the kidneys, which had undergone a cystic degeneration, the parenchyma of these organs being almost wholly destroyed. Cultures taken from the liver and heart blood failed to reveal the presence of *B. cholerae suis*.

Hog 747, inoculated with Chamberland filtrate, died twenty-seven days after inoculation. There was no inflammation at the point of injection, but the areolar tissue in that region was stained a faint yellow colour. The right inguinal gland was considerably enlarged and quite red. The gland on the left side was much smaller and not so red. The liver exhibited on its surface a number of minute points of coagulation necrosis; spleen somewhat enlarged and dark; both kidneys presented a number of punctate hæmorrhages on their surfaces; in the neighbourhood of the ileocæcal valve in the cæcum and colon there was a number of comparatively recent ulcers. No cultures were taken from this animal because of *post-mortem* changes which were far advanced when the autopsy was made.

TABLE 15.

No. of Hog.	Material Injected.	Inoculation		Autopsy.	Remarks.
		Date.	Result.		
749	10 cc. diluted unfiltered serum hog 704	1903 May 27	1903 Died June 26	Hæmorrhages	<i>B. cholerae suis</i> present
748	11 cc. diluted filtered serum hog 704	Do.	Died June 15	Do.	<i>Micrococcus</i> from liver. <i>B. cholerae suis</i> not present.
747	Do.	Do.	Died June 23	Hæmorrhages and ulcers	No cultures taken.

Each of these hogs was kept in a separate pen.

In this experiment the features of especial interest are: (1) The absence of *B. cholerae suis* from the serum of hog 704, both before and after filtration, as determined by cultures and by the inoculation of small animals; (2) the production of fairly well-defined lesions of hog cholera in at least two of the hogs inoculated with this serum; (3) the failure of rabbits and guinea-pigs to succumb to doses of the serum which proved fatal to hogs; (4) the presence of *B. cholerae suis* in cultures from hog 749, although a culture test and the

inoculation of a rabbit both indicated the absence of this organism from the serum with which the hog was inoculated.

Filtration Experiment No. 2.—Hog 751 was inoculated with 5 cc. of defibrinated blood from hog 703. Hog 703 was carrying the herd I. disease and was quite sick at the time blood was drawn. Hog 751 showed decided symptoms of illness fifteen days after inoculation and became gradually worse. Eighteen days after inoculation the animal was etherised and blood was drawn and defibrinated. The lesions found at autopsy were not marked, and did not seem sufficiently grave to account for the severe illness of the animal at the time it was killed. The spleen was enlarged and soft. The mucosa of the cæcum and colon was congested and exhibited a number of small erosions, and the intestinal contents were bile stained. The other organs were normal in appearance.

The defibrinated blood, which was collected in a sterile bottle, was kept on ice for two days, when the clear serum that had separated from the red cells was withdrawn with a sterile pipette, and diluted with sterile physiological salt solution. After reserving a portion of the diluted unfiltered serum the remainder was divided into three parts and filtered as follows: One portion was filtered through a sterile Berkefeld laboratory cylinder No. 7 (the filtrate was designated "751 Bd"); a second portion of the diluted serum was passed through a sterile Chamberland cylinder marked "F" (filtrate designated "751 Ch. F"); the third portion was passed through a sterile Chamberland cylinder marked "B" (filtrate designated "751 Ch. B"). In the case of the Chamberland filtrations the cylinders were arranged in the manner shown in Plate 2. From each of these three filtrates 25 cc. was removed by means of a sterile pipette and added to a flask containing 400 cc. of neutral beef broth. At the same time, 2 cc. of the undiluted serum of hog 751 was added to 400 cc. of sterile beef broth. All four of these flasks were placed together in a thermostat at 37.5° C.

The flasks inoculated with the unfiltered serum and with the Chamberland "F" filtrates became clouded, while those inoculated with the Berkefeld and Chamberland "B" filtrates remained clear after being in the incubator for nine days. An examination of the culture from the unfiltered serum showed it to contain a micrococcus in pure culture, while the flask inoculated with the Chamberland "F" filtrate contained a pure culture of a large bacillus which resembled *B. subtilis*. No attempt was made to identify these organisms, the examination being carried only so far as was necessary to ascertain that *B. cholerae suis* was absent. The presence of the large bacillus in the culture from the Chamberland "F" filtrate, in view of its absence from the unfiltered serum, can only be accounted for by accidental contamination from the air, for which there was abundant opportunity during the removal of the 25 cc. used for the culture.

Hog 766 was inoculated with defibrinated blood of 751 immediately after it was drawn, and placed alone in a pen; was first noticed to be sick in eleven days, and died in fourteen days. The autopsy revealed numerous small hæmorrhages and some œdema in the lungs. The liver and spleen were enlarged and congested; the kidneys hæmorrhagic. There was considerable pigmentation, and there were also numerous small hæmorrhages in the intestine; a few recently formed ulcers in the colon.

Hog 788 was inoculated with diluted, not filtered, serum and placed alone in a pen; was first noticed to be quite sick in nine days, and remained so for eighteen days, when improvement began. This improvement continued for thirty days, when the animal had apparently completely recovered, so it was placed in the exposure pen to test its immunity. The hog was killed after remaining in the exposure pen for about seven months, where other hogs were dying of hog cholera. It was in an unthrifty condition, but no lesions of hog cholera could be found.

Hog 783 was inoculated with Berkefeld filtrate; was first noticed to be

sick in eight days. This hog was in a moribund condition when killed fifteen days after inoculation. The legs and ears were reddened and there were many red areas over the ventral surface of the body. There was no lesion at the point of inoculation. The lymph glands were œdematous and there were several areas of red collapse in the lungs. The kidneys were hæmorrhagic; the intestines appeared normal, although their contents were bile-stained.

Hog 789, inoculated with Berkefeld filtrate, showed symptoms of illness in eight days and died in eleven days. There was extreme reddening of the ventral surface of the body and of the head and ears; the lungs were sprinkled throughout with ecchymoses varying from 3 to 15 mm. in diameter; spleen enlarged and engorged with blood; kidneys presented a few punctiform hæmorrhages; some ecchymoses and ulcers in the mucous membrane of the intestines. Hog 783 and 789 were placed in a pen by themselves.

Hog 790, inoculated with Chamberland "F" filtrate, was slightly sick in eleven days and became gradually worse and died twenty-eight days after inoculation. The ventral surface of the body was extensively reddened; the lymphatic glands were enlarged and red; the kidneys hæmorrhagic, and cæcum and colon extensively ulcerated.

Hog 791, a mate to hog 790, was injected on the same date with same lot of serum; was seen to be sick in seven days, became rapidly worse, and died in twelve days. There was extreme reddening of the ventral surface of the body and of the ears; no lesion at the seat of infection, but the adjacent lymph glands were enlarged and hæmorrhagic; the lungs were sprinkled throughout with ecchymoses; the spleen enlarged and congested; the kidneys presented punctiform hæmorrhages in the cortices; both large and small intestines were flecked with hæmorrhages of varying size, but no ulcers could be found. Hogs 790 and 791 were placed in the same pen.

Hog 784, inoculated with Chamberland "B" filtrate, became sick six days after inoculation and died in twelve days. The autopsy revealed lesions which belong exclusively to the hæmorrhagic type of disease. There was extensive reddening of the skin, reddening and swelling of the lymphatic glands, but no inflammation at the seat of injection. Ecchymoses of varying size and number were present in the lungs, spleen, liver, kidneys, stomach, and large and small intestines.

Hog 786, inoculated at the same time and in the same manner as hog 784, became slightly sick eight days after inoculation, and being hopelessly sick on the fifteenth day, was killed. The lesions found belonged to the chronic intestinal type, and therefore differed quite markedly from those met with in its mate, hog 784. The only explanation of the wide difference in the lesions found in these two hogs seems to lie in a variation in resisting power possessed by the two animals. Hog 784 and 786 had been placed in a pen by themselves.

TABLE 16.

No. of Hog.	Material Injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
766	5 cc. defibrinated blood hog 751	1903 June 15	1903 Sick June 23; died June 29	Hæmorrhages	No cultures taken.
788	20 cc. unfiltered serum hog 751	June 18	Very sick; recovered	—	Immune when exposed subsequently
783	20 cc. serum 751 "Bd."	Do.	Sick June 26; killed July 3	Hæmorrhages	In dying condition when killed.
789	Do.	Do.	Sick June 26; died June 29	Do.	No cultures taken.
790	20 cc. serum 751 "Ch. F."	Do.	Sick June 29; died July 16	Hæmorrhages and ulcers	Do.
791	Do.	Do.	Sick June 25; died June 30	Hæmorrhages	Do.
784	20 cc. serum 751 "Ch. B."	Do.	Sick June 24; died June 30	Do.	Do.
786	Do.	Do.	Sick June 26; killed July 3	Ulcers	In dying condition when killed.

The results of Filtration Experiment No. 2 are even more striking than those obtained in Filtration Experiment No. 1. Flasks inoculated with 25 cc. of the diluted serum—5 cc. more than was employed for inoculating a hog—failed to reveal the presence of *B. cholerae suis* in either the filtered or unfiltered serum. It seems extremely improbable that *B. cholerae suis* could have been present in all three of the filtrates and also in the unfiltered serum in sufficient numbers to produce acute hog cholera by subcutaneous injection and yet have failed to grow in the cultures from any of them. The possibility of accidental infection from without or from the pens or syringes also seems to be highly improbable in view of the uniformity of the results, and the great rarity of such accidental infection in other experiments where hogs were inoculated with substances other than the blood or tissues of hogs sick of hog cholera.

Notwithstanding the unlikelihood of the disease in these hogs having been caused by any other agent than the injected serum, plans were made for another set of experiments which would eliminate still further the chances of error mentioned above.

Filtration Experiment No. 3.—Hog 797, the animal which furnished the blood for this experiment, was inoculated subcutaneously with 5 cc. of blood from hog 792, which was carrying herd I. disease. Hog 797 became very sick eleven days after inoculation, and was etherised and blood drawn from the heart and defibrinated. The autopsy on hog 797 disclosed hæmorrhagic kidneys and a number of recent ulcerations in the cæcum and colon.

The blood obtained from hog 797 was kept on ice for three days. The serum was then withdrawn with a sterile pipette and mixed with sterilised physiological salt solution. Different portions of this diluted serum were then filtered through three sterilised filters—a Berkefeld laboratory cylinder No. 7 (filtrate designated "797 Bd."), a Chamberland cylinder "F" (filtrate designated "797 Ch. F."), a Chamberland cylinder "B" (filtrate designated "797 Ch. B.").

The Chamberland cylinders were so arranged that the filtration should take place from within outward; the Berkefeld cylinder was arranged in the usual manner. After completing the several filtrations, cultures were made as follows:—

To flasks containing about 400 cc. of sterile neutral bouillon 25 cc. of each of the filtrates was added, and to a similar flask 4 cc. of the original undiluted serum. All cultures were placed immediately in the incubator at 37.5° C. Three days after these cultures were prepared, the one made from the undiluted and unfiltered serum showed a dense clouding, and the cultures from the Chamberland "F" and "B" filtrates were both slightly cloudy. The culture from the Berkefeld filtrate remained clear. In order to ascertain whether *B. cholerae suis* was present in either of these cultures, guinea pigs were inoculated with $\frac{1}{2}$ cc. of each. The guinea-pig inoculated with the culture from the unfiltered and undiluted serum remained well. The same is true of the guinea-pig inoculated from the Chamberland "B" culture. The one inoculated with the Chamberland "F" culture died twenty-five days after inoculation. A careful bacteriological examination, however, failed to reveal the presence of *B. cholerae suis*, but indicated that death was due to infection by an entirely different organism.

Immediately after defibrination the blood of hog 797 was injected subcutaneously into hogs 819 and 820, 5 cc. into each. The inoculations with the diluted serum of hog 797 were made after the serum had been in the refrigerator overnight.

The numbers of the hogs concerned and their arrangement in the pens are as follows:—

[TABLE.

Hog 819.	Received 5 cc. defibrinated blood of hog 797	} Placed in same pen.
820.	Do.	
837.	Received 22 cc. diluted, unfiltered serum of hog 797	} Do.
827.	Uninoculated pen check	
832.	Received 22 cc. serum hog 797, filtered through Berkefeld	} Do.
834.	Do.	
830.	Uninoculated pen check	} Do.
828.	Received 22 cc. serum hog 797, filtered through Chamberland "F"	
829.	Do.	} Do.
836.	Uninoculated pen check	
824.	Received 22 cc. serum hog 797, filtered through Chamberland "B"	} Do.
833.	Do.	
838.	Uninoculated pen check	} Do.
825.	Received in same manner, 22 cc. sterile bouillon	
826.	Do.	} Do.
831.	Uninoculated pen check	

Hog 819 was inoculated with 5 cc. of defibrinated blood of hog 797; was desperately sick in eight days and was etherised in nine days in order that blood might be drawn for other experiments. The autopsy revealed minute hæmorrhages in the lungs, enlarged spleen, congested kidneys; intestinal contents were bile-stained, but no ulcers could be found.

Hog 820 was inoculated at the same time and with the same material as hog 819. This hog became desperately ill and was etherised nine days after inoculation in order that blood might be obtained for other experimental work. The autopsy was quite similar to that of hog 819, the only noticeable difference being that in the case of hog 820 the intestines were congested and some hæmorrhages were found on the mucous coat.

Hog 837 was inoculated with diluted, not filtered, serum of hog 797; showed no symptoms of illness for seven days, when it appeared to be very sick, and the symptoms increased in severity till death, eleven days after inoculation. There was no inflammation at the point of inoculation. The inguinal glands were slightly reddened; there were a few small hæmorrhagic areas in the lungs. The spleen was enlarged and dark; a few punctiform hæmorrhages were seen in the kidneys. The intestinal contents were fluid and faintly tinged with blood. The mucous membrane of the cæcum and colon presented a few small ecchymoses, and in the cæcum some small ulcers were found, evidently of recent formation. From the spleen of this animal a pure culture of a micro-organism resembling *B. cholerae suis* in all of its cultural characters except in its action upon saccharose was obtained. This sugar underwent fermentation with the production of gas.

Hog 827 was not inoculated, but placed as a check in the pen with hog 837; showed no signs of illness for fourteen days, when symptoms similar to those seen in 837 developed, and the hog died four days later. The subcutaneous areolar tissue over the abdomen and thorax was sprinkled with small ecchymoses. The inguinal, lumbar, mesenteric, and mediastinal glands were enlarged and reddened. Numerous petechiæ on the surface of the heart muscle. The spleen was somewhat enlarged and quite dark; kidneys were dotted with numerous punctiform hæmorrhages; the peritoneum contained a number of discrete hæmorrhages. A few small hæmorrhages were found on the serous surface of the stomach, and also on the serous and mucous surfaces of the small intestines, cæcum, and colon. No ulcers could be found. Cultures from the spleen revealed the presence of *B. cholerae suis*.

Hog 832 was inoculated with the Berkefeld filtrate from serum of hog 797; was quite sick in seven days and died in twelve days. There was no lesion at

the seat of injection. The lungs were sprinkled throughout with minute hæmorrhages. The spleen was greatly enlarged and engorged with blood; the kidneys thickly sprinkled with punctiform hæmorrhages. The small intestine exhibited numerous circumscribed hæmorrhages in the mucosa. The mucosa of the cæcum and colon contained many small ulcers and the intestinal contents were blood-stained. No cultures were taken.

Hog 834 was injected with the same material at the same time as hog 832; was quite sick seven days after inoculation, became rapidly worse, and died on the twelfth day. There were no lesions at the seat of injection. The inguinal glands were enlarged and reddened; there were a few small ecchymoses on the surfaces of the lungs; the spleen was enlarged and congested; the kidneys were also congested and in addition were dotted with minute sharply defined hæmorrhagic points. A few superficial erosions were found on the mucous surface of the cæcum and colon. Cultures from the spleen revealed the presence of *B. cholerae suis*.

Hog 830 was not inoculated, but served as a pen check on hogs 832 and 834; seemed to be slightly sick in eleven days; was desperately sick in fifteen days and was killed. There were no lesions at the seat of inoculation. The inguinal, mediastinal, and mesenteric glands were all enlarged and very red. There were diffuse hæmorrhages in the epicardium and a large number of minute, sharply defined hæmorrhages on the surfaces of the lungs. The spleen was very much enlarged; the kidneys showed the characteristic punctiform hæmorrhages in their cortices. The peritoneum was dotted with numerous small ecchymoses, as were the serous surfaces of the stomach, cæcum, and colon.

Hog 828 was inoculated with Chamberland "F" filtrate from serum of hog 797; first sick in seven days and was found dead in twelve days. The inguinal glands were somewhat reddened. On the surfaces of the lungs a few small hæmorrhagic points could be seen. The spleen was enlarged, cæcum and colon congested, and on the mucous surface certain small areas were covered with a tenacious yellow exudate which, upon being removed, revealed a rather superficial erosion of the mucosa.

TABLE 17.

No. of Hog.	Material Injected.	Inoculation.		Autopsy.	Remarks.
		Date.	Result.		
819	5 cc. defibrinated blood hog 797	1903 July 14	1903 Sick July 22; killed July 23	Hæmorrhages	No cultures taken.
820	Do.	Do.	Do.	Do.	Do.
837	22 cc. unfiltered serum hog 797	July 20	Sick July 27; died July 31	Do.	Atypical <i>B. cholerae suis</i> from spleen.
827	Not inoculated; pen check on hog 837	Exp'd July 20	Sick Aug. 3; died Aug. 7	Do.	No cultures taken.
832	22 cc. serum hog 797 "Bd"	July 20	Sick July 27; died Aug. 1	Do.	Do.
834	Do.	Do.	Do.	Do.	<i>B. cholerae suis</i> from spleen.
830	Not inoculated; pen check on hogs 832 and 834	Exp'd July 20	Sick July 31; killed Aug. 3	Do.	Do.
828	22 cc. serum hog 797 "Ch. F."	July 20	Sick July 27; killed Aug. 1	Hæmorrhages and ulcers	No cultures taken.
829	Do.	Do.	Do.	Do.	<i>B. cholerae suis</i> from spleen.
836	Not inoculated; pen check on hogs 828 and 829	Exp'd July 20	Sick Aug. 1 died Aug. 6	Do.	No cultures taken.
824	22 cc. serum hog 797 "Ch. B."	July 20	Sick July 27; died Aug. 1	Do.	<i>B. cholerae suis</i> from spleen.
833	Do.	Do.	Sick July 27; died July 31	Do.	Atypical <i>B. cholerae suis</i> from spleen.
838	Not inoculated; pen check on hogs 824 and 833	Exp'd July 20	Sick Aug. 3; died Aug. 11	Do.	<i>B. cholerae suis</i> from spleen.
825	22 cc. sterile bouillon	July 20	Remained well	—	—
826	Do.	Do.	Do.	—	—
831	Not inoculated; pen check on hogs 825 and 826	Exp'd July 20	Do.	—	—

Hog 829 was treated in the same manner as hog 828; first signs of sickness in seven days; was found dead in twelve days. The autopsy was almost identical with that of hog 828. Cultures from the spleen revealed *B. cholerae suis*.

Hog 836 was not inoculated, but served as a pen check on hogs 828 and 829. The first symptoms of illness were noted in twelve days and the animal died in seventeen days. A few small hæmorrhages were found on the auricular appendages of the heart. The spleen was enlarged and quite dark; the kidneys were very much congested and exhibited some punctiform hæmorrhages on their surfaces. Some small petechiæ on the serous surface of the stomach. The small intestine presented on its serous surface a large number of small, rather sharply defined ecchymoses. The serous surfaces of the cæcum and colon were less affected than the same portion of the small intestine. The mucous coat of the latter presented many small erosions, the centres of which were of a reddish colour. The contents of the cæcum were semi-fluid and contained some blood.

Hog 824 was inoculated with Chamberland "B" filtrate from serum of hog 797; was first noticed to be sick in seven days; became rapidly worse and died twelve days after inoculation. The entire ventral surface of the body was reddened. The inguinal, lumbar, and mesenteric glands were enlarged and red. There were numerous scattered hæmorrhagic areas on the surfaces of the lungs and the cephalic lobe of the left lung was hepatised. The spleen was enlarged and dark with irregular diffuse hæmorrhagic areas on its surface. The kidneys were thickly dotted with punctiform hæmorrhages. There were numerous small petechiæ on the serous and mucous surfaces of both the large and small intestines. The mucous membrane of the cæcum and colon contained a number of small ulcers.

Hog 833 was inoculated at the same time with the same material as hog 824; was quite sick in seven days and became rapidly worse, dying twelve days after inoculation. There were no lesions found at the seat of inoculation. The inguinal glands were enlarged and red, and there were many sharply defined hæmorrhages on the surfaces of the lungs; the spleen was somewhat enlarged; the kidneys congested and dotted with numerous punctiform hæmorrhages. Some hæmorrhages were found on the surfaces of the stomach and also on the serous and mucous surfaces of the intestines. The intestinal contents were blood-stained.

Hog 838 was not inoculated, but placed as check in the pen with hogs 824 and 833. The animal remained well for fourteen days, when it became suddenly ill and died twenty-two days after exposure. The lungs contained, besides several areas of red collapse, numerous small hæmorrhages. Both of the auricles of the heart were sprinkled with petechiæ. The spleen was enlarged, kidneys hæmorrhagic, and innumerable minute hæmorrhages were found on the mucosa of the small intestine; a number of ulcers were present in the mucosa of the cæcum and colon. Cultures from the spleen contained pure growths of *B. cholerae suis*.

Hogs 825, 826, and 831: Two of these hogs, 825 and 826, were injected with sterile bouillon, the other, 831, serving as a check. All three remained perfectly well for forty days. Hogs 825 and 826 were then injected with blood from a hog sick with herd II. disease, hog 831 being again used as a check. All three died, thus showing that their failure to contract disease when used in Filtration Experiment No. 3 was not due to natural immunity.

It is evident that the results obtained in Filtration Experiment No. 3 substantiate completely the work described under Filtration Experiments Nos. 1 and 2. *B. cholerae suis* could not be found in any of the material used for injection either before or after filtration, and yet the hogs inoculated with this material sickened promptly and the majority of them died, typical lesions of hog cholera being found in every instance. Filtration Experiment No. 3 was of greater value than the two preceding experiments on account of

the check animals which were used in connection with the different inoculations. A glance at the accompanying table will show that in every instance the injected animals became sick from four to seven days sooner than the accompanying pen checks, thus indicating in a direct way that the infection arose from the injected material and not from the pens in which the animals were kept. Hogs 825 and 826, which were handled in the same manner as the remainder of the animals in this experiment, but which were injected with sterile bouillon instead of with the filtered diseased serum, remained perfectly well, thus furnishing proof that the disease in the hogs which sickened could not be attributed to an infection through careless handling or through the introduction of diseased material into the pens subsequent to inoculation. We would call especial attention to the fact that the subcutaneous injections of filtered blood not only caused the death of the injected animals, with typical lesions of hog cholera, but that the disease thus produced was contagious for susceptible animals placed in the pen with those which were injected.

Discussion of Filtration Experiments.—The results of the subcutaneous injections of hog cholera blood filtered through Chamberland and Berkefeld cylinders show that these injections produced disease quite as uniformly as those with the unfiltered blood. On the whole, it is true, hogs inoculated with the unfiltered blood became sick more quickly after inoculation than the hogs inoculated with the same serum after filtration, but this was not always the case. It is also true that a greater percentage of deaths occurred among the hogs inoculated with the unfiltered serum than among the hogs inoculated with the filtered serum. Of the thirty-six hogs inoculated with the unfiltered serum, all became sick, twenty-four died, ten were killed in a moribund condition, and two recovered; while of the sixty-one hogs injected with filtered serum, fifty-eight became sick, thirty-nine died, ten were killed in a moribund condition, nine recovered, and three showed no perceptible sickness. So although the mortality was less in the hogs injected with filtered serum, there was practically no difference in the production of disease in the case of unfiltered and of filtered blood; for the three hogs which failed to show noticeable symptoms of sickness after the injection of filtered blood must have possessed great resisting power, since two of them remained well on exposure to natural infection, and the other lived for many weeks in contact with sick hogs but died eventually, though it is not certain that death was due to hog cholera.

The resisting power of these three hogs may have been natural, or, as will appear from what is said later on, it may have been acquired from the serum injections. The possibility that they may have been really sick, but not have visible evidence of this, should not be lost sight of.

It is not surprising, however, that the blood after filtration should show less potency than blood before filtration; for whatever the nature of the infectious agent, it is but natural to suppose that some of it is removed by the filter. Nevertheless, diminution of potency is not observed in all cases, as already remarked, and when no diminution is observed it is probable that there is such a large amount of the infectious agent present originally, that although a part is removed by filtration, there still remains a sufficient amount to cause disease.

It will be observed that these results show not only that the filtered blood produces disease with great regularity, but that hogs which recover from the disease produced in this way have immunity from natural infection. Of the nine hogs that recovered after being sick, only one died in the exposure pen, but from the history of this case it seems probable that this hog, 1137, may not have fully recovered from the sickness produced by the filtered serum.

The results furthermore show that the disease produced by injection of filtered blood is contagious; for uninoculated hogs placed in the pens with the hogs inoculated with the filtered blood remained well until the inoculated animals became sick, and then contracted disease more or less promptly.

There were twenty-two such check hogs in the pens with the hogs inoculated with filtered serum, and seventeen of the twenty two contracted the disease, while five did not show noticeable symptoms of illness.

Of the seventeen that became sick, fourteen died, two were killed in a moribund condition, and one recovered and remained well on exposure to natural infection. Of the five which showed no disease from association, one remained well in the exposure pen and the others died. So the disease produced by the filtered blood proved contagious in at least seventy-seven per cent of cases, and four of the five failures may possibly be accounted for by the fact, which can be verified from the records, that the disease in the inoculated animals to which these checks were exposed was of a mild type. The other failure was probably a case of natural immunity, since the hog did not contract the disease on exposure to natural infection.

The experiments made to test the infectiousness of the blood and tissues of animals sick as a result of the inoculation of filtered blood, show that the blood of such animals is infectious and that the viscera cause the disease on feeding. The urine was tested and found to be infectious in one case. This urine contained *B. cholerae suis*, but, since this organism does not appear to affect the virulence of the blood, it could hardly be regarded as the cause of the pathogenic property of the urine.

It is evident that there is no essential difference between the disease produced by filtered diseased blood, on the one hand, and that acquired in natural outbreaks, on the other. The symptoms and lesions differ in no particular in the one case from those in the other; but still more striking and convincing evidence of identity is the fact that hogs recovered from sickness produced by the filtered blood have immunity from the natural disease.

We would emphasize the fact that the filtered blood was shown to be free from *B. cholerae suis* by culture tests in all cases, and by the inoculation of rabbits or guinea-pigs in most cases.

General Conclusions.—We have called attention to the fact that acute hog cholera, at least the form of that disease encountered in south-western Iowa, possesses in addition to those symptoms and lesions generally recognised, certain essential features which have not been considered as fully in previous work as, in our opinion, their importance deserves. These features upon which we wish to lay special stress are:

(1) Contagiousness: The disease should be communicable from sick to well animals by association. (2) Infectiousness: The blood of sick animals should cause disease upon subcutaneous injection into healthy non-immunes. (3) Immunity: After recovery the animals should resist subsequent exposure to the disease.

Our experiments have shown that pure cultures of *B. cholerae suis* injected subcutaneously into hogs usually produce but slight disturbance, although after intravenous injections or feeding, a severe illness frequently results. The disease produced in this manner may present the symptoms and lesions seen in acute hog cholera, but does not possess the characteristics of contagiousness nor of infectiousness of the blood; nor are those hogs which have recovered from such illness immune when exposed subsequently to the natural disease. In regard to the experiments with pure cultures of *B. cholerae suis*, therefore, we may say that they demonstrate the very considerable pathogenic power which that organism possesses for hogs, but they also show that the disease produced by that organism lacks several of the essential features of acute hog cholera.

The experiments with blood serum derived from hogs sick of hog cholera and proven to be free from *B. cholerae suis* show, on the contrary, that such serum produces illness in hogs with great regularity upon subcutaneous injection, and, furthermore, that the disease thus produced possesses all of the characteristics of the natural disease, including symptoms, lesions, contagiousness, infectiousness of the blood, and immunity in those animals which recover.

These results are in such striking contrast to those obtained when cultures of *B. cholerae suis* are used, and they are in such perfect harmony with those obtained by the use of unfiltered blood from sick hogs, that we are forced to conclude that there exists in the blood of hogs suffering from acute hog cholera some virus other than *B. cholerae suis*, and that this virus is necessary for the production of that disease.

This virus, present in the blood of hogs sick of acute hog cholera, but absent from pure cultures of *B. cholerae suis*, is known to us only by the effects it produces. We have failed completely in all attempts to discover by microscopic examination or by the usual cultural methods any visible micro-organism in these filtrates. That the pathogenic power of the filtered blood is due to some living agent endowed with the power of reproduction, and not to the presence of a toxine alone, there can be no doubt, from the fact that the disease induced by the filtered serum is communicated from sick to healthy animals by association, and, moreover, from the fact that we have transferred the disease induced by filtered serum to a second and even a third animal by subcutaneous injections, the serum being filtered each time previous to inoculation.

While our experiments establish beyond question that the filterable virus was present in all the outbreaks of hog cholera studied experimentally by us, it is also true that *B. cholerae suis* was present almost as uniformly. So it would be impossible to overlook, even if we were inclined to do so, the part which this organism may have played. But from the results of our inoculations with the filterable virus, on the one hand, and our results and those of others with cultures, on the other, we are compelled to conclude that the prime cause in our cases was the filterable virus, and that *B. cholerae suis* was at most an accessory factor.

The exact rôle played by *B. cholerae suis* in outbreaks of acute hog cholera is difficult to define. That the fatal result in many instances is materially influenced by the presence of that organism cannot be doubted, and, in addition, we would emphasise the fact that although the filterable virus appears to have been the primary invader, in those cases of acute hog cholera which we have studied, we do not deny the possibility of independent disease being caused by *B. cholerae suis*. In fact it is difficult to avoid a belief in such a possibility, on account of the very considerable pathogenic power for hogs exhibited by many cultures of that organism when fed or administered intravenously. The filterable virus seems not only to cause disease by itself, as shown by the results of many of our experiments, but it seems also to be able to lower the resisting power of the hog and thus enable *B. cholerae suis* to invade the body, and this we believe takes place in the majority of cases in natural outbreaks. If the filterable virus is capable of lowering the resisting power of hogs for *B. cholerae suis*, then it is easily conceivable that other agencies may also lessen the resisting power of these animals.

So it must be admitted that a disease in hogs may exist which is due to *B. cholerae suis*, and having no connection with filterable virus found by us in the outbreaks we have studied. From the knowledge we have of the pathogenic powers of *B. cholerae suis*, however, we would expect that any disease in which it is the chief pathogenic factor would be possessed of a low degree of contagiousness.

The fact that *B. cholerae suis* was found in a large proportion of the cultures taken from hogs inoculated with filtered serum seems to indicate strongly that that organism has its normal habitat on or in the bodies of healthy hogs, and that in the case of lowered resistance on the part of the hogs this organism enters the circulation. A careful study of the bacterial flora of the hog's alimentary tract might throw considerable light upon this subject.

We have begun investigations of this nature, but as yet other more pressing

work has prevented their completion. In a few preliminary examinations, guinea-pigs and rabbits were inoculated subcutaneously with small amounts of the intestinal contents of normal hogs and a few agar plates were made from the same material. In one case only, when the method of Drigalski and Conrad for the isolation of typhoid bacilli was used, did we succeed in discovering an organism which possessed the cultural characters of *B. cholerae suis*. The only way in which this organism from the normal intestinal contents differed from the classical type of *B. cholerae suis* was in its lack of pathogenic power for guinea-pigs. It will be readily understood that the isolation of *B. cholerae suis* from material containing an immense number of colon bacilli is a matter of unusual difficulty, and it is therefore not surprising that we should fail to discover it even though it may have been present in very considerable numbers. The isolation of a non-virulent form of *B. cholerae suis* from the intestines is, therefore, considered very important on account of the support it lends to the hypothesis that *B. cholerae suis* may be frequently an inhabitant of the intestine of the normal hog.

If such a hypothesis be true, the conditions would be entirely analogous to those under which many pathogenic micro-organisms exist, as, for example, the pneumococcus in the mouths of healthy individuals, and the swine plague bacillus (*B. suis*) on the tonsils of healthy hogs, and in the pens where healthy hogs are kept, the supposition being that micro-organisms present in small numbers or possessing a low degree of virulence are successfully resisted until certain influences either heighten the virulence of the bacteria or lower the resisting power of the animal body. The production of disease by feeding small quantities of *B. cholerae suis* might seem to indicate that that organism could not remain in the hog's intestine without giving rise to more or less serious disturbance, but it should be remembered that these small quantities of culture ($\frac{1}{10}$ to $\frac{1}{2}$ cc.) contain enormous numbers of bacilli and probably many more than the hog under ordinary circumstances is able to resist.

The toxin present in artificial cultures may also play some part in causing illness when cultures are fed, or it may be—and this last supposition seems not unlikely—that *B. cholerae suis*, if it exists in the bodies of normal hogs, possesses a very low degree of virulence, and that it is only through a lowered resistance on the part of the hog that it is enabled to enter the circulation and cause disease. The isolation from a healthy hog of an organism indistinguishable from *B. cholerae suis* except by absence of pathogenic power for guinea-pigs lends strength to the explanation last mentioned of the way in which hogs are liable to attacks by *B. cholerae suis*. *B. coli communis* is a familiar example of an organism constantly present in health, and yet assuming under certain conditions very great pathogenic power.

Whatever may be the port of entry or the influences which bring about an invasion of *B. cholerae suis*, we are convinced that the filterable virus was responsible for the high degree of infectiousness and, therefore, for the spread of the disease encountered in the several outbreaks of acute hog cholera described in previous pages of this bulletin. It seems reasonable to suppose that what has been found to be true in these eight outbreaks is also true of other epidemics of acute hog cholera, and therefore that the extensive losses occasioned by outbreaks of that disease are to be attributed, in the main, to the filterable virus. In many of the individual hogs, the fatal termination may have been in large measure due to *B. cholerae suis*, but the probabilities are that, without the filterable virus, comparatively few hogs would have been attacked.

It follows from all that has been demonstrated in the preceding pages that, if a practical method of protecting hogs from the filterable virus should be discovered, the problem of combating hog cholera, at least the highly infectious form of that disease, will have been solved.