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A. D. MELVIN, CHIEF OF BUREAU.

FURTHER EXPERIMENTS CONCERNING THE PRODUCTION OF IMMUNITY FROM HOG CHOLERA.

BY

M. DORSET, M. D., C. N. MCBRYDE, M. D.,
AND
W. B. NILES, D. V. M.,
Of the Biochemic Division.



WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN 102.

A. D. MELVIN, CHIEF OF BUREAU.

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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., September 25, 1907.

SIR: I have the honor to transmit herewith a paper entitled "Further Experiments Concerning the Production of Immunity from Hog Cholera," by Doctors M. Dorset, C. N. McBryde, and W. B. Niles, of the Biochemic Division of this Bureau, and to recommend its publication as a bulletin in the Bureau series.

This paper embodies the records of experiments carried out during the seasons of 1903, 1904, 1905, and 1906, this work being a continuation of that reported in Bulletin 72 of this Bureau. The results obtained show quite clearly that a comparatively certain method of protecting hogs from hog cholera has been secured. The experiments, however, have not as yet been extensive enough, nor have they been applied under the varying conditions of practice to a sufficient extent to warrant claims concerning the practical value of the methods described in this paper, though it is strongly believed that they should be of great service in combating hog cholera in this country.

Very respectfully,

A. D. MELVIN,
Chief of Bureau.

HON. JAMES WILSON,
Secretary of Agriculture.

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FURTHER EXPERIMENTS CONCERNING THE PRODUCTION OF IMMUNITY FROM HOG CHOLERA.

INTRODUCTION.

In a previous publication from this Bureau^a it has been shown that the disease known as hog cholera, encountered in epizootic form in Iowa and other parts of the United States, is caused by a filterable and probably ultramicroscopic virus which exists in the blood of sick hogs, and that *Bacillus cholerae suis*, although present in most cases of hog cholera, in all likelihood plays the part of a secondary invader. This modification of our views regarding the etiology of hog cholera necessarily carried with it changes in plans for combating the disease.

Most of the methods for combating the disease which have heretofore been proposed were very naturally based upon the general belief that *B. cholerae suis* was the cause of hog cholera, and many different methods of preparing vaccines and antitoxic sera through the use of that organism were devised and subjected to extensive laboratory as well as practical tests. Although a certain degree of success was apparently attained in some instances, none of these methods have been found to act with sufficient reliability to warrant their use in practice.

In the case of vaccines prepared from cultures of *B. cholerae suis* a certain amount of immunity against that organism may have been at times induced in the treated hogs, and a similar result may have been secured by the administration of antitoxins prepared through the use of the same organism, for in the earlier publication above referred to it has been shown that *B. cholerae suis* usually invades the body of the hog after injection with the filterable virus, and, as stated there, it is reasonable to believe that the final result of the disease is in many cases materially influenced by the presence of that organism. Any vaccine or serum which protects against *B. cholerae suis* would therefore be expected to aid the body in its struggle against the twofold invasion of filterable virus and *B. cholerae suis*. We believe that the partial success attained at times by the use of sera and vaccines prepared from *B. cholerae suis* is to be explained upon this ground.

In view of the fact, however, that the filterable virus is the primary invader, and that it appears to be quite sufficient in itself to cause

^a Bulletin 72, Bureau of Animal Industry, U. S. Dept. of Agriculture.

the death of most hogs, any substance which protects only from *B. cholerae suis* must necessarily be regarded simply as a palliative, which, in the light of past experiences, is without a value that is in any way commensurate with the cost of applying it.

It thus seems evident that all efforts looking to the prevention or treatment of hog cholera should be directed primarily toward securing some means of protecting the hog from invasion by the filterable virus, or of overcoming that virus if invasion has already taken place.

Since it was first definitely determined that *B. cholerae suis* is not the primary cause of hog cholera the work of the Bureau of Animal Industry has been along the lines just suggested. Much difficulty has been experienced in carrying out this work, due in great measure to our inability to cultivate the filterable virus artificially and to the fact that of all animals exposed to this virus only hogs have proven susceptible. We have thus been restricted to the use of the blood and tissues of sick hogs in our attempt to secure a satisfactory serum or vaccine.

As stated in Circular 43 of this Bureau, issued February 12, 1904, "the basis of the immunity experiments, therefore, has been the use of attenuated and disease-producing liquid or dried blood, or the use of this blood mixed with blood obtained from immune animals, in which animals the immunity has been increased by the injection of large doses of disease-producing blood obtained from hogs known to have the disease; or, in other words, disease-producing blood and anti-toxic blood separate and combined have been successfully used." ^a

The efforts to attenuate the virus in blood from diseased hogs by drying, by heat, and by chemical agents have not led to satisfactory results. This appears to be due in part at least to the unequal resistance of the virus in different lots of diseased blood to these agencies. At times the protection afforded by diseased blood attenuated artificially has appeared to be complete; at others a vaccine prepared in identically the same manner has either caused death, thus showing an entire lack of attenuation, or else the hogs have shown no ill effects from the vaccination but have succumbed promptly when exposed to hog cholera, showing that the supposed vaccine had no protective power whatever.

It may be that later investigations will show that it is possible to obtain a satisfactory vaccine for hog cholera by attenuating the virus, but our own results were so discouraging that efforts in this direction were abandoned and our entire attention turned to the production of a serum in the manner hereinafter described.

^a Subsequent to the publication of Circular 43, Boxmeyer and McClintock (*Journal of Infectious Diseases*, vol. 2, No. 2, March, 1905), described a few experiments along these lines and seemed to obtain better results by using a combination of disease-producing blood and immune serum than by using an artificially attenuated virus.

PRELIMINARY EXPERIMENTS.

The earlier experiments, which had for their object the production of a serum which would protect from the filterable virus, were not wholly successful, some of them indeed resulting in almost complete failure; but as these failures are quite instructive on account of the light they throw upon the details of producing the serum, it seems desirable to describe them very briefly before taking up the more recent work.

As it was known that immune hogs could withstand perfectly injections of disease-producing blood^a which were more than sufficient to destroy nonimmunes, the plan originally adopted was to inject hogs immune from hog cholera with disease-producing blood in increasing doses, the object of this treatment being, of course, to impart a protective power to the blood of the immune, previous experiments having shown that blood from nonhyperimmunized immunes possessed very little if any such power. The records of two experiments carried out with serum from immune hog S44 treated in this manner are given below.

EXPERIMENTS OF 1903 AND 1904.

Immune hog S44 was an adult animal weighing approximately 150 pounds, which had recovered from an attack of hog cholera. This immune was injected subcutaneously with increasing doses of disease-producing blood which had been shown to contain the filterable virus.^b

The amount of disease-producing blood injected was increased to 400 c. c. before blood was drawn from the immune.

The following statement shows the dates and amounts of disease-producing blood used for injection of immune hog S44,^c with records of blood drawings:

July 24, 1903, 1 c. c. disease-producing blood injected.
August 4, 1903, 4 c. c. disease-producing blood injected.
August 19, 1903, 12 c. c. disease-producing blood injected.
September 1, 1903, 30 c. c. disease-producing blood injected.
September 26, 1903, 50 c. c. disease-producing blood injected.
October 14, 1903, 250 c. c. disease-producing blood injected.
October 20, 1903, 250 c. c. disease-producing blood injected.
October 29, 1903, 400 c. c. disease-producing blood injected.
November 9, 1903, 100 c. c. blood drawn from tail.

^aThe term "disease-producing blood" is applied in this paper to blood from hogs affected with hog cholera, which blood was shown to be capable of causing the disease when injected into nonimmune hogs.

^bSee experiments with blood from Herd E, Bulletin 72, Bureau of Animal Industry, pp. 43-65.

^cWe acknowledge our indebtedness to Dr. E. C. Schroeder, who had these injections, as well as those described under experiments A and B, made for us at the Experiment Station of this Bureau at Bethesda, Md.

November 19, 1903, 400 c. c. disease-producing blood injected.
 December 10, 1903, 480 c. c. disease-producing blood injected.
 January 4, 1904, 150 c. c. blood drawn from tail.
 January 5, 1904, 490 c. c. disease-producing blood injected.
 January 28, 1904, 480 c. c. disease-producing blood injected.
 February 9, 1904, 185 c. c. blood drawn from tail.

It is interesting to note that even the largest doses of disease-producing blood did not seriously disturb the general health of the immune, the only ill effects noted after the injection of the blood being swelling at the point of injection and occasionally a temporary loss of appetite.

Two experiments to test the protective value of the blood drawn from hog 844 were carried out.

TABLE 1.—*Experiment A with blood drawn November 9, 1903, from immune hog 844.*

No. of hog.	Material injected.	Inoculation.		Exposure.	
		Date.	Result.	Date.	Result.
1002....	20 c. c. defibrinated blood of hog 844, subcutaneously.	Nov. 11, 1903	Remained well.	Dec. 18, 1903	Died; hog cholera.
1004....	20 c. c. defibrinated blood of hog 844+1 c. c. disease-producing blood.do.....do.....do.....	Remained well.
1003....	1 c. c. disease-producing blood.....do.....	Died; hog cholera.do.....do.....

TABLE 2.—*Experiment B with blood drawn February 9, 1904, from immune hog 844.*

No. of hog.	Material injected.	Inoculation.		Exposure.	
		Date.	Result.	Date.	Result.
1086....	20 c. c. blood of hog 844+1 c. c. disease-producing blood.	Feb. 11, 1904	Remained well.	Mar. 11, 1904	Died; hog cholera.
1087....do.....do.....do.....do.....	Remained well.
1088....do.....do.....do.....do.....	Sick; recovered.
1089....do.....do.....do.....do.....	Died; hog cholera.
1090....	1 c. c. disease-producing blood alone.....do.....	Died; hog cholera.do.....do.....
1091....do.....do.....do.....do.....do.....

Experiment A.—By referring to Table 1 it will be seen that only three hogs were used in this experiment. Of these, after injection, the one that received only immune blood remained well; the one which received both immune blood and disease-producing blood also remained well, while the third animal which received only disease-producing blood died of hog cholera. When the two surviving hogs were injected approximately thirty days later with disease-producing blood to test their immunity the one which had been treated previously with immune blood alone died, while the other which had received the mixture of immune blood and disease-producing blood remained well.

Experiment B.—This experiment was designed as a control on the results of experiment A, except that it was considered unnecessary to use the immune serum alone, as it appeared not to produce any

lasting immunity. One point of difference between experiments A and B should be noted: Experiment B was carried out with immune blood drawn February 9, 1904, whereas in experiment A the drawing of November 9, 1903, was used.

The two experiments agree in showing that the serum from the hyperimmunized immune was capable of protecting hogs from hog cholera when the infection took place simultaneously with the administration of the immune serum. It is evident, however, that the duration of the immunity secured by these injections was not of sufficient length to render the method, as used at that time, suitable for practical purposes. Other experiments with blood from immune hog 844 and from other hyperimmunized hogs about the same time did not lead to more promising results, and the experiments were therefore laid aside temporarily on account of the pressure of other work.

EXPERIMENTS OF 1905.

In the spring of 1905 this work was resumed with the intention of determining whether it was possible to secure by means of the above-described method, or some variation of it, an immunity which would be more lasting than that obtained in previous experiments.

Four immunes were used to supply serum. These hogs were all adult animals, more than a year old, and had all passed through exposure to hog cholera without exhibiting noticeable symptoms of disease. It is difficult to classify these hogs as possessing either "natural" immunity or "acquired" immunity, as they may have suffered a light attack of disease which passed by unnoticed. It is quite certain, however, that they possessed considerably greater powers of resistance than is ordinarily the rule in hogs.

Two methods were resorted to for hyperimmunizing these hogs. For the sake of convenience the first plan has been designated the "slow method" and the second the "quick method," depending upon the manner of injecting the disease-producing blood. The slow method consisted of three successive injections of disease-producing blood, followed by a drawing of blood from the immune, and then more injections of disease-producing blood, followed by more drawings from the immune; in other words, it followed in a general way the plan used in raising the potency of the blood serum of immune cattle for securing a protective serum for "rinderpest." The quick method is along the line of Nicolle's so-called "méthode brutale" of producing an antirinderpest serum, and was planned to consist of one very large dose of disease-producing blood administered subcutaneously, and followed after an interval of ten days or more by blood drawings from the immune. It was planned to use a different strain of disease-producing blood for injecting each immune, but this could not be done owing to the impossibility of securing a

sufficient quantity of blood from different outbreaks at the time the injections were to be made. All of the immunes, therefore, were injected with diseased blood which might be considered a mixture of several different strains, but which was virulent in all cases (as proven by the injection of controls) unless otherwise stated.

In view of the virulence of this blood the behavior of the immunes after injection seems truly remarkable, for not one of them showed any ill effects whatever other than a transitory stiffness, due undoubtedly to the local inflammation caused by the large injections. Immune hog Q No. 2 received 1,440 times the usual lethal dose of hog-cholera blood without any effect whatever being noticeable except a little stiffness. As far as the immune is concerned, therefore, there seemed to be no reason why the single large dose should not be used instead of the much slower process of administering the disease-producing blood in increasing doses.

IMMUNES USED FOR PRODUCTION OF SERUM IN 1905.

A very brief history of each of the immunes is given below. It should be noted that the virulence of the disease-producing blood injected was tested each time by the injection of one or more non-immune hogs, and in each case it proved to be of high pathogenic power unless otherwise stated.

HYPERIMMUNIZED IMMUNE Q NO. 1 (QUICK METHOD).

This hog passed through an exposure to hog cholera in the summer of 1904 without showing any symptoms of disease, and was not subjected to further exposure until the summer of 1905, when it weighed approximately 130 pounds. On September 12, 1905, it was injected subcutaneously with 900 c. c. of blood that was supposed to be virulent, but nonimmune hogs that were given small doses of the same blood did not sicken, so the treatment had to be repeated. On October 16, 1905, this hog was given 1,275 c. c. of virulent blood. No reaction worthy of note followed this injection. Blood drawings from this immune were made as follows:

November 1, 1905, 140 c. c. defibrinated blood obtained.

November 9, 1905, 125 c. c. defibrinated blood obtained.

November 16, 1905, 150 c. c. defibrinated blood obtained.

December 27, 1905, 200 c. c. defibrinated blood obtained.

The first drawing of this blood was used in the vaccination of hogs in the 1905 experiment.

HYPERIMMUNIZED IMMUNE Q NO. 2 (QUICK METHOD).

This hog passed through an exposure to hog cholera in 1904 without showing any symptoms of disease. On November 1, 1905, when the hog weighed 145 pounds, it was injected subcutaneously with 1,440 c. c. of disease-producing blood. Some soreness and stiffness followed this injection, but otherwise the animal remained well.

Drawings of blood were made from hog Q No. 2 as follows:

- November 24, 1905, 225 c. c. of defibrinated blood obtained.
- December 1, 1905, 230 c. c. of defibrinated blood obtained.
- December 8, 1905, 200 c. c. of defibrinated blood obtained.
- December 27, 1905, 350 c. c. of defibrinated blood obtained.

The first drawing of blood from this hog was used in the vaccination of hogs in the 1905 experiments, and the second drawing was used in the 1906 experiment to test the keeping quality of immune serum.

HYPERIMMUNIZED IMMUNE 1234 (SLOW METHOD).

This hog was first exposed to hog cholera on November 9, 1904, when it was placed in a pen with sick hogs; no illness resulted. On September 29, 1905, at which time it weighed 168 pounds, hog 1234 was injected subcutaneously with 175 c. c. of disease-producing blood. This was followed on October 21 by 420 c. c. of disease-producing blood, and the third dose, 840 c. c., was given November 5, 1905. About two months later, or, to be exact, on January 13, 1906, the hog received the last dose of disease-producing blood. The virulence of the blood used for each of these injections was proven by the production of disease in nonimmune hogs. Drawings of blood were made from the tail of this hog as follows:

- November 14, 1905, 100 c. c. defibrinated blood obtained.
- November 21, 1905, 130 c. c. defibrinated blood obtained.
- November 28, 1905, 125 c. c. defibrinated blood obtained.
- January 24, 1906, 450 c. c. defibrinated blood obtained.
- February 2, 1905, 525 c. c. defibrinated blood obtained.
- August 31, 1906, 60 c. c. defibrinated blood obtained.

The first drawing of serum from hog 1234 was used in the 1905 experiments, while the second drawing and last drawing were used in 1906 Experiments XXVIII and XXIX, hereinafter described.

HYPERIMMUNIZED IMMUNE 1212 (SLOW METHOD).

Hog 1212 was injected with blood from a sick hog on August 27, 1904, and was later placed in the exposure pen, but in neither instance did the hog become sick.

On September 29, 1905, this immune weighed approximately 160 pounds and was injected with 175 c. c. of disease-producing blood. The injection of disease-producing blood was repeated as follows:

- October 21, 1905, 420 c. c.
- November 5, 1905, 820 c. c.
- January 13, 1906, 840 c. c.

Practically no symptoms followed these injections, although non-immunes that received only 5 c. c. of the same blood contracted hog cholera and in most instances died.

Drawings of blood were made from immune hog 1212 as follows:

- November 14, 1905, 100 c. c. defibrinated blood obtained.
- November 21, 1905, 140 c. c. defibrinated blood obtained.
- November 28, 1905, 10 c. c. defibrinated blood obtained.
- January 24, 1906, 75 c. c. defibrinated blood obtained.

The first drawing of this serum was the only one used for experimental purposes, and this was used in the 1905 experiments only.

VACCINATION EXPERIMENTS IN 1905.

Only the first drawings of hyperimmune blood were used in the experiments described below. The blood was defibrinated, separated from the clot, and mixed with sufficient 5 per cent carbolic acid solution to give a 0.5 per cent solution in the defibrinated blood.

In so far as the method of vaccination is concerned, the 1905 experiments practically repeat those of 1903 and 1904, though of course the mode of hyperimmunizing the immunes was modified as explained above. It was also hoped to determine whether different immunes varied in their power to yield a potent serum, whether the "quick" or the "slow" method of hyperimmunization was preferable, and whether by increasing the amount of disease-producing blood given to the immunes the duration of the immunity in treated hogs could be prolonged. The amount of disease-producing blood administered with the immune blood for the purposes of vaccination was also varied for similar reasons. In all treated hogs the diseased blood and the hyperimmune serum were injected simultaneously on different sides of the body beneath the skin. Control animals of the same size and age, and of the same litters also, if available, were injected with the same dose of the same disease-producing blood alone in order to demonstrate the protective value of the immune blood.

Several weeks after vaccination the hogs which withstood the treatment were placed in an "exposure pen."^a This pen contained hogs sick of hog cholera, and as the pen was comparatively small the exposure to which the treated hogs were subjected was quite as severe as any that could be encountered under natural conditions by association. The severity of this exposure is made plain by the death of the control animals.

It seems unnecessary to give detailed records of the hogs used in this and succeeding experiments. It may be stated, however, that in cases where hogs became sick the clinical records and the autopsy findings in all cases were such as are usually met with in hog cholera, unless otherwise stated. The salient features are set down in Table 3.

^a The term "exposure pen" as used in this paper refers to a pen where hogs sick of hog cholera were kept and hogs placed therein were subjected at all times to close association with the sick animals. The exposure was entirely by association, as the hogs were not allowed to feed upon the bodies of those that died.

TABLE 3.—Results of hog vaccinations in experiments of 1905.

No. of hog.	Weight.	Material injected.	Inoculation.		Exposure.		Autopsy.
			Date.	Result.	Date.	Result.	
1397	Pounds. 60	10 c. c. first drawing of serum from hog Q No. 1; 1 c. c. disease-producing blood of hog 1394.	Dec. 2, 1905	Sick; recovered.	Dec. 27, 1905	Remained well.	
1398	60	20 c. c. first drawing of serum from hog Q No. 1; 1 c. c. disease-producing blood of hog 1394.	do.	do.	do.	do.	
1422	50	10 c. c. first drawing of serum from hog Q No. 1; 2 c. c. disease-producing blood of hog 1406.	Dec. 16, 1905	Died Dec. 29, 1905.			Engorged spleen, etc.
1423	50	20 c. c. first drawing of serum from hog Q No. 1; 2 c. c. disease-producing blood of hog 1406.	do.	Died Jan. 22, 1906.			Ulcers.
1399	60	10 c. c. first drawing of serum from hog Q No. 2; 1 c. c. disease-producing blood of hog 1394.	Dec. 2, 1905	Remained well.	Dec. 27, 1905	Remained well.	
1400	60	20 c. c. first drawing of serum from hog Q No. 2; 1 c. c. disease-producing blood of hog 1394.	do.	do.	do.	do.	
1424	45	10 c. c. first drawing of serum from hog Q No. 2; 2 c. c. disease-producing blood of hog 1406.	Dec. 16, 1905	do.	Jan. 9, 1906	do.	
1425	45	20 c. c. first drawing of serum from hog Q No. 2; 2 c. c. disease-producing blood of hog 1406.	do.	do.	do.	do.	
1401	60	10 c. c. first drawing of serum from hog 1294; 1 c. c. disease-producing blood of hog 1394.	Dec. 2, 1905	do.	Dec. 27, 1905	do.	
1402	60	20 c. c. first drawing of serum from hog 1294; 1 c. c. disease-producing blood of hog 1394.	do.	do.	do.	do.	
1426	50	10 c. c. first drawing of serum from hog 1294; 2 c. c. disease-producing blood of hog 1406.	Dec. 16, 1905	do.	Jan. 9, 1906	do.	
1427	50	20 c. c. first drawing of serum from hog 1294; 2 c. c. disease-producing blood of hog 1406.	do.	do.	do.	do.	
1403	60	10 c. c. first drawing of serum from hog 1212; 1 c. c. disease-producing blood of hog 1394.	Dec. 2, 1905	do.	Dec. 27, 1905	do.	
1404	60	20 c. c. first drawing of serum from hog 1212; 1 c. c. disease-producing blood of hog 1394.	do.	do.	do.	do.	

TABLE 3.—Results of hog vaccinations in experiments of 1905—Continued.

No. of hog.	Weight. Pounds.	Material injected.	Inoculation.		Exposure.		Autopsy.
			Date.	Result.	Date.	Result.	
1428	50	10 c. c. first drawing of serum from hog 1212; 2 c. c. disease-producing blood of hog 1406.	Dec. 16, 1905	Remained well	Jan. 9, 1906	Remained well	
1429	50	20 c. c. first drawing of serum from hog 1212; 2 c. c. disease-producing blood of hog 1406.	do	do	do	do	
1405	60	1 c. c. disease-producing blood of hog 1394.	Dec. 2, 1905	Sick Dec. 7; died Dec. 12, 1905.			Hemorrhagic lesions.
1406	60	do	do	Sick Dec. 7; Killed Dec. 18, 1905.			Do.
1407	60	do	do	Sick Dec. 7, 1905; died Feb. 7, 1906.			Ulcers.
1408	60	do	do	Sick; recovered.			
1430	50	2 c. c. disease-producing blood of hog 1406.	Dec. 16, 1905	Died Jan. 3, 1906.			Hemorrhagic lesions.
1431	50	do	do	Died Jan. 4, 1906.			Hemorrhages and ulcers.
1432	50	do	do	Died Jan. 11, 1906.			Do.
1433	50	do	do	Died Jan. 12, 1906.			Do.
1400	60	Check in exposure pen.	do	do	Dec. 27, 1905	Died Feb. 10, 1906.	Do.
1410	60	do	do	do	do	Remained well	Do.
1411	60	do	do	do	do	Very sick; killed	Do.
1412	60	do	do	do	do	do	Do.
1455	35	do	do	do	Jan. 8, 1906	Died Jan. 17, 1906.	Do.
1456	40	do	do	do	do	Died Jan. 20, 1906.	Do.
1457	40	do	do	do	do	Remained well.	Do.

As will be seen from Table 3, 16 hogs were vaccinated by the use of a combination of hyper-immune blood and disease-producing blood, blood from each immune serving for the treatment of 4 hogs in doses of 10 c. c. and 20 c. c. Half of the treated hogs received 2 c. c. of disease-producing blood and the remainder 1 c. c., simultaneously with the immune blood. For the purpose of control, 8 hogs were injected with disease-producing blood alone, half of these receiving 2 c. c. and the remainder 1 c. c., all injections being made subcutaneously.

Of the 16 hogs which were vaccinated, 2 died, 2 became sick but recovered, and 12 showed no symptoms of illness whatever as a result of the treatment. The animals which became sick were all treated with serum from immune hog Q No. 1, the serum from this immune appearing to be barely sufficient to protect from 1 c. c. of disease-producing blood, but not potent enough to protect from 2 c. c. Serum from the other 3 immunes gave perfect protection from 2 c. c. as well as from 1 c. c. of

disease-producing blood when injected simultaneously with it, and the protection afforded by 10 c. c. of this serum seemed to be quite as satisfactory as that secured by the use of twice that amount.

The protective power of the serum from hyperimmunized hogs Q No. 2, 1234, and 1212 is made strikingly apparent by the behavior of the control animals, which were injected with disease-producing blood alone. Of the 8 hogs thus injected, 6 died of hog cholera, 1 was killed when in a moribund condition, and the eighth hog also contracted the disease but recovered. From these facts there seems to be no reason for doubting the protective power of the serum from these 3 immunes when injected simultaneously with disease-producing blood.

In order to determine whether or not this immunity was of more than fleeting duration the survivors from the vaccinating dose were placed in the exposure pen together with 7 untreated healthy hogs, as shown in Table 3. This exposure to disease by association took place twenty-four days after vaccination in the case of 6 of the treated hogs and twenty-five days after the vaccination of the remaining 8 hogs. The interval between vaccination and exposure was not as long as was desired, but owing to the lateness of the season it was considered best to expose at that time rather than wait until the following summer.

It will be seen from the table that not one of the vaccinated hogs contracted disease as a result of this exposure. Of the 7 control hogs which were placed in the exposure pen at the same time as the vaccinated hogs to prove the virulence of the exposure, 5 contracted hog cholera, while 2 remained well. Of the 5 which contracted the disease, 3 died and 2 were killed when desperately sick to secure blood for other experiments.

From the results given above there seems to be no reason to doubt the power of serum from hyperimmunized immunes to protect non-immunes from a fatal dose of disease-producing blood administered simultaneously with the hyperimmune serum. It appears also that in the case of serum from 3 of the immunes, at least, a dose of 10 c. c. was quite as effective as 20 c. c. The serum from both immunes which were hyperimmunized by the slow method proved potent, whereas serum from immune hog Q No. 1 treated by the quick method failed to give satisfactory results. The low protective power of the serum from this immune would appear to be due to some peculiarity on the part of the immune itself, inasmuch as blood from immune hog Q No. 2 treated by the same method proved quite as effective as blood from either of the "slow" immunes. Without more extended trials, therefore, with other immunes, preference could not be given to either one of the methods of hyperimmunization over the other. These experiments indicate also that large doses of disease-producing blood

may be expected to confer upon the blood of the immune the power to protect nonimmunes for a greater length of time than blood from immunes which receive considerably smaller doses, for in the experiments carried out up to this time the immunity exhibited by the vaccinated hogs after treatment may have been due (in some of the treated hogs at least) to the action of the immune blood alone and not necessarily to the combined action of immune and disease-producing blood.

It will be remembered that in the experiments of 1903 and 1904 the immune blood protected the treated animals from the disease-producing blood which was administered simultaneously with it, but some of the vaccinated animals succumbed when exposed to hog cholera about thirty days later. The most rational explanation of this occurrence appeared to lie in the supposition that too much immune blood was administered with the diseased blood. The animals would thus be completely protected from the simultaneous injection of the virus, but owing to this complete protection from the virus the animal body would have no stimulus to bring about a reaction and a consequent production of an active immunity which was aimed at by the simultaneous method of vaccination. Under such circumstances it seemed reasonable to suppose that the vaccinated hogs possessed only a passive and transitory immunity, such as might be expected from the injection of an antitoxin.

In the experiments of 1905 we were unable to clear up this point, owing to difficulties experienced in securing suitable material for the work. The experiments were greatly delayed and it was only possible to carry out those which have just been described. Inasmuch as no hogs were injected with the immune serum alone in these experiments, the question as to the nature of the immunity afforded by our injections could not be definitely determined. There is reason to believe, however, that this may have been due to the serum alone, at least in the case of those which received 20 c. c. of immune serum, while there can be no doubt that the 2 hogs 1397 and 1398, which became sick but recovered, secured an active immunity. It is entirely possible that the other hogs which received 10 c. c. of immune blood with the disease-producing blood suffered a very mild attack of disease and thus secured an active, permanent immunity. It would be natural to suppose, however, that if 10 c. c. of immune serum gave sufficient protection from the disease-producing blood to prevent any outward manifestation of disease the protection afforded by 20 c. c. would be complete, and that subsequent immunity in these hogs would presumably be due to the immune blood alone.

EXPERIMENTS OF 1906.

The experiments thus far described show quite clearly that blood from certain hyperimmunized immunes possesses the power to protect nonimmune hogs from a fatal dose of disease-producing blood administered simultaneously with the serum. It has also been shown that hogs which survive a simultaneous injection of serum and disease-producing blood are rendered immune for at least twenty-five days thereafter, even though they show no reaction after the injection. Notwithstanding the success which attended most of these experiments, many questions affecting the practicability of this method for combating hog cholera yet remained undetermined. In conducting the experiments during the year 1906, therefore, we have endeavored to decide, in so far as time and material would permit, these other questions in connection with experiments designed to substantiate the results already obtained.

PLAN OF THE EXPERIMENTS.

The experiments which are to be described hereafter will perhaps be best understood by indicating the general plan which was followed. The questions to be decided concerned first, the immunes, and second, the hogs that were to be vaccinated.

Questions concerning the immunes.—Without attempting to go into the plans in detail, the 1906 experiments may be said to have had the following objects in view: To determine (1) whether natural immunes or those which have acquired immunity are best suited for serum production; (2) what percentage of either natural or acquired immunes may be expected to yield a potent serum; (3) whether the "quick method" or the "slow method" of hyperimmunization yields the best results; (4) whether disease-producing blood from different outbreaks of hog cholera is equally well suited for the purposes of hyperimmunization; (5) whether the amounts of disease-producing blood used for hyperimmunization might be reduced without affecting the potency of the serum, and (6) how long after the last injection with disease-producing blood the immunes retain the power to furnish a potent serum.

Questions concerning vaccination. Many questions arise in connection with the practical application of the serum, but it has not thus far been possible to attempt to answer all of them. The principal points we had in mind during the 1906 experiments were as follows: To determine (1) the dose of serum required to protect a small hog (25 to 50 pounds) from a simultaneous injection of a fatal dose of disease-producing blood; (2) the duration of the immunity which follows this simultaneous injection; (3) whether it is necessary to

produce a reaction (i. e., visible illness or fever, or both) in order to secure a lasting immunity; (4) whether the administration of sufficient serum to prevent a reaction will tend to shorten the duration of the immunity in vaccinated hogs; (5) whether hogs vaccinated by a simultaneous injection of serum and disease-producing blood are likely to be injured or stunted in growth; (6) whether hogs vaccinated by this "serum-simultaneous" method may communicate hog cholera to unvaccinated hogs by association with them; (7) whether a lasting immunity may be produced by the injection of the serum alone; (8) whether the serum alone can be used successfully as a cure; (9) how long the serum will retain its potency, and (10) whether satisfactory results may be obtained by vaccinating suckling pigs.

GENERAL PLAN FOR THE PRODUCTION OF SERUM IN 1906.

The immunes used.—Eight immune hogs were to be hyperimmunized. If possible, 4 of these were to be hogs that had acquired immunity, while the remainder were to be natural immunes. Four of these 8 hogs (2 natural immunes and 2 that possessed acquired immunity) were to be hyperimmunized by the use of one strain of disease, while the remaining 4 were to be given disease-producing blood from an entirely different source.

Method of hyperimmunization.—There were certain general features in the process of immunization which applied alike to all immunes treated by the same method. First of all, in order to make sure of a firm immunity and to avoid losses later, a preliminary injection of 20 c. c. of disease-producing blood was given each immune. After this the regular process of hyperimmunization was begun. Four of the immunes were hyperimmunized by the quick method and 4 by the slow method described under the 1905 experiments.

The immunes treated by the quick method were injected with one dose of disease-producing blood, the amount administered being equal to 1,000 c. c. of disease-producing blood to each 100 pounds of body weight. Exactly three weeks after this injection blood was drawn from the tails of these immunes and preserved for experimental use. This blood drawing from the immunes was repeated at intervals of seven or eight days until three drawings had been made, and in most cases, as shown by the detailed records which follow, a fourth drawing was made approximately a month after the third drawing.

The immunes treated by the slow method were injected three times. The first dose was in the proportion of 100 c. c. of disease-producing blood to 100 pounds of body weight; the second dose, which followed the first after from ten to fourteen days, was in the proportion of 250 c. c. per 100 pounds body weight, and the third and last dose, which was given approximately twelve days after the

second, was in the proportion of 500 c. c. per 100 pounds of body weight. Blood drawings from the immunes hyperimmunized by the slow method were begun nine or ten days after the last injection of disease-producing blood and repeated at intervals of seven days.

The object of these repeated blood drawings from the immunes was of course to secure blood for the purpose of ascertaining how long after the last injection of disease-producing blood the immune retained the power to furnish potent serum. These different drawings of immune blood—that is, some of them—were tested on non-immune hogs, as shown in the experiments hereinafter described.

Method of collecting and preserving the immune blood.—The immune blood was drawn in all cases from the tails of the immunes and was collected in a wide, sterilized dish. It was allowed to coagulate, and the clot which formed was then subjected to pressure in order that all of the serum might be separated from it. Instead of a clear serum, therefore, we secured always a serum containing large numbers of red blood corpuscles and which might be more properly called defibrinated blood. The time required for the red cells to settle and the very considerable loss of serum which would have resulted if we had attempted to use clear serum only, owing to lack of facilities for using a large centrifuge, made it necessary to employ the serum containing red cells. In applying a similar method to cattle the use of a serum containing red cells would no doubt be considered objectionable, owing to the danger of transmitting some intercurrent disease, such as Texas fever, through such injections. Such contingencies were not likely to occur in the case of hogs, however, and, furthermore, any possible danger of this kind was no doubt avoided through the addition of an antiseptic to the serum immediately after it was drawn. After the serum had been completely separated from the clot by pressure and by straining through a sterilized cloth it was mixed with a 5 per cent aqueous solution of carbolic acid in the proportion of 9 parts of serum to 1 of carbolic-acid solution, thus giving a 0.5 per cent solution of carbolic acid in the serum. This served to keep the serum perfectly, provided it was placed at once in sterilized bottles until used. It should be remembered that all of the hyperimmune serum used in the experiments described hereafter consisted, in reality, of 1 part of carbolic-acid solution and 9 parts of serum and red cells.

Sources of disease-producing blood with details of the hyperimmunization.—As already stated, disease-producing blood from two entirely distinct sources was used for the treatment of the immunes. One of these strains of disease was obtained near Scribner, Nebr., and is referred to as the "Scribner disease." The other strain of disease was secured near Alexandria, Va., and has been designated the "Syphax disease." Blood from both of these sources proved quite virulent

when administered to nonimmune hogs; the blood from the Scribner outbreak, however, appeared to produce a somewhat more acute type of disease than the blood from the Syphax outbreak. The immunes hyperimmunized with blood from the Scribner outbreak are designated "Scribner immunes," and, depending upon the method used for hyperimmunization, the individuals have been called "Scribner quick immunes" or "Scribner slow immunes," as the case might be. The same plan is followed in speaking of the "Syphax immunes."

DESCRIPTION OF THE SCRIBNER OUTBREAK.

This outbreak of hog cholera occurred near Scribner, Nebr., in the fall of 1905. When this locality was first visited by one of us it was found that hog cholera of a virulent type existed on some half dozen farms. Several of these were visited and the sick animals were found to exhibit the usual symptoms of hog cholera, and at autopsy the usual lesions, such as intestinal ulcers and ecchymoses in the various organs, were noted. From one of these infected herds, in which the final loss was about 74 per cent, blood was secured for experimental purposes. This blood was collected in a sterile vessel, defibrinated, and kept on ice until it was used at the Bureau experiment farm near Ames, Iowa, for the injection of hogs 1395, 1393, and 1394, which received, respectively, 1 c. c., 2.5 c. c., and 5 c. c., subcutaneously on November 18, 1905. These hogs became sick six days after injection, and hogs 1393 and 1395 died on December 3, 1905. The other hog, 1394, was killed on December 1 to secure blood for other experiments. Autopsies which were held on 2 of these hogs revealed the usual lesions of hog cholera.

The blood of hog 1394, after dilution, was filtered through a Chamberland bougie, and the filtered blood was proven to possess the same degree of virulence as the unfiltered blood.

A portion of the unfiltered blood of hog 1394 was placed in sealed glass bulbs and preserved on ice until May, 1906, when, upon injection, it proved to be quite virulent, and hogs inoculated with this disease or exposed to it furnished all of the blood used in hyperimmunizing the so-called "Scribner immunes."

SCRIBNER IMMUNES.

HYPERIMMUNIZED IMMUNE 1286 (QUICK METHOD).

This hog was exposed to hog cholera on August 17, 1905, together with another of about the same weight (50 pounds). As a result of this exposure hog 1286 exhibited only slight indications of sickness and soon recovered, although the other hog exposed at the same time died, showing at autopsy the usual lesions of hog cholera. In order to test further the immunity of hog 1286 it was placed in the station

exposure pen where a number of sick hogs were kept. This hog failed to show symptoms of disease and was not exposed further until the summer of 1906, when it weighed 125 pounds.

June 13, 1906, hog 1286 received subcutaneously 20 c. c. of Scribner disease-producing blood, and at the same time a control hog was injected with 10 c. c. of the same blood. The control died, while hog 1286 remained well.

June 25, 1906, hog 1286 was injected subcutaneously with 1,250 c. c. of Scribner disease-producing blood secured from several hogs. At the same time 5 nonimmune hogs were injected to serve as checks on the virulence and also to furnish blood for treating other immunes. All of these checks became very sick and were destroyed, while hog 1286 remained well except for slight soreness following the injection.

Blood was drawn from the tail of hog 1286 as follows:

July 16, 1906, 356 c. c. defibrinated blood obtained.

July 23, 1906, 300 c. c. defibrinated blood obtained.

July 30, 1906, 250 c. c. defibrinated blood obtained.

This blood was used in Experiments I and II described in another part of this paper.

HYPERIMMUNIZED IMMUNE 1313 (QUICK METHOD).

This hog was injected subcutaneously with 25 c. c. of diluted diseased blood on September 30, 1905. Hog 1313 did not become noticeably sick as a result of the injection, although the blood was of a fair degree of virulence. A later exposure to disease in the exposure pen was likewise without effect upon this hog. No further exposure to hog cholera was made until the summer of 1906, as described below.

June 13, 1906, hog 1313 received subcutaneously 20 c. c. of Scribner disease-producing blood; a nonimmune hog received at the same time 10 c. c. of the same blood. The check died of hog cholera, but hog 1313 remained well.

July 5, 1906, hog 1313, which weighed 130 pounds, was injected subcutaneously with 1,300 c. c. of Scribner disease-producing blood. Two nonimmune hogs injected at the same time with 2 c. c. each of the same disease-producing blood became very sick and were killed to furnish blood for the other experiments. Hog 1313 was a little sore on the day following the injection; this soon passed away, but on July 8 and for several days thereafter the hog was quite sluggish and disinclined to eat. By July 13, however, recovery was complete. Blood was drawn from the tail of hog 1313 as follows:

July 26, 1906, 400 c. c. defibrinated blood obtained.

August 2, 1906, 465 c. c. defibrinated blood obtained.

August 9, 1906, 450 c. c. defibrinated blood obtained.

September 6, 1906, 400 c. c. defibrinated blood obtained.

The blood from this immune was used in Experiments III and IV, which are described in the following pages:

HYPERIMMUNIZED IMMUNE 1383 (SLOW METHOD).

This hog was injected subcutaneously on November 17, 1905, with 5 c. c. of blood from a hog sick of hog cholera. This blood as a result of a number of inoculations was found to cause the death of somewhat more than 50 per cent of the inoculated hogs. Hog 1383 showed slight sickness after injection, but soon recovered and also remained well when placed in the exposure pen about three weeks later. In June, 1906, this hog was used for the production of serum, as described below.

June 13, 1906, hog 1383 was injected subcutaneously with 20 c. c. of Scribner disease-producing blood. A nonimmune hog was injected at the same time with 10 c. c. of the same blood. The non-immune died, but hog 1383 remained well.

June 25, 1906, hog 1383 weighed 105 pounds and was injected subcutaneously with 105 c. c. of Scribner disease-producing blood. Five nonimmune hogs injected at the same time with 10 c. c. each of the same blood became very sick and were killed to furnish blood for other experiments; hog 1383 remained well. The blood used for this injection was the same as that used for the hyperimmunization of hog 1286, and the same checks served for both immunes.

July 5, 1906, hog 1383 received a second injection consisting of 265 c. c. of Scribner disease-producing blood. The blood used was the same as that used for the injection of immune 1313 on the same date, and, as stated previously, produced hog cholera in nonimmune animals in doses of 2 c. c. Hog 1383 was made somewhat sore by the injection, but was well again after two or three days.

July 14, 1906, hog 1383 was given a third injection of Scribner disease-producing blood, 525 c. c. being administered subcutaneously. Two nonimmunes injected with 2 c. c. each of the same blood as controls on the virulence of the blood died of hog cholera. Hog 1383 remained well.

This injection completed the hyperimmunization of hog 1383, and blood drawings were made from this animal as follows:

- July 24, 1906, 392 c. c. defibrinated blood obtained.
- July 31, 1906, 360 c. c. defibrinated blood obtained.
- August 7, 1906, 400 c. c. defibrinated blood obtained.
- August 14, 1906, 360 c. c. defibrinated blood obtained.
- September 4, 1906, 82 c. c. defibrinated blood obtained.

The blood from this immune proved to be exceptionally potent, and it was therefore used in a number of different experiments.

HYPERIMMUNIZED IMMUNE 1403 (SLOW METHOD).

By referring to Table 3 it will be seen that this hog was one of those vaccinated by the serum-simultaneous method in the 1905 experiments. The hog showed no symptoms of disease, either as a result of vaccination or of the subsequent exposure to hog cholera in the exposure pen on December 27, 1905. No further exposure to disease was made until June, 1906, when hyperimmunization was begun. The weight of the hog at this time was 90 pounds.

June 13, 1906, hog 1403 was injected subcutaneously with 20 c. c. of Scribner disease-producing blood. A control injected at the same time with 10 c. c. of the same blood died, whereas hog 1403 remained well.

June 25, 1906, hog 1403 was injected subcutaneously with 90 c. c. of the same disease-producing blood as that used for injecting immunes 1286 and 1383 on the same date. Hog 1403 was not visibly affected by the injection.

July 5, 1906, hog 1403 was injected with 225 c. c. of Scribner disease-producing blood. The same blood was used at the same time for injecting immunes 1313 and 1383 and four control hogs as previously stated. Immune 1403 was sluggish and disinclined to eat for several days following the injection, but by July 9 had apparently recovered.

July 14, 1906, hog 1403 was injected for the last time with 450 c. c. of Scribner disease-producing blood, subcutaneously, blood from the same source being used at the same time for the injection of immune 1383 and two controls, as stated above. Hog 1403 showed about the same disturbance as was noted after the second injection, but was well again in four or five days.

Blood drawings were made from immune 1403 as follows:

- July 24, 1906, 395 c. c. defibrinated blood obtained.
- July 31, 1906, 390 c. c. defibrinated blood obtained.
- August 7, 1906, 400 c. c. defibrinated blood obtained.
- August 14, 1906, 360 c. c. defibrinated blood obtained.
- September 14, 1906, 360 c. c. defibrinated blood obtained.

DESCRIPTION OF THE SYPHAX OUTBREAK.

This outbreak of hog cholera occurred near Alexandria, Va. At the time the first symptoms of illness were observed, on September 6, 1905, there were 37 hogs in the herd. The disease did not progress very rapidly, but nevertheless all of the hogs were finally attacked by the disease. At the time the herd was first visited by representatives of this Bureau only 9 hogs remained alive, and some of these were killed, while others died, the final result being that 3 hogs out of the 37 that were attacked recovered. The lesions found at autopsy

were such as are usually met with in hog cholera, except that the pulmonary lesions in some of the animals were rather more prominent than we have ordinarily found to be the case.

Blood from one of the sick animals in this herd served as the starting point for the strain of disease from which blood was secured to hyperimmunize the "Syphax immunes." Filtered blood from this Syphax herd produced hog cholera when injected into nonimmune hogs, even though it was found to be free from all of the ordinary bacteria.

SYPHAX IMMUNES.

HYPERIMMUNIZED IMMUNE 1392 (QUICK METHOD).

This hog, with 5 others, was injected subcutaneously on November 17, 1905, with 5 c. c. of hog-cholera blood. All became sick as a result of this inoculation and 4 died, hog 1392 and one other finally recovering. Hog 1392 was placed in the exposure pen in December, 1905, and remained well. This hog exhibited quite typical symptoms of hog cholera after the first blood injection, and the immunity subsequently exhibited must be regarded as having been acquired through this attack of disease.

June 13, 1906, hog 1392 received 20 c. c. of virulent Scribner disease-producing blood in common with all of the immunes which were later hyperimmunized with blood from either the Scribner or Syphax outbreaks. Hog 1392 remained well.

July 13, 1906, hog 1392, which at that time weighed 130 pounds, received subcutaneously 1,300 c. c. of Syphax disease-producing blood. Five control hogs which were injected at the same time with 5 c. c. of the same blood became very sick and were killed to furnish blood for other experiments. Hog 1392 was somewhat stiff and sore on the two days following the injection, but otherwise remained well. Drawings of blood from immune 1392 were made as follows:

August 3, 1906, 375 c. c. defibrinated blood obtained.

August 10, 1906, 435 c. c. defibrinated blood obtained.

August 17, 1906, 500 c. c. defibrinated blood obtained.

September 17, 1906, 100 c. c. defibrinated blood obtained.

HYPERIMMUNIZED IMMUNE 1274 (QUICK METHOD).

This hog was first exposed to hog cholera on July 22, 1905, by the subcutaneous injection of 0.25 c. c. of hog-cholera blood. Another hog injected at the same time died, but hog 1274 did not become sick, nor did this hog show symptoms of disease when placed in the exposure pen some weeks later. The records indicate that this hog possessed quite a high degree of natural immunity.

June 13, 1906, hog 1274 was injected subcutaneously with 20 c. c. of Scribner disease-producing blood along with the other immunes. No ill effects were noticed as a result of this inoculation.

July 26, 1906, this hog, which weighed at that time 125 pounds, was injected subcutaneously with 1,250 c. c. of Syphax disease-producing blood, and 4 control hogs were injected at the same time with from 2 c. c. to 5 c. c. each of the same blood. All of the controls became very sick and were killed to furnish blood for other experiments. Hog 1274 showed no marked effects from the injection. Drawings of blood from this hog were made as follows.

August 16, 1906, 450 c. c. defibrinated blood obtained.

August 23, 1906, 450 c. c. defibrinated blood obtained.

September 1, 1906, 378 c. c. defibrinated blood obtained.

October 1, 1906, 700 c. c. defibrinated blood obtained.

HYPERIMMUNIZED IMMUNE 1310 (SLOW METHOD).

This hog, with one other, was injected with hog-cholera blood on September 29, 1905; the mate to hog 1310 showed slight indisposition after the injection, but hog 1310 remained well. Both hogs were later placed in the exposure pen. Hog 1310 did not thrive there, but never became plainly sick. After removal from the exposure pen this hog regained its vigor, and on July 13, 1906, at which time the hyperimmunization was begun, its weight was 180 pounds.

June 13, 1906, the preliminary injection of 20 c. c. of virulent Scribner disease-producing blood was made. No visible reaction followed this injection.

July 13, 1906, hog 1310 was injected subcutaneously with 180 c. c. of Syphax disease-producing blood from the same source as that used for the injection of immune hog 1392 on the same date. Hog 1310 remained well, although the control hogs injected at the same time contracted hog cholera.

July 26, 1906, hog 1310 was injected subcutaneously with 400 c. c. of Syphax disease-producing blood from the same source, as that used for the injection of immune hog 1274 on the same date. Hog 1310 showed practically no disturbance of health after this injection, although the control animals injected with a much smaller amount of the disease-producing blood contracted hog cholera.

August 7, 1906, hog 1310 was injected subcutaneously with 900 c. c. of Syphax disease-producing blood. Two nonimmune hogs injected subcutaneously with 2 c. c. and 5 c. c. of the same blood contracted hog cholera and died. With the exception of a little soreness, hog 1310 remained well.

Blood was drawn from this immune as follows:

August 16, 1906, 450 c. c. defibrinated blood obtained.

August 23, 1906, 450 c. c. defibrinated blood obtained.

September 1, 1906, 540 c. c. defibrinated blood obtained.

October 1, 1906, 450 c. c. defibrinated blood obtained.

HYPERIMMUNIZED IMMUNE 1297 (SLOW METHOD).

This hog weighed approximately 55 pounds on September 13, 1905, at which time it was injected subcutaneously with hog-cholera blood. No sickness followed this injection and a later exposure in the exposure pen was likewise without result as far as could be observed, though a mild attack at this time may have passed unobserved.

June 13, 1906, this hog received, with the other immunes, a preliminary injection of 20 c. c. of Scribner disease-producing blood without any symptoms of disease being caused.

June 29, 1906, hog 1297 was injected subcutaneously with 175 c. c. of Syphax disease-producing blood. Two nonimmune hogs injected at the same time with 2 c. c. each of the same blood died of hog cholera. Hog 1297 remained well.

July 13, 1906, hog 1297 was injected subcutaneously with 440 c. c. of Syphax disease-producing blood from the same source as that used for the injection of immunes 1310 and 1392 on the same date. All of the checks injected with the same lot of blood became sick and either died or were killed to furnish blood for other experiments. Hog 1297 remained well except for slight soreness following the injection.

July 26, 1906, hog 1297 was again injected with Syphax disease-producing blood, the dose being 850 c. c. of blood from the same source as that used for the injection of immunes 1274 and 1310 on the same date. As previously stated, controls injected at the same time became sick and were killed to furnish blood for other experiments. Blood was drawn from immune 1294 as follows:

August 4, 1906, 390 c. c. defibrinated blood obtained.

August 11, 1906, 300 c. c. defibrinated blood obtained.

August 18, 1906, 500 c. c. defibrinated blood obtained.

September 17, 1906, 360 c. c. defibrinated blood obtained.

It has been previously stated that it was intended to use, for the production of hyperimmune serum, 4 naturally immune hogs and 4 which had acquired immunity through an attack of hog cholera. When it came to the actual working out of this plan, however, as is shown in the preceding pages, we were only able to secure 1 hog (1392) that had recovered from an undoubted attack of hog cholera; 2 others (1286 and 1383) had shown slight though not characteristic symptoms of that disease, while the fourth (1403), among those that were considered as having acquired immunity, had been vaccinated in 1905, but had not shown symptoms of illness at any time after being vaccinated. In regard to this last-mentioned hog, it should be noted that it has been found to be frequently the case that susceptible hogs may show no symptoms following vaccination and yet may secure through this treatment a firm and lasting immunity, and we believe this to have been the case with hog 1403. The remaining 4 immunes, 1274, 1297, 1310, and 1313, were not vaccinated, but

nevertheless they showed no symptoms of disease when exposed to hog cholera, and we may therefore with a reasonable degree of assurance look upon these hogs as being naturally immune. From what has just been said it becomes evident that the plan to test the relative capability of "natural" and "acquired" immunes to produce a potent protective serum could not be carried out on as extensive a scale as was desired, but nevertheless it was hoped that considerable information might be acquired from a comparison of serum from hog 1392 with serum from the hogs that appeared to possess a natural immunity.

VACCINATION EXPERIMENTS IN 1906.

The experiments as originally planned were necessarily modified more or less owing to the existence of conditions which could not be foreseen, but notwithstanding the fact that the original plan was not carried out in all instances it may perhaps lead to a better understanding of the experiments which are now to be described if this plan is briefly outlined.

The experiments with serum from each of the hyperimmunized immunes were to consist of two sections, (1) preliminary experiments and (2) secondary experiments.

Preliminary experiments.—These experiments were designed in the main to determine the potency of the immune serum. A number of nonimmune hogs were to be injected with 2 c. c. each of disease-producing blood. A few of these were to be left untreated as controls, while the others were to receive simultaneously with the disease-producing blood varying doses of the immune serum. Uninoculated hogs were to be placed in the pens with the treated as well as the untreated hogs to determine the likelihood of disease being transmitted by the treated hogs. Any survivors from the preliminary experiments were to be reserved for exposure to hog cholera at a later date.

Secondary experiments.—These were intended to determine many questions of practical interest, the preliminary experiments having already established the degree of potency of the different sera when injected simultaneously with diseased blood. In these secondary experiments four lots of hogs were to be treated.

Lot 1. These hogs were to receive disease-producing blood with the minimum dose of serum required to protect.

Lot 2. These were to receive disease-producing blood with double the minimum amount of serum required to protect.

Lot 3. These were to receive the minimum amount of serum alone.

Lot 4. This lot was to receive the maximum amount of serum alone.

Controls were of course to be injected with disease-producing blood alone, and, as in the preliminary experiments, hogs were to be placed

in the pens with the treated as well as the untreated ones to determine the likelihood of disease being conveyed to others by contact. The survivors from these experiments were to be divided into two lots, one to be exposed within thirty days after vaccination, the other to be placed in a quarantine pasture and not subjected to exposure until the expiration of three or more months. As will be explained later, the exposure to hog cholera could not be carried out in the manner planned, owing to an outbreak of disease among the hogs in the quarantine pasture.

In addition to carrying out these preliminary and secondary experiments, serum from certain of the immunes was to be used in special experiments with suckling pigs, and also for the purpose of testing the curative value and the keeping quality of the serum, and other questions of more or less practical importance. Except in the case of these special experiments the tests with the serum from the Syphax and Scribner immunes are described separately.

TESTS OF SERUM FROM HOGS HYPERIMMUNIZED WITH BLOOD FROM THE SCRIBNER OUTBREAK.

The description of the outbreak from which this strain of disease was derived has already been given on page 20, and the history of the immunes with the details of hyperimmunization and time of the various blood drawings has also been given.

SERUM FROM IMMUNE 1286 (QUICK METHOD).

EXPERIMENT I.—*Preliminary experiment with first drawing of serum.*

This experiment was carried out in accordance with the plan originally decided upon for all preliminary experiments. Eight hogs were injected; 2 of these received disease-producing blood simultaneously with 5 c. c. of serum, 2 received disease-producing blood with 10 c. c. of serum, 2 disease-producing blood with 15 c. c. of serum, and 2 disease-producing blood alone. These four lots of hogs were placed in separate pens with an uninoculated check in each pen. The hogs which received only 5 c. c. and 10 c. c. of immune serum with the diseased blood became distinctly sick, but recovered; the uninoculated checks in the pens with these did not contract disease, however, in either case; the 2 hogs which received 15 c. c. of immune serum with the disease-producing blood did not become sick, and their pen check also remained well. The 2 hogs which received disease-producing blood alone sickened and died promptly after injection, and at autopsy exhibited the usual lesions of hog cholera; the uninoculated pen check with these last 2 hogs contracted disease from them and showed lesions of hog cholera when destroyed on August 4, 1906.

TABLE 4. Preliminary experiment with first drawing of serum from hog 1286 ("quick" immune—Scribner).

No. of hog.	Weight. Pounds.	Material injected.		Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
		Date.	Result.	Date.	Result.	Date.	Result.	Date.	Result.	
1520	30	5 c. c. serum and 2 c. c. disease - producing blood of hog 1505.	July 18, 1906	Sick July 28 to Aug. 12, 1906; recovered.	Oct. 15, 1906	Became unthrifty	Nov. 16, 1906	Died Dec. 8, 1906.	Negative.	
1521	40	do.	do.	do.	do.	do.	do.	Became unthrifty and was killed.	Do.	
1523	35	10 c. c. serum and 2 c. c. disease - producing blood of hog 1505.	do.	Sick July 28 to Aug. 8, 1906; recovered.	do.	do.	do.	Became unthrifty and died Dec. 18, 1906.	Do.	
1524	35	do.	do.	Sick July 28 to Aug. 4, 1906; recovered.	do.	do.	do.	Became unthrifty and died Dec. 10, 1906.	Do.	
1526	40	15 c. c. serum and 2 c. c. disease - producing blood of hog 1505.	do.	Remained well.	do.	do.	do.	Remained well.	Do.	
1527	35	do.	do.	do.	do.	do.	do.	do.	Hemorrhagic lesions.	
1522	30	Pen check with hogs 1520 and 1521.	do.	do.	do.	Sickened and died Nov. 6, 1906.	do.	do.	Do.	
1525	40	Pen check with hogs 1523 and 1524.	do.	do.	do.	do.	do.	do.	Do.	
1528	30	Pen check with hogs 1526 and 1527.	do.	do.	do.	Not exposed.	do.	do.	Hemorrhages and ulcers.	
1529	25	2 c. c. disease-producing blood of hog 1505.	July 18, 1906	Sickened July 26; died Aug. 4, 1906.	do.	do.	do.	do.	Do.	
1530	30	do.	do.	Sickened July 26; died July 30, 1906.	do.	do.	do.	do.	Hemorrhagic lesions.	
1531	30	Pen check with hogs 1529 and 1530.	do.	Sickened Aug. 1; killed Aug. 4.	do.	do.	do.	do.	Do.	

The protective power of 1286 serum is well shown by this experiment, in which all the vaccinated hogs survived, while those which received disease-producing blood alone died promptly. It is evident also that 5 c. c. of this serum is probably the least amount that would protect, as even those which received 10 c. c. showed some signs of illness. In accordance with the original plan, the survivors from this experiment were placed in a large quarantine pasture to be reserved for exposure at a later date.

Many other hogs which had been treated in various ways with different sera, together with a considerable number of uninoculated control animals, were placed in this pasture from time to time for the same purpose. In some way hog cholera was accidentally introduced into this pasture toward the end of October, 1906, as shown by the sickness and death of some of the hogs, the autopsies revealing typical lesions of hog cholera. The first hogs to show symptoms of hog cholera in this pasture were the unvaccinated controls about October 15, and the first death among these occurred October 25. This accidental exposure, to which a large number of our vaccinated hogs and unvaccinated control hogs were subjected, resulted in the death of 80 per cent of all the unvaccinated hogs.

Table 4 shows that pen checks 1522 and 1525, which were placed in the quarantine pasture with the vaccinated hogs, both died of hog cholera as a result of this exposure, while none of the vaccinated hogs were made sick. Although this exposure was accidental, it occurred approximately three months after vaccination, and therefore in this instance did not interfere with our plans. As the treated hogs did not become sick from the pasture exposure, they were placed in the exposure pen where hogs sick of hog cholera (Scribner disease) were kept. Conditions in this pen were extremely unfavorable for the general health of the hogs exposed, and the disease was very virulent, as shown by the death of all the check animals. It will be seen from Table 4 that treated hogs 1520, 1521, 1523, and 1524 all became unthrifty. One of these was killed and 3 died about thirty days after being placed in the exposure pen, while 1526 and 1527, the 2 remaining vaccinated hogs, remained well. None of the first 4 hogs mentioned showed any of the lesions of hog cholera at autopsy, and there seems little room to doubt that these hogs died of unthriftiness and impaired vitality caused by the original vaccinating dose. It will be remembered that they became sick after vaccination, and they should therefore have been subsequently much more resistant to hog cholera than the 2 hogs (1526 and 1527) which showed no such symptoms. We consider it quite evident, therefore, that the attack of hog cholera brought on by vaccination weakened the constitution of those which received serum in doses of 5 c. c. and 10 c. c. to such an extent that they could not withstand the unfavorable conditions existing in the

exposure pen.. The deaths of the checks in the quarantine pasture shows that the pasture exposure was sufficient to cause the death of nonimmune hogs. The fact that all the vaccinated hogs survived shows that when given the same exposure at the end of three months the immunity remained quite firm.

EXPERIMENT II.—*Secondary experiment with first drawing of serum.*

In the secondary experiment with serum from immune 1286 the doses of serum used with disease-producing blood were 10 c. c., which was considered the minimum safe dose, and 20 c. c., double the amount needed to surely protect, as planned. A number of hogs in this experiment were also injected with serum alone. The results which followed vaccination are in complete agreement with those obtained in Experiment I. The 3 hogs injected with disease-producing blood alone contracted hog cholera and died, and the uninoculated hog in this same pen also died of hog cholera contracted from those which received the disease-producing blood alone. Of 6 hogs injected with diseased blood plus 10 c. c. of serum, all became sick and 1 died; the remaining 5 finally recovered. None of the 6 hogs injected with disease-producing blood plus 20 c. c. of immune serum showed any illness after vaccination, and those injected with serum alone likewise remained well. One of the 2 pen checks exposed to the lot of vaccinated hogs which sickened after vaccination contracted hog cholera and was killed; the other remained well.

Nineteen days after vaccination, as shown by Table 5, half of the vaccinated hogs which had received 20 c. c. of serum with disease-producing blood were placed in the Scribner exposure pen. About the same time 2 hogs which had received 20 c. c. of serum alone, 2 which received 10 c. c. of serum alone, and 1 uninoculated pen check were placed in the same exposure pen. Of the hogs which were made sick by vaccination with 10 c. c. of serum plus diseased blood, 3 were placed in the exposure pen thirty-four days after vaccination, together with 1 pen check.

Both of the pen checks (1569 and 1576) which were placed in the exposure pen with the treated hogs died of hog cholera, although not one of the 10 vaccinated hogs showed any visible signs of illness.

As the Scribner strain of disease had been used exclusively for the hyperimmunization of hog 1286, which furnished the serum, and as the diseased blood used in the simultaneous vaccination was of the Scribner strain, and furthermore as the exposure pen in which these hogs were placed to test their immunity contained only the Scribner strain of disease, it was deemed desirable to ascertain the effect of a different strain of disease on the vaccinated hogs. An exposure pen containing only the Syphax strain of disease was therefore prepared, and hogs 1570, 1571, 1573, 1578, 1579, 1582, and 1584 were placed in

TABLE 5.—Secondary experiment with first drawing of serum from hog 1286 ("quick," immune—*Scribner*).

No. of hog.	Weight. <i>Pounds.</i>	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
1562 a . . .	40	10 c. c. serum and 2 c. c. disease-producing blood of hog 1531.	Aug. 4, 1906	Slightly sick Aug. 13 to 21, 1906; recovered.			Sept. 7, 1906	Remained well.	
1563 a . . .	55	do.	do.	do.			do.	do.	
1564 a . . .	55	do.	do.	do.			do.	do.	
1565	35	do.	do.	Slightly sick; recovered.	Oct. 15, 1906	Remained well.	do.	Not exposed.	
1566	30	do.	do.	Slightly sick Aug. 13 to 23, 1906; recovered.	do.	do.	do.	do.	
1567	55	do.	do.	Sickened Aug. 13; died Aug. 20, 1906.					Hemorrhagic lesions.
1570 a . . .	35	20 c. c. serum and 2 c. c. disease-producing blood of hog 1531.	do.	Remained well.			Aug. 23, 1906	Remained well.	
1571 a . . .	40	do.	do.	do.			do.	do.	
1572	35	do.	do.	do.	Oct. 15, 1906	Remained well.	do.	do.	
1573 a . . .	50	do.	do.	do.	do.	do.	Nov. 19, 1906	do.	
1574	40	do.	do.	do.	Oct. 15, 1906	Remained well.	do.	do.	
1575	65	do.	do.	do.	do.	do.	Nov. 16, 1906	do.	
1578 a . . .	35	20 c. c. serum alone.	do.	do.	do.	do.	Nov. 19, 1906	do.	
1579 a . . .	40	do.	do.	do.	do.	do.	Aug. 21, 1906	do.	
1580	40	do.	do.	do.	Oct. 15, 1906	Sick and died Nov. 7, 1906.	do.	do.	Do.
1581	25	do.	do.	do.	do.	Sick and died Nov. 14, 1906.	do.	do.	Do.
1582 a . . .	35	10 c. c. serum alone.	do.	do.			Aug. 21, 1906	Remained well.	No pathological lesions.
1583	45	do.	do.	do.	Oct. 15, 1906	Killed by other hogs.			
1584 a . . .	40	do.	do.	do.			Aug. 21, 1906	Remained well.	No marked lesions.
1585	35	do.	do.	do.	Oct. 15, 1906	Unthrifty; died Nov. 14, 1906.			Hemorrhagic lesions.
1568	55	Pen check with hogs 1562 to 1567.	do.	Sickened Aug. 29; killed Sept. 3, 1906.			Sept. 7, 1906	Sickened; died Sept. 23, 1906.	Do.
1569	40	do.	do.	Remained well.			Aug. 23, 1906	Sickened; died Sept. 15, 1906.	Do.
1576	20	Pen check with hogs 1570 to 1575.	do.	do.					Hemorrhagic and ulcers.
1577	35	do.	do.	do.	Oct. 15, 1906	Sickened; died Nov. 14, 1906.			Do.
1558	20	2 c. c. disease-producing blood of hog 1531.	Aug. 4, 1906	Sickened Aug. 9; died Aug. 16, 1906.					Hemorrhagic lesions.

	Do.	Hemorrhages and ulcers. Do.	Hemorrhagic lesions.
1579	50	do.	do.
1580	40	do.	do.
1581	30	do.	do.
1712	50	do.	do.

^a These animals were given double exposure to Scribner and Syphax strains of disease.

it about September 15, 1906. On October 30, 1906, the 3 remaining hogs which had withstood exposure in the Scribner exposure pen were also placed in the Syphax exposure pen. None of the vaccinated hogs became sick as a result of this second exposure, although an unprotected hog exposed at the same time contracted hog cholera and died. This would indicate that the serum alone served to confer immunity which might last for six weeks, although the possibility of the immunity being heightened by the exposure in the Scribner exposure pen can not be overlooked.

The remainder of the vaccinated hogs in Experiment II, together with pen check 1577, none of which were exposed in the exposure pen, were placed in the quarantine pasture to await a later exposure. As previously explained, however, our plans in this respect were interfered with by an unforeseen outbreak of hog cholera among the control hogs which were being kept with the vaccinated hogs in this quarantine pasture. This outbreak occurred approximately two and one-half months after vaccination of the hogs in Experiment II. The vaccinated hogs in this experiment which were exposed in this manner consisted of 2 that had received 10 c. c. of serum with 2 c. c. of diseased blood, 3 that had received 20 c. c. of serum with diseased blood, and 2 each of those which received 20 c. c. and 10 c. c. of immune serum alone. (See Table 5.) One of those that had received 10 c. c. of serum alone was killed by other hogs the day it was placed in the pasture. Check hog 1577 died of hog cholera as a result of this exposure. Of the vaccinated hogs, both of those injected with 20 c. c. of serum alone died of hog cholera; the remaining one injected with 10 c. c. of serum alone died, but no marked lesions were found at autopsy. The hogs which were given 10 c. c. and 20 c. c. of serum simultaneously with diseased blood remained well. The 3 hogs vaccinated with 20 c. c. of serum plus diseased blood which remained well in the quarantine pasture were later placed in the Scribner exposure pen, where they again remained well.

In summarizing the experiments with the first drawing of serum from immune 1286, we find that 18 hogs were treated by the serum-simultaneous

method, the dose of serum varying from 5 c. c. to 20 c. c., and that only one of these died from the vaccination, although all that received less than 15 c. c. of serum became sick. Of the 5 hogs which were injected with diseased blood alone, all died of hog cholera. Eight hogs were injected with serum alone, no ill effects whatever following the injection.

In regard to the exposure to hog cholera after vaccination, we find that all vaccinated hogs, whether treated with serum alone or with a combination of serum and disease-producing blood, were rendered completely immune from hog cholera for at least three weeks. It appears, however, from the death of hogs 1580 and 1581 that the serum of immune 1286 when given alone, even in doses of 20 c. c., does not afford protection to hogs weighing from 25 to 40 pounds for as long as two and one-half months.

In so far as the first drawing of serum from immune 1286 is concerned, we may conclude that (1) when given in sufficient dose it protects hogs completely from a fatal dose of disease-producing blood administered simultaneously with the serum; (2) that the hogs which survive vaccination by the serum-simultaneous method are rendered immune for a period of at least two and one-half months; (3) that hogs which show no visible reaction after this treatment are rendered quite as resistant as those which were made distinctly sick by the protective inoculation; (4) if a sufficient dose of serum is not given with the disease-producing blood the vitality of the vaccinated hog is apt to be impaired; (5) hogs vaccinated with serum alone are rendered immune for a period of three weeks, but this immunity does not last two and one-half months; (6) uninoculated hogs placed in pens with others vaccinated by the serum-simultaneous method did not contract hog cholera from them unless the vaccinated hogs themselves became distinctly sick.

SERUM FROM IMMUNE 1313 (QUICK METHOD).

EXPERIMENT III.—*Preliminary experiment with first drawing of serum.*

This experiment was carried out in the same manner as Experiment I, the doses of immune serum being 5 c. c., 10 c. c., and 15 c. c.; at the same time each hog was injected subcutaneously with 2 c. c. of disease-producing blood. Controls were injected at the same time with an equal amount of the same disease-producing blood alone. Of the 6 hogs treated with both serum and disease-producing blood, none showed any symptoms of sickness after vaccination, although both of the hogs which were injected with an equal amount of the same disease-producing blood alone became very sick and one of them died. The pen checks which were placed with each lot of

vaccinated hogs did not contract disease from them, but the pen check with the controls 1751 and 1752 contracted disease from them and died.

Approximately three weeks after vaccination all of the treated hogs with their pen checks were placed in the quarantine pasture. (See Table 6.) The accidental outbreak of hog cholera in this pasture began about this time, so that these hogs may be considered as having been exposed to disease about the time that they were placed in the pasture. None of the vaccinated hogs became sick as a result of this exposure; 2 of the pen checks became sick and 1 died; the remaining check did not show symptoms of sickness. At a later date, in order to further test their immunity, the vaccinated hogs, with the check which did not become sick in the pasture, were placed in the Scribner exposure pen, but none of them became sick.

EXPERIMENT IV.—*Secondary experiment with first drawing of serum.*

The second experiment with this serum was carried out in the manner planned for secondary experiments except that the minimum dose of serum used was 10 c. c., even though 5 c. c. had given protection in the preliminary experiment. That this dose was not too great is shown by the sickness and death of one of the serum-simultaneous vaccinated hogs that received 10 c. c. of serum. As may be seen from Table 7, 6 hogs were injected with 10 c. c. of serum plus 2 c. c. of disease-producing blood, while 6 received 20 c. c. of serum with the disease-producing blood. Four hogs were injected with 10 c. c. of serum alone and a like number received 20 c. c. of serum alone. Two hogs were injected with disease-producing blood alone. In order to test the likelihood of hog cholera being conveyed through the agency of vaccinated hogs, 2 untreated healthy hogs were placed in each pen with the serum-simultaneous vaccinated hogs, and as a control 1 was placed in the pen with the 2 hogs that received disease-producing blood only.

Of the 6 hogs that received 10 c. c. of serum with disease-producing blood, 1 became sick and died, showing at autopsy the lesions of hog cholera. One of the hogs that was given 20 c. c. of serum with disease-producing blood died nearly seven weeks after vaccination, but the symptoms and lesions exhibited by this hog were such that a positive diagnosis of hog cholera could not be made. The other vaccinated hogs remained well, as did all of the checks in the pens with the vaccinated hogs. Both of the hogs that were given disease-producing blood alone died of hog cholera, and their check contracted the disease from them and died.

All of the surviving serum-simultaneous vaccinated hogs were exposed to infection at the same time, 4 being placed in the pasture

TABLE 6.—*Preliminary experiment with first drawing of serum from hog 1313 ("quick" immune—Scribner).*

No. of hog.	Weight. <i>Pounds.</i>	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
1733....	40	5 c. c. serum and 2 c. c. disease-producing blood of hog 1664.	Sept. 26, 1906	Remained well.....	Oct. 15, 1906	Remained well.....	Dec. 22, 1906	Remained well.....	
1734....	34	do	do	do	do	do	do	do	
1736....	45	10 c. c. serum and 2 c. c. disease-producing blood of hog 1664.	do	do	do	do	do	do	
1737....	40	do	do	do	do	do	do	do	
1739....	50	15 c. c. serum and 2 c. c. disease-producing blood of hog 1664.	do	do	do	do	do	do	
1740....	50	do	do	do	do	do	do	do	
1735....	35	Pen check with hogs 1733 and 1734.		do	do	Died Nov. 7, 1906.			Hemorrhagic lesions.
1738....	40	Pen check with hogs 1736 and 1737.		do	do	Sickened, but recovered.		Not exposed.	
1741....	40	Pen check with hogs 1739 and 1740.		do	do	Remained well.....	Nov. 19, 1906	Remained well.....	
1751....	40	2 c. c. disease-producing blood of hog 1664.	Sept. 26, 1906	Sickened Oct. 8; died Oct. 13, 1906.					Do.
1752....	40	do	do	Sick Oct. 8 to Nov. 5, 1906; recovered.				Not exposed	Do.
1753....	35	Pen check with hogs 1751 and 1752.		Sickened Oct. 18; died Nov. 1, 1906.					Do.

TABLE 7.—Secondary experiment with first drawing of serum from hog 1313 ("quick", immune—Scribner).

No. of hog.	Weight. Pounds.	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
177	45	10 c. c. serum and 2 c. c. disease-producing blood of hog 1711.	Oct. 10, 1906	Remained well			Oct. 25, 1906	Remained well	
178	45	do.	do.	do.			do.	do.	
179	45	do.	do.	do.			do.	do.	
180	40	do.	do.	do.			Oct. 25, 1906	Remained well, but unthrifty.	Upper extrem. Hemorrhages and ulcers.
181	35	do.	do.	Sickened Oct. 20; died Nov. 19, 1906.			Dec. 22, 1906	Remained well	
182	30	20 c. c. serum and 2 c. c. disease-producing blood of hog 1711.	do.	Remained well			Oct. 25, 1906	do.	
183	35	do.	do.	do.			Dec. 22, 1906	do.	
184	40	do.	do.	do.			Oct. 25, 1906	do.	
185	25	do.	do.	do.			Oct. 25, 1906	Not exposed.	
186	45	do.	do.	do.			Oct. 25, 1906	Remained well	
187	40	do.	do.	do.			Oct. 25, 1906	do.	
188	45	do.	do.	do.			Oct. 25, 1906	do.	
189	45	do.	do.	do.			Oct. 25, 1906	do.	
190	45	do.	do.	do.			Oct. 25, 1906	do.	
191	60	10 c. c. serum alone.	do.	Became unthrifty and died Nov. 27, 1906.			Jan. 31, 1907	Died Feb. 21, 1907.	No marked lesions.
192	60	do.	do.	Remained well.			Oct. 25, 1906	Remained well	Hemorrhagic lesions.
193	45	do.	do.	do.			do.	Sickened and died Nov. 17, 1906.	Do
194	45	do.	do.	do.			do.	do.	
195	40	do.	do.	do.			Jan. 31, 1907	Died Feb. 16, 1907.	No marked lesions.
196	30	20 c. c. serum alone.	do.	do.			Oct. 25, 1906	Remained well	
197	65	do.	do.	do.			Jan. 31, 1907	Died Feb. 19, 1907.	Hemorrhagic lesions.
198	75	do.	do.	do.			Oct. 25, 1906	Sickened and died Nov. 17, 1906.	Hemorrhages and ulcers.
199	40	do.	do.	do.			Jan. 31, 1907	Died Feb. 20, 1907.	Hemorrhagic lesions.
200	35	Pen check with hogs 177, 182.	do.	do.			do.	Died Feb. 17, 1907.	Do.
201	35	do.	do.	do.			Oct. 25, 1906	Sickened and died Nov. 16, 1906.	Eaten by other hogs; no autopsy.
202	25	Pen check with hogs 185, 190.	do.	do.			do.	Sickened and died Nov. 14, 1906.	Hemorrhagic lesions.
203	25	do.	do.	do.			Nov. 23, 1906	Became unthrifty; died Dec. 15, 1906.	No marked lesions.
204	50	2 c. c. disease-producing blood of hog 1711.	Oct. 10, 1906	Sickened Oct. 15; died Oct. 28, 1906.					Hemorrhages and ulcers.
205	35	do.	do.	Sickened Oct. 16; died Oct. 25, 1906.					Hemorrhagic lesions.
206	30	Pen check with hogs 187 and 188.	do.	Sickened Oct. 26; died Oct. 31, 1906.					Do.

and 6 in the Scribner exposure pen fifteen days after vaccination. None of these hogs became distinctly sick, though hogs 1780 and 1788, which were exposed in the pasture, became unthrifty. Three of the hogs that survived the exposure in the pasture were placed in the Scribner exposure pen two months and twelve days after vaccination in order to further test their immunity. Of these 3 hogs 1, No. 1780, finally died more than three months after vaccination. The only lesion of hog cholera found at the autopsy of this hog was a single button ulcer in the cecum. In view of the lack of acute lesions and the unthriftiness which preceded the last exposure, it seems probable that this hog had contracted hog cholera before being placed in the Scribner exposure pen, and indeed the unthriftiness of this hog in the pasture may have been caused by a light attack of hog cholera following vaccination, the one ulcer found at autopsy being the only trace left of that attack.

Four of the hogs that were treated with serum alone were placed in the Scribner exposure pen nineteen days after vaccination, while the remaining 4 were first exposed three months and twenty-one days after vaccination. Of the first lot, 2 remained well, while 2 died of hog cholera; 2 checks exposed at the same time died of hog cholera. The amount of serum injected did not seem to affect the result of this exposure, as one of the survivors had received 10 c. c. and the other 20 c. c. of serum. Of the 4 that were first exposed three months and twenty-one days after injection of the serum, all died, and the pen check exposed at the same time also died of hog cholera.

Summary of experiments with serum from immune 1313.

In all, 18 hogs were treated by the serum-simultaneous method, the serum being used in doses of 5 c. c., 10 c. c., 15 c. c. and 20 c. c. Of these, 2 became sick and died, though it was impossible to make a positive diagnosis of hog cholera in the case of one of them. In the case of one other hog (1780) there is also some doubt whether death resulted from vaccination or from subsequent exposure to disease, but for convenience in summarizing, and because no symptoms of illness were observed after vaccination, the death of the hog is counted as due to exposure.

To determine the virulence of the disease-producing blood used in the serum-simultaneous vaccinations, 4 hogs were injected with disease-producing blood alone. All of these became sick and 3 died. Seven uninoculated hogs were placed in pens with the vaccinated hogs, and all remained well. Two similar unvaccinated hogs which were placed in pens with those injected with disease-producing blood alone died of hog cholera. None of the hogs injected with serum alone were made sick by the injection.

In testing the immunity of the treated hogs, 16 that were vaccinated by the serum-simultaneous method were exposed to hog cholera

from fifteen to nineteen days after vaccination, and all remained well except 1780, which became unthrifty. Three of these hogs which had been exposed originally in the pasture were again exposed by being placed in the Scribner exposure pen seventy-three days after vaccination. One of these (1780) finally died as described above; the other 2 remained well.

Of 8 hogs that were treated with serum alone, none became sick from treatment, but 6 died when exposed to infection from nineteen days to three months and twenty-one days after vaccination. It is worthy of note that the 2 survivors were, with 2 others that succumbed, exposed only nineteen days after vaccination. Of 7 unvaccinated check hogs exposed along with the treated hogs, 6 became sick and 5 died.

The following conclusions may be drawn from the experiments with first drawing of serum from immune 1313:

(1) Serum from immune 1313 was sufficiently potent to protect the great majority of the injected hogs from a fatal dose of disease-producing blood administered simultaneously with the serum.

(2) Hogs treated by the serum-simultaneous method, even though they became slightly sick, did not convey the disease to nonimmunes by association.

(3) Hogs vaccinated by a simultaneous injection of serum and disease-producing blood remained immune for at least fifteen days after vaccination.^a

(4) Immunity subsequent to vaccination in hogs which received the maximum dose of serum with disease-producing blood was quite as firm as in those that received the minimum dose in the same manner.

(5) The immunity conferred by the serum of hog 1313 when administered alone in doses of 10 c. c. and 20 c. c. did not persist in full force for as long as nineteen days, and at the end of three months and twenty-one days it seemed to have completely disappeared.

SERUM FROM IMMUNE 1383 (SLOW METHOD).

EXPERIMENT V.—*Preliminary experiment with first drawing of serum.*

This first experiment with the first drawing of blood from immune 1383 was carried out in exactly the same manner as the preliminary experiments with the serum-simultaneous method, the serum being given in doses of 5 c. c., 10 c. c., and 15 c. c. Two hogs were injected with disease-producing blood alone, and 1 un inoculated hog was placed in each of the four pens used in this experiment. Table 8 shows that none of the vaccinated hogs became sick after treatment, nor did any of the check hogs in the same pens contract disease from

^a Other experiments have shown that immunity following serum-simultaneous injections lasts much longer than this.

TABLE 8.—Preliminary experiment with first drawing of serum from hog 1383 ("slow" immune—Scribner).

No. of hog.	Weight. <i>Pounds.</i>	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
1536	30	5 c. e. serum and 2 c. e. disease-producing blood of hog 1519.	July 30, 1906	Remained well.....	Oct. 25, 1906	Remained well.....	Nov. 16, 1906	Remained well.....	
1538	30	do.	do.	do.	do.	do.	do.	do.	
1539	30	10 c. e. serum and 2 c. e. disease-producing blood of hog 1519.	do.	do.	do.	do.	do.	do.	
1540	35	do.	do.	do.	do.	do.	do.	do.	
1542	40	15 c. e. serum and 2 c. e. disease-producing blood of hog 1519.	do.	do.	do.	do.	do.	do.	
1543	30	do.	do.	do.	do.	do.	do.	do.	
1537	30	Pen cheek with hogs 1536 and 1538.	do.	do.	do.	Sickened and died Oct. 25, 1906.	Nov. 19, 1906	Remained well.....	Hemorrhagic lesions. Do.
1541	25	Pen cheek with hogs 1539 and 1540.	do.	do.	do.	Sickened and died Oct. 27, 1906.	do.	do.	Hemorrhagic lesions. Do.
1544	25	Pen cheek with hogs 1542 and 1543.	do.	do.	do.	Sickened and died Nov. 16, 1906.	do.	do.	Hemorrhagic lesions. Do.
1545	35	2 c. e. disease-producing blood of hog 1519.	July 30, 1906	Sickened Aug. 5; died Aug. 14, 1906.	do.	do.	do.	do.	
1546	30	do.	do.	Sickened Aug. 7; died Aug. 21, 1906.	do.	do.	do.	do.	Hemorrhagic lesions.
1547 ^a	25	Pen cheek with hogs 1545 and 1546.	do.	Sick Aug. 15 to 25, 1906; recovered.	do.	do.	Aug. 25, 1906	Remained well.....	

^a This hog received double exposure to Scribner and Syphax strains of disease.

them. Both of the hogs which were injected with the same dose of the same disease-producing blood as that used on the vaccinated hogs but without the serum, contracted hog cholera and died, and the pen check with these 2 hogs contracted hog cholera from them, but recovered.

The 6 vaccinated hogs with their 3 pen checks were placed in the quarantine pasture and were there accidentally exposed to disease about two and one-half months after vaccination; as a result of this exposure all 3 of the checks died of hog cholera, while the vaccinated hogs remained well.

Five of the vaccinated hogs had their immunity further tested by being placed in the Scribner exposure pen a little more than three and one-half months after vaccination. They again remained well, although the disease to which they were exposed was quite virulent.

The protection afforded against a fatal dose of disease-producing blood by even 5 c. c. of serum from immune 1383 was apparently complete. Instead of proceeding with a secondary experiment in the usual manner it was decided to determine the minimum amount of this serum required to protect against a fatal dose of diseased blood.

EXPERIMENT VI.—*Second experiment with first drawing of serum.*

No hogs were injected with serum alone in this experiment, all vaccinations being made by the serum-simultaneous method. The doses of serum used were reduced to $2\frac{1}{2}$ c. c., 5 c. c., and $7\frac{1}{2}$ c. c., and only 1 c. c. of disease-producing blood was used. This dose of blood was only half of that heretofore employed, but notwithstanding this it proved to be quite sufficient to infect unprotected hogs, for all of the hogs which received 1 c. c. of the disease-producing blood alone died of hog cholera, and the pen check exposed to them likewise contracted the disease and died.

Of the 6 vaccinated hogs, Nos. 1600 and 1601, which received only $2\frac{1}{2}$ c. c. of serum with the disease-producing blood, became slightly sick after vaccination; the remaining vaccinated hogs which received larger doses of serum showed no symptoms of disease, and none of the pen checks exposed to the vaccinated hogs became sick. All of the vaccinated hogs with the pen checks were moved to the quarantine pasture and were there exposed to hog cholera approximately two months after vaccination. Two of the 3 pen checks died as a result of this exposure and at autopsy typical lesions of hog cholera were found. The third check evidently possessed a high degree of natural resistance to the disease, for it did not become distinctly sick in the pasture nor in the exposure pen, where it was placed later.

PRODUCTION OF IMMUNITY FROM HOG CHOLERA.

TABLE 9.—*Second preliminary experiment with first drawing of serum from hog 1383 ("slow" immune—Scribner).*

No. of hog.	Weight.	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
1600	Pounds. 35	2½ e. c. serum and 1 e. c. disease-producing blood of hog 1389.	Aug. 18, 1906	Sick Aug. 25 to 27, 1906, but apparently recovered. Slightly sick, but recovered.	Oct. 15, 1906	Sickened latter part of November and died Dec. 8, 1906. ^a	Dec. 22, 1906	Remained well.	Intestinal ulcers.
1601	30	do.	do.	Remained well.	do.	do.	Nov. 19, 1906	do.	
1603	50	5 e. c. serum and 1 e. c. disease-producing blood of hog 1389.	do.	do.	do.	Sickened and died Oct. 29, 1906.	Nov. 19, 1906	Remained well.	No typical lesions.
1604	35	do.	do.	do.	do.	do.	do.	do.	
1606	40	7½ e. c. serum and 1 e. c. disease-producing blood of hog 1389.	do.	do.	do.	do.	Nov. 22, 1906	do.	Hemorrhages and ulcers.
1607	40	do.	do.	do.	do.	Sickened and died Oct. 27, 1906.	Nov. 16, 1906	Became unthrifty.	Do.
1602	35	Pen cheek with hogs 1600 and 1601.	do.	do.	do.	do.	do.	do.	Lesions indicating infection with gas bacillus.
1605	35	Pen cheek with hogs 1603 and 1604.	do.	do.	do.	do.	do.	do.	Hemorrhages and ulcers.
1608	40	Pen cheek with hogs 1606 and 1607.	Aug. 18, 1906	Sickened Aug. 24; died Aug. 27, 1906.	do.	do.	do.	do.	Do.
1609	35	do.	do.	do.	do.	do.	do.	do.	Lesions indicating infection with gas bacillus.
1610	35	do.	do.	Sickened Aug. 24; died Sept. 1, 1906.	do.	do.	do.	do.	Hemorrhages and ulcers.
1611	35	do.	do.	Sickened Aug. 24; died Sept. 4, 1906.	do.	do.	do.	do.	Do.
1612	35	do.	do.	do.	do.	do.	do.	do.	Do.
1613	30	Pen cheek with hogs 1609, 1610, 1611, and 1612.	do.	Sickened Aug. 30; died Sept. 5, 1906.	do.	do.	do.	do.	Do.

^a Probably died from vaccination.

Two of the 6 vaccinated hogs died while in the quarantine pasture; the remaining 4 did not become sick. From the autopsy findings there seems to be little room for doubt that the death of hog 1600, 1 of the 2 that died in the quarantine pasture, was due to hog cholera. It has been stated that this hog showed symptoms of illness shortly after vaccination. If this was due to a mild attack of hog cholera, as was probably the case, then it is hardly conceivable that the hog could have contracted hog cholera in the pasture, unless indeed this be a rare instance of failure to acquire immunity through an attack of the disease. It seems to us more likely that hog 1600 never completely recovered from the mild attack of hog cholera which followed vaccination, and that when placed in the rather crowded quarantine pasture it succumbed. The autopsy showed only the lesions which are found in the chronic type of hog cholera. The death of hog 1604, the other vaccinated animal which died in the pasture, can not be explained in the same way, for this hog did not show symptoms of illness following vaccination, and its death, if caused by hog cholera, was no doubt due to a failure of the vaccine to afford protection for two months, the time which intervened between vaccination and exposure. The fact, however, that hog 1603 (treated in the same manner as hog 1604), hog 1601, which received half as much serum, and hogs 1606 and 1607, which received more serum, all remained well, though subjected to the same exposure as hog 1604, points strongly to the likelihood that the death of hog 1604 was due to unusual susceptibility, or else to some cause other than hog cholera. This latter possibility must not be overlooked, as the lesions found at autopsy were not typical of that disease and as the conditions in the pasture were not conducive to thriftiness. Three of the surviving vaccinated hogs—1601, 1603, and 1606—were later placed in the Scribner exposure pen, where they again remained well.

Experiments V and VI show that the minimum amount of serum from hog 1383 required to protect with certainty from a simultaneous injection of a lethal dose of disease-producing blood probably lies in the neighborhood of 5 c. c. for hogs weighing from 30 to 35 pounds.

EXPERIMENT VII. *Second drawing of serum from immune 1383.*

It will be remembered that blood was drawn from each of the immunes at different times after hyperimmunization, but in the experiments heretofore described only serum from the first drawings of blood was used. In Experiments VII and VIII serum from the second and third drawings of blood from hog 1383 were tested, and although no attempt was made to determine accurately the potency of this serum, doses of 10 c. c. were found to give full protection from a lethal dose of disease-producing blood.

TABLE 10.—*Preliminary experiment with second drawing of serum from hog 1383 ("slow" immune—Scribner).*

No. of hog.	Weight. Pounds.	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
1647.	75	10 c. e. serum + 2 c. e. disease-producing blood from hogs 1568 and 1614.	Sept. 3, 1906	Remained well.	Oct. 15, 1906	Remained well.	Dec. 22, 1906	Remained well.	
1648.	50	20 c. e. serum + 2 c. e. disease-producing blood from hogs 1568 and 1614.	do.	do.	do.	do.	do.	do.	
1649.	75	do.	do.	do.	do.	do.	do.	do.	
1650.	55	do.	do.	do.	do.	do.	do.	do.	
1651 ^a	90	20 c. e. serum alone.	do.	do.	do.	do.	do.	do.	
1652 ^a	55	10 c. e. serum alone.	do.	do.	do.	do.	do.	do.	
1661.	55	2 c. e. disease-producing blood from hogs 1568 and 1614.	do.	Sickened Sept. 9; died Sept. 14, 1906.	do.	do.	Nov. 19, 1906 Sept. 20, 1906	do. do.	Hemorrhages and ul- cers.
1662.	45	do.	do.	Sickened Sept. 9; died Sept. 17, 1906.	do.	do.	do.	do.	Hemorrhagic lesions.
1663.	45	do.	do.	Sickened Sept. 9; died Oct. 6, 1906.	do.	do.	do.	do.	Hemorrhages and ul- cers.
1664.	40	Pen check with hogs 1661, 1662, and 1663.	do.	Sickened Sept. 19; killed Sept. 26, 1906.	do.	do.	do.	do.	Do.

^a These hogs were given double exposure to Scribner and Syphax strains of disease.

Table 10 shows that 4 hogs vaccinated by a simultaneous injection of disease-producing blood and the second drawing of serum remained well, although 3 checks which received the disease-producing blood alone all died of hog cholera. In this experiment 2 hogs were also injected with serum alone. These 2 were placed in the Scribner exposure pen seventeen days after receiving the serum injection. They remained well there and were removed to the Syphax exposure pen four weeks later, and here they again remained well. The virulence of the disease to which they were exposed in the Syphax pen is shown by the death of unprotected checks exposed to the same infection. (See Experiment XVI.)

The 4 hogs vaccinated by the serum-simultaneous method were placed in the quarantine pasture, and were there, with a number of others, accidentally exposed to infection approximately six weeks after vaccination. They all remained well and were transferred to the Scribner exposure pen three and one-half months after vaccination. No symptoms of disease followed this second exposure.

TABLE 11.—Preliminary experiment with third drawing of serum from hog 1383 ("slow" immune—Scribner).

No. of hog.	Weight. Pounds.	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
1653	50	10 c. c. serum and 2 c. c. disease-producing blood of hogs 1648 and 1614.	Sept. 3, 1906	Remained well.	Oct. 15, 1906	Remained well.	Dec. 22, 1906	Remained well.	
1654	50	do.	do.	do.	do.	do.	Nov. 16, 1906	do.	
1655	50	20 c. c. serum and 2 c. c. disease-producing blood of hogs 1648 and 1614.	do.	do.	do.	do.	Dec. 22, 1906	do.	
1656	40	do.	do.	do.	do.	do.	do.	do.	Hemorrhages and ulcers.
1661	35	2 c. c. disease-producing blood of hogs 1648 and 1614.	do.	Sickened Sept. 9; died Sept. 14, 1906.	do.	do.	do.	do.	Hemorrhagic lesions.
1662	45	do.	do.	Sickened Sept. 9; died Sept. 17, 1906.	do.	do.	do.	do.	Hemorrhages and ulcers.
1663	45	do.	do.	Sickened Sept. 9; died Oct. 6, 1906.	do.	do.	do.	do.	Hemorrhages and ulcers.
1664	40	Pen check with hogs 1661, 1662, and 1663.	do.	Sickened Sept. 19; killed Sept. 26, 1906.	do.	do.	do.	do.	Do.

EXPERIMENT VIII.—Third drawing of serum from immune 1383.

This experiment with the third drawing of serum was conducted in the same manner as the experiment with the second drawing, except that no hogs were vaccinated with serum alone. The results were in entire accord with those of Experiment VII. The 4 hogs vaccinated by the serum-simultaneous method, the serum being given in doses of 10 c. c. and 20 c. c. remained perfectly well. The death of the 3 control hogs, 1661, 1662, and 1663, shows the virulence of the diseased blood used in the experiment. The vaccinated hogs were exposed to hog cholera in the quarantine pasture six weeks after vaccination, and were given a later exposure in the Scribner exposure pen; none of them showed any symptoms of illness following either exposure.

Summary of experiments with serum from immune 1383.

In summarizing the results obtained with the serum from immune 1383, it should be noted first of all that in so far as our results indicate there is no difference in the protective power of the first, second, and third drawings, although it is true that no attempt was made to determine the minimum dose of

serum of the second and third drawings required to protect from a fatal dose of diseased blood.

In all, 20 hogs were treated by the serum-simultaneous method, the doses of serum used varying from $2\frac{1}{2}$ c. c. to 20 c. c., and the dose of disease-producing blood being from 1 c. c. to 2 c. c. to each hog. At the same time 12 hogs were injected with equivalent doses of the same disease-producing blood alone. Of the 20 vaccinated hogs only 1 (1600) died from the treatment, and this hog received but $2\frac{1}{2}$ c. c. of serum. Of the 12 hogs which received the same disease-producing blood without any serum all died.

In the pens with the hogs vaccinated by the serum-simultaneous method, 6 checks were used to determine the likelihood of the disease being transmitted by vaccinated hogs. None of these checks became sick, but of 4 checks placed in the pens with hogs injected with disease-producing blood only, all contracted disease.

Of the 19 hogs which survived vaccination by the serum-simultaneous method, all were exposed to hog cholera in the quarantine pasture from six weeks to two and one-half months after vaccination. Only 1 died from this exposure (1604), and, as previously explained, we can not be quite certain that this death was really due to hog cholera. Six pen checks, which had remained well during exposure to the vaccinated hogs, were exposed in the pasture with the vaccinated animals, and as a result 5 died of hog cholera.

Only 2 hogs were vaccinated with serum alone. These were first exposed to disease seventeen days after vaccination and remained well. Later exposure to disease in the Scribner and Syphax exposure pens did not produce sickness in these hogs.

From the results of the experiments with serum from hog 1383 we may draw the following conclusions:

1. Serum from hog 1383, when given in sufficient dose, protects nonimmunes from an otherwise fatal dose of disease-producing blood administered simultaneously with the serum.

2. The serum alone in doses of 10 c. c. protects from infection occurring within 17 days after the administration of the serum.

3. Immunity conferred by the serum-simultaneous method lasts for two and one-half months at least.

4. Hogs treated by the serum-simultaneous method, and which are not made ill by vaccination, do not communicate hog cholera to non-immunes by association.

5. The duration of immunity produced by the serum-simultaneous method of vaccination does not seem to be shortened by increasing the dose of the serum considerably above the amount necessary to prevent the occurrence of a distinct reaction.

SERUM FROM IMMUNE 1403 (SLOW METHOD).

Although two separate experiments were carried out with serum from immune 1403, very small doses of serum were not used, the experiments previously noted having shown that except in the case of unusually potent serum, such as that from immune 1383, 10 c. c. was as small a dose as could be relied upon.

EXPERIMENT IX.—*Preliminary experiment with first drawing of serum from immune 1403.*

In this experiment only the serum-simultaneous method of vaccination was used. Six hogs were vaccinated, the dose of serum being 10 c. c., 15 c. c., and 20 c. c. The same amount of disease-producing blood, 2 c. c., was used in all cases, and controls were inoculated with 2 c. c. of the same disease-producing blood alone. Only 1 (1553) of the 6 treated hogs showed signs of sickness after vaccination, while all of the hogs that were injected with the disease-producing blood alone died of hog cholera. The pen checks exposed to the vaccinated hogs did not become sick, although the check exposed to the hogs which received disease-producing blood alone contracted hog cholera and died.

Several weeks after vaccination 5 of the treated hogs with their 3 pen checks were placed in the quarantine pasture, and 4 of them were there exposed to hog cholera approximately two and one-half months after vaccination. It will be remembered that this outbreak of disease in the quarantine pasture occurred about October 15, 1906, so that hog 1549, which died September 16 (see Table 12), was not exposed to hog cholera after vaccination. Of the 4 vaccinated hogs which were exposed in the pasture, none showed symptoms of hog cholera, though 1 became unthrifty. All 3 of the pen checks died in the pasture and at autopsy showed distinct lesions of hog cholera.

The 4 vaccinated hogs which survived this exposure were placed in the Scribner exposure pen three and one-half months after vaccination. One of these 4 hogs (1556) was unthrifty when placed in the exposure pen and died about two weeks after exposure. The autopsy did not disclose lesions of hog cholera and the death was probably due to other causes. Hog 1552, another one of those placed in the Scribner exposure pen, also died a short while after exposure, but did not show positive lesions of hog cholera at autopsy. This hog when exposed was small and unthrifty, and while we can not state the cause of death with certainty, there is a strong probability that, as in the case of hog 1556, the death was not due to hog cholera. The other 2 vaccinated hogs remained well in the exposure pen.

TABLE 12.—*Preliminary experiment with first drawing of serum from hog 1403 ("slow" immune—Scribner).*

No. of hog.	Weight. Pounds.	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
1549	30	10 c. c. serum and 2 c. c. disease - producing blood of hog 1531.	Aug. 4, 1906	Remained well.		Sickened and died Sept. 16, 1906. ^a			Death due to intestinal perforation caused by worms. No lesions of cholera. Bile-duct occupied by intestinal worms.
1550	40	do.	do.	do.	Oct. 15, 1906	Remained well.	Nov. 19, 1906	Remained well.	No marked lesions.
1552	25	15 c. c. serum and 2 c. c. disease - producing blood of hog 1531.	do.	do.	do.	do.	Nov. 16, 1906	Became unthrifty; died Nov. 27, 1906. ^b	
1553	25	do.	do.	Slightly sick Aug. 14 to 18, 1906; recovered.					
1555	40	20 c. c. serum and 2 c. c. disease - producing blood of hog 1531.	do.	Remained well.	Oct. 15, 1906	Remained well.	Nov. 19, 1906	Remained well.	
1556	45	do.	do.	do.	do.	do.	do.	do.	
1551	25	Pon cheek with hogs 1549 and 1550.	do.	do.	do.	Became unthrifty.	Nov. 17, 1906	Sickened and died Nov. 30, 1906. ^b	Do.
1554	20	Pon cheek with hogs 1552 and 1553.	do.	do.	do.	Sickened and died Oct. 31, 1906.			Hemorrhages and ulcers.
1557	20	Pon cheek with hogs 1555 and 1556.	do.	do.	do.	Sickened and died Nov. 3, 1906.			Do.
1558	20	2 c. c. disease-producing blood of hog 1531.	Aug. 8, 1906	Sickened Aug. 9; died Aug. 16, 1906.					Hemorrhagic lesions.
1559	50	do.	do.	Sickened Aug. 9; died Aug. 22, 1906.					Do.
1560	40	do.	do.	Sickened Aug. 9; died Aug. 16, 1906.					Hemorrhages and ulcers.
1561	30	Pon cheek with hogs 1558, 1559, and 1560.	do.	Sickened Aug. 18; died Sept. 3, 1906.					Do.

^a Died before being exposed to hog cholera.^b Probably did not die of hog cholera.

EXPERIMENT X.—*Second experiment with first drawing of serum from immune 1403.*

In Experiment X 12 hogs were treated by the serum-simultaneous method, half of these being given 10 c. c. and the other half 20 c. c. of serum. Eight hogs were given serum alone in doses of 10 c. c. and 20 c. c., and 4 hogs were given disease-producing blood alone in the same dose as was used for the serum-simultaneous vaccinations. Checks were placed in the pens with both of the serum-simultaneous lots of hogs and also with those injected with disease-producing blood alone. Table 13 shows that none of the vaccinated hogs became sick, nor did they communicate disease to the exposed pen checks. All of the hogs which received disease-producing blood alone died, and they communicated hog cholera to the check which was placed in the pen with them.

For the purpose of testing the subsequent immunity of the vaccinated hogs, these were divided into two lots. One lot was to be exposed to hog cholera within a few weeks after vaccination, while the other was not to be exposed for several months. The first lot was exposed in the Scribner exposure pen, 4 of the hogs treated by serum alone being placed there thirteen days after vaccination. These were followed eleven days later by 6 of those treated by the serum-simultaneous method (see Table 13) and 1 of the pen checks. This pen check died of hog cholera; 1 of the hogs vaccinated with serum alone became very slightly sick, but recovered. The other vaccinated hogs remained well, but in order to secure further data concerning the behavior of hogs immune from one strain of disease when exposed to another all of these vaccinated hogs were later placed in the Syphax exposure pen, where they all remained well.

The other lot of vaccinated hogs in this experiment, with 2 checks, was placed in the quarantine pasture and there exposed to hog cholera seven weeks after vaccination. Both checks contracted hog cholera; one died, and the other was killed when in a moribund condition, and at autopsy showed typical lesions of that disease. One of the hogs (1638) treated with serum alone also became sick following this exposure and died of hog cholera, as did hog 1628, which was treated with 20 c. c. of serum plus 2 c. c. of disease-producing blood. None of the other vaccinated hogs became sick, and all of them, except hog 1620, which was unthrifty, were placed in the Scribner exposure pen about four months after vaccination. None of them contracted hog cholera from this exposure.

Summary of experiments with serum from immune 1403.

Only the first drawing of serum was used. In all 18 hogs were treated by the serum-simultaneous method, the doses of serum given in conjunction with 2 c. c. of disease-producing blood being 10 c. c.,

TABLE 13.—Secondary experiment with first drawing of serum from hog 1403 ('slow', immune—Scribner).

No. of hog.	Weight (pounds.)	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
1616 a	40	10 e. c. serum and 2 e. c. disease, produced in blood of hog 1598.	Aug. 25, 1906	Remained well.		Sept. 18, 1906	Remained well.		
1617 a	35	do.	do.	do.		do.	do.		
1618 a	35	do.	do.	do.		do.	do.		
1619	40	do.	do.	do.	Oct. 15, 1906	Remained well.	Became unthrifty.	Dec. 22, 1906	Became unthrifty.
1620	35	do.	do.	do.	do.	do.	do.	Dec. 22, 1906	Remained well.
1621	35	do.	do.	do.	do.	do.	do.	Sept. 18, 1906	do.
1624 a	40	20 e. c. serum and 2 e. c. disease, produced in blood of hog 1598.	do.	do.		do.	do.		
1625 a	40	do.	do.	do.		do.	do.		
1626 a	40	do.	do.	do.	Oct. 15, 1906	Remained well.	Sickened and died	Dec. 22, 1906	do.
1627	45	do.	do.	do.	do.	do.	do.	do.	do.
1628	40	do.	do.	do.	do.	do.	do.	do.	do.
1629	40	do.	do.	do.	do.	do.	do.	do.	do.
1632 a	50	10 e. c. serum alone.	do.	do.		do.	do.	Dec. 22, 1906	Remained well.
1633 a	45	do.	do.	do.		do.	do.	Sept. 7, 1906	Slightly sick, but recovered.
1634	45	do.	do.	do.	Oct. 15, 1906	Remained well.	do.	do.	do.
1635	45	do.	do.	do.	do.	do.	do.	do.	do.
1636 a	60	20 e. c. serum alone.	do.	do.		do.	do.	do.	do.
1637	40	do.	do.	do.	Oct. 15, 1906	Remained well.	do.	do.	do.
1638	50	do.	do.	do.	do.	do.	do.	do.	do.
1639 a	45	do.	do.	do.		do.	do.	Sept. 7, 1906	Remained well.
1622	35	Pen check with hogs 1616 to 1621.	do.	do.	Oct. 15, 1906	Sickened and died	do.	do.	do.
1623	25	do.	do.	do.	do.	do.	do.	do.	do.
1630	35	Pen check with hogs 1624 to 1629.	do.	do.		do.	do.	do.	do.
1631	40	do.	do.	do.	Oct. 15, 1906	Sickened and was killed Nov. 1, 1906.	do.	do.	do.
1640	40	2 e. c. disease—producing blood of hog 1598.	Aug. 25, 1906	Sickened Sept. 1; died Sept. 9, 1906.				Sept. 18, 1906	Sickened and died Oct. 10, 1906.
1641	40	do.	do.	do.					
1642	40	do.	do.	do.					
1643	35	do.	do.	do.					
1644	25	Pen check with hogs 1640 to 1643.	do.	do.					

^a These hogs were given double exposure to Scribner and Syphax strains of disease.

15 c. c., and 20 c. c. Of these 18 hogs 1 became slightly sick as a result of vaccination but soon recovered, while the others remained well. As controls on the virulence of the disease-producing blood, as well as on the protective power of the serum, 7 hogs were injected with disease-producing blood alone in the same dose as that given the vaccinated hogs. All of these controls died of hog cholera. To test the danger of vaccinated hogs conveying the disease to nonimmunes, 6 checks were exposed in the pens with those treated by the serum-simultaneous method. None of these checks became sick, although the 2 checks exposed to hogs injected with disease-producing blood alone contracted hog cholera, as was to be expected.

Of the 18 hogs treated by the serum-simultaneous method which were exposed to infection at various times after vaccination, 1 contracted hog cholera and died; none of the others became sick.

Of 8 hogs treated with the serum alone, 1 contracted hog cholera when exposed seven weeks after vaccination; the others remained well.

Of 6 unvaccinated hogs exposed along with the treated animals, all died of hog cholera.

These experiments warrant the following conclusions:

(1) Serum from immune 1403 when given in sufficient doses affords perfect protection from an otherwise fatal dose of disease-producing blood administered simultaneously.

(2) Hogs vaccinated by the serum-simultaneous method, with rare exceptions, remain immune for at least seven weeks after vaccination.

(3) Hogs treated by the serum-simultaneous method, if not made ill by the treatment, do not convey hog cholera to others by association.

(4) For seven weeks after vaccination immunity in hogs treated with serum alone is as firm as in those treated by the serum-simultaneous method.

(5) Hogs immunized from one strain of hog cholera are also immune from other strains of the same disease.

TESTS OF SERUM FROM HOGS HYPERIMMUNIZED WITH BLOOD FROM THE SYPHAX OUTBREAK.

SERUM FROM IMMUNE E392 (QUICK METHOD).

EXPERIMENT XI.—*First drawing of serum.*

The results obtained in Experiment XI did not differ materially from those of similar experiments carried out with serum from immunes treated with the Scribner disease-producing blood. The smallest dose of serum used was 10 c. c., and although the 2 hogs given this dose did not become visibly sick from the serum-simultaneous vaccination, the fact that 1 of those given 15 c. c. as well as 1

TABLE 14.—Preliminary experiment with first drawing of serum from hog 1392 ("quick," immune—*Syphax*).

No. of hog.	Weight. <i>Pounds.</i>	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
1588.....	40	10 c. c. serum and 2 c. c. disease-producing blood of hog 1560.	Aug. 14, 1906	Remained well.....	Oct. 15, 1906	Remained well.....	Dec. 16, 1906	Remained well.....	
1589.....	35	do.	do.	do.	do.	do.	do.	do.	
1591.....	35	15 c. c. serum and 2 c. c. disease-producing blood of hog 1560.	do.	Sick Aug. 19 to 23, 1906; recovered.	do.	do.	do.	do.	
1592.....	30	do.	do.	do.	do.	do.	do.	do.	
1594.....	40	20 c. c. serum and 2 c. c. disease-producing blood of hog 1560.	do.	Remained well.....	do.	do.	do.	do.	
1595.....	40	do.	do.	do.	do.	do.	do.	do.	
1590.....	40	Pen check with hogs 1588 and 1589.	do.	do.	do.	Sickened and died	do.	do.	Hemorrhages and ulcers. Do.
1593.....	35	Pen check with hogs 1591 and 1592.	do.	do.	do.	do.	do.	do.	
1596.....	40	Pen check with hogs 1594 and 1595.	do.	do.	do.	do.	do.	do.	
1597.....	35	2 c. c. disease-producing blood of hog 1560.	Aug. 14, 1906	Sickened Aug. 21; died Aug. 31, 1906.	do.	do.	do.	do.	Lung lesions indicating pneumonia.
1598.....	40	do.	do.	Sickened Aug. 21; died Aug. 29, 1906.	do.	Became unthrifty and died Dec. 28, 1906.	do.	do.	Hemorrhages and ulcers.
1599.....	40	Pen check with hogs 1597 and 1598.	do.	Sickened Aug. 27; died Sept. 19, 1906.	do.	do.	do.	do.	Hemorrhagic lesions. Do.

TABLE 15.—Secondary experiment with first drawing of serum from hog 1392 ("quick," immune—*Syphax*).

No. of hog.	Weight. <i>Pounds.</i>	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
1685.....	45	10 c. c. serum and 2 c. c. disease-producing blood of hog 1562.	Sept. 15, 1906	Remained well.....			Oct. 2, 1906	Remained well.....	
1686.....	30	do.	do.	do.	do.	do.	do.	do.	
1687.....	45	do.	do.	do.	do.	do.	Dec. 22, 1906	Killed by hogs.....	Badly mutilated; no autopsy.

1688	35	.do.	.do.	.do.	.do.	.do.	Dec. 22, 1906	Remained well.	
1689	33	.do.	.do.	.do.	.do.	.do.	Oct. 2, 1906	.do.	
1690	43	.do.	.do.	.do.	.do.	.do.	.do.	.do.	
1693	45	20 c. c. serum and 2 c. c. disease-producing blood of hog 1662.	.do.	.do.	.do.	.do.	.do.	.do.	
1694	50	.do.	.do.	.do.	.do.	.do.	.do.	.do.	Ulceration of cecum.
1695	40	.do.	.do.	.do.	.do.	.do.	.do.	.do.	
1696	45	.do.	.do.	Sick (C).	Oct. 15, 1906	Sickened; died Nov. 7, 1906, a	.do.	.do.	
1697	45	.do.	.do.	Remained well.	.do.	Remained well.	Dec. 22, 1906	Remained well.	
1698	35	10 c. c. serum alone.	.do.	.do.	.do.	.do.	.do.	.do.	
1701	40	.do.	.do.	.do.	.do.	.do.	.do.	.do.	
1702	40	.do.	.do.	.do.	.do.	.do.	.do.	.do.	No pathological lesions.
1703 ^b	45	.do.	.do.	.do.	.do.	.do.	Sept. 26, 1906	Remained well.	No marked lesions.
1704	40	.do.	.do.	.do.	.do.	.do.	.do.	Sickened; died Nov. 18, 1906.	
1705	55	20 c. c. serum alone.	.do.	.do.	Oct. 15, 1906	Became unthrifty; killed Jan. 2, 1907.	.do.	.do.	No pathological lesions.
1706 ^b	30	.do.	.do.	.do.	.do.	.do.	Sept. 26, 1906	Remained well.	
1707	50	.do.	.do.	.do.	Oct. 15, 1906	Sickened; died Nov. 14, 1906.	.do.	.do.	Hemorrhages and ulcers.
1708 ^b	50	.do.	.do.	.do.	.do.	.do.	Sept. 26, 1906	Remained well.	
1691	35	Pen check with hogs 1685 to 1690.	.do.	.do.	.do.	.do.	Oct. 2, 1906	Sickened; died Oct. 17, 1906.	Do.
1692	35	.do.	.do.	.do.	Oct. 15, 1906	Sickened; died Oct. 30, 1906.	.do.	.do.	Hemorrhagic le- sions.
1699	35	Pen check with hogs 1693 to 1698.	.do.	.do.	.do.	.do.	Oct. 2, 1906	Sickened; died Oct. 25, 1906.	Do.
1700	30	.do.	.do.	.do.	Oct. 15, 1906	Sickened; died Nov. 3, 1906.	.do.	.do.	Hemorrhages and ulcers.
1706	40	2 c. c. disease-producing blood.	Sept. 15, 1906	Sickened Sept. 25; died Sept. 28.	.do.	.do.	Oct. 2, 1906	Sickened; died Oct. 25, 1906.	
1710	40	.do.	.do.	Sick Sept. 24 to Oct. 13, 1906; re- covered.	.do.	.do.	Oct. 25, 1906	Remained well.	
1711	35	Pen check with hogs 1709 and 1710.	.do.	Sickened Oct. 8; died Oct. 14, 1906.	.do.	.do.	.do.	.do.	Do.

^a Thus hog probably died from vaccination.

^b These hogs were given double exposure to Scribner and Syphax strains of disease.

given 20 c. c. of serum did become sick makes it quite probable that less than 10 c. c. of this serum could not be used with 2 c. c. of virulent blood with safety. (See Table 14.)

None of the hogs treated by the serum-simultaneous method died, although 2 became sick, as already stated; notwithstanding the sickness of the 2 vaccinated hogs, the untreated pen checks exposed to them did not become sick. Both of the hogs injected with disease-producing blood alone became sick and died of hog cholera, and they also communicated disease to their pen check.

Following out the usual plan, the surviving hogs from this experiment were placed in the quarantine pasture. These hogs had been vaccinated two months when the accidental outbreak of disease occurred in the pasture, but none of them became sick from the exposure. Two of the 3 checks which were placed in the pasture with the treated animals died of hog cholera; the third check became unthrifty and died, but apparently did not suffer from hog cholera. Four of the vaccinated hogs were later transferred to the Scribner exposure pen and again remained well.

EXPERIMENT XII.—*Second experiment with first drawing of serum from immune 1392.*

In Experiment XII 12 hogs were treated by the serum-simultaneous method, 8 were treated with serum alone, and 2 were injected with disease-producing blood alone. Four uninoculated hogs were placed in the pens with the serum-simultaneous hogs, and 1 with the 2 that received disease-producing blood only. As a result of the treatment, as far as could be observed none of the vaccinated hogs showed symptoms of illness, and the checks in the pens with them also remained well. With the hogs that received disease-producing blood alone the result was quite different, for both contracted hog cholera and one died; the pen check that was exposed to these hogs also became sick and died of hog cholera. (See Table 15.)

For the purpose of testing the immunity possessed by the vaccinated hogs at different lengths of time after vaccination, these hogs were divided into two lots, 6 of the serum-simultaneous vaccinated hogs being placed in the Syphax exposure pen seventeen days after vaccination and 4 of the hogs treated with serum alone in the same pen eleven days after vaccination. Table 15 shows the results of these exposures. Of the 6 hogs treated by the serum-simultaneous method and exposed seventeen days after vaccination, all remained well; of the 4 hogs vaccinated with serum alone and exposed in the Syphax exposure pen eleven days after vaccination, 1 (1704), after being sick a long time, died about two months later, probably of hog cholera, although the lesions found at autopsy were slight and not such as would be expected from a chronic case of hog cholera. The other 3 hogs (1703, 1706, and 1708), vaccinated with serum alone,

remained well and were later exposed to the Scribner disease without being made sick. Two of the uninoculated check hogs were placed in the exposure pen with the serum-simultaneous hogs, and both died of hog cholera.

The exposure of the second lot of hogs in this experiment took place in the quarantine pasture thirty days after vaccination. Of the 6 serum-simultaneous hogs that were exposed there, 1 (1696) did not thrive, and finally died of hog cholera seven weeks after vaccination, while the others remained well. The death of hog 1696 was probably due to a mild form of disease caused by the original serum-simultaneous vaccination, as the hog at autopsy showed the lesions of the chronic type of hog cholera, though it had been exposed to disease in the pasture only three weeks at the time of its death. Of the 4 hogs treated with serum alone, which were accidentally exposed in the quarantine pasture thirty days after vaccination, 1 (1707) died of hog cholera. The others did not contract the disease. Both of the pen checks that were placed in the pasture with the vaccinated hogs died of hog cholera.

Summary of experiments with serum from immune 1392.

A summary of the results obtained through the use of the first drawing of serum from immune 1392 shows that of 18 hogs treated by the serum-simultaneous method, the serum being used in doses of 10 c. c., 15 c. c., and 20 c. c., 1 became sick and 1 (1696; see above) died from the treatment. Of 4 hogs injected with the same amount of the same disease-producing blood as that used for the serum-simultaneous treatment, all became sick and 3 died.

All of the surviving treated hogs with the surviving checks were exposed to infection after vaccination. All 17 of the serum-simultaneous hogs, exposed seventeen to thirty days after vaccination, remained well; 2 of the 8 hogs treated with serum alone died of hog cholera when exposed eleven to thirty days after treatment, while of 4 checks exposed in the same manner as the treated hogs, all died of hog cholera.

As a result of these experiments it will be seen that —

(1) The serum of hog 1392, in doses of 10 c. c. or more, was sufficient to protect hogs from a fatal dose of disease-producing blood administered simultaneously with the serum.

(2) Hogs treated by simultaneous injections of serum from hog 1392, together with disease-producing blood, remained immune for at least thirty days after vaccination.

(3) Hogs treated by the serum-simultaneous method, even when they became distinctly sick, did not convey disease to unprotected animals which associated with them.

TABLE 16.—*Preliminary experiment with first drawing of serum from hog 1274 ("quick," immune—Syphax).*

No. of hog.	Weight. <i>Pounds.</i>	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
1715....	30	5 c. c. serum and 2 c. c. disease - producing blood of hog 1680.	Sept. 21, 1906	Remained well....	Oct. 15, 1906	Died Nov. 18, 1906....			Doubtful lesions of hog cholera.
1716....	40	do.	do.	do.	do.	Remained well....			
1718....	40	10 c. c. serum and 2 c. c. disease - producing blood of hog 1680.	do.	Sick Sept. 30 to Oct. 8, 1906; recovered.	do.	Became unthrifty and killed by hogs.	Dec. 12, 1906	Remained well....	No autopsy.
1719....	50	do.	do.	do.	do.	Became unthrifty and was not exposed.			
1721....	45	15 c. c. serum and 2 c. c. disease - producing blood of hog 1680.	do.	Remained well....	Oct. 15, 1906	Remained well....	Dec. 22, 1906	Remained well....	
1722....	40	do.	do.	do.	do.	do.	do.	do.	
1717....	40	Pen check with hogs 1715 and 1716.	do.	do.	do.	Sickened but recovered.	Dec. 12, 1906	Died Jan. 17, 1907.	Congestion of lungs and pleura.
1720....	40	Pen check with hogs 1718 and 1719.	do.	do.	do.	Became unthrifty and killed by hogs.			No autopsy.
1723....	35	Pen check with hogs 1721 and 1722.	do.	do.	do.	Died Nov. 11, 1906....			Hemorrhages and ulcers.
1730....	40	2 c. c. of disease-producing blood of hog 1680.	Sept. 21, 1906	Sickened Sept. 27; died Oct. 1, 1906.	do.	do.			Hemorrhagic lesions.
1731....	50	do.	do.	Sickened Sept. 27, 1906; killed when moribund.	do.	do.			Do.
1732....	40	Pen check with hogs 1730 and 1731.	do.	Sickened Oct. 4; died Oct. 13, 1906.	do.	do.			Hemorrhages and ulcers.

(4) The immunity produced by injections of serum alone from hog 1392 did not seem to be as complete as that produced by a simultaneous injection of serum and disease-producing blood.

(5) Hogs immunized with serum prepared through the use of one strain of disease were immune when exposed to another strain of disease.

SERUM FROM IMMUNE 1274 (QUICK METHOD).

EXPERIMENT XIII.—*Preliminary experiment with first drawing of serum.*

The preliminary experiment with the first drawing of serum from immune 1274 consisted of serum-simultaneous vaccinations only, a certain number of controls being, of course, used. The results of this experiment were not entirely successful, as may be seen from Table 16.

First of all, we find that serum from hog 1274 probably has distinct protective power when injected simultaneously with a fatal dose of disease-producing blood. This is indicated by the death of the 2 hogs that received disease-producing blood alone, and the survival of all those treated with serum. At the same time it is surprising that the 2 hogs that received 10 c. c. of serum with the disease-producing blood should have become distinctly sick, whereas the 2 that received only 5 c. c. of serum remained well. This result could only be explained on the ground of unusual susceptibility on the part of hogs 1718 and 1719, or because hogs 1715 and 1716, which received only 5 c. c. of serum, were exceptionally resistant to the disease. In the light of this experiment only, the latter supposition seems to be the most reasonable explanation of the conflicting results, for when the vaccinated hogs with their pen checks were exposed to hog cholera in the quarantine pasture three weeks after vaccination only one of the three pen checks (1723) died of hog cholera. It is true that one other sickened, but it recovered, and the third check never became distinctly sick in the quarantine pasture. The subsequent death of this check (1717) in the exposure pen can not be attributed to hog cholera. It appears, therefore, that the lot of hogs used in this experiment taken as a whole were probably more than normally resistant to hog cholera, and for this reason too great stress should not be laid upon the results. At the same time the serum must have given the vaccinated hogs a certain degree of protection, for both of the controls injected with disease-producing blood alone became very sick, one of them died, and the other was killed to secure blood for other experiments, and at autopsy both showed characteristic lesions of hog cholera. In contrast to this is the behavior of the hogs which received the same disease-producing blood in combination with the serum, not one of these having died from vaccination. When this lot of hogs was exposed to disease in the quarantine pasture one of the pen checks died of hog cholera, one became sick but recovered, while the other never showed marked symptoms of illness. With regard to the treated hogs in the quarantine pasture, we find that one of those which had

received but 5 c. c. of serum with the disease-producing blood died, but a positive diagnosis of hog cholera could not be made from the autopsy findings. The 2 hogs that were made sick by vaccination remained more or less unthrifty, but neither contracted hog cholera; the other 3 vaccinated hogs remained well in the quarantine pasture as well as in the Syphax exposure pen where they were placed after removal from the pasture.

EXPERIMENT XIV.—*Second experiment with first drawing of serum from immune 1274.*

The second experiment with serum from hog 1274 is incomplete, as the vaccinated hogs were not exposed to natural infection after vaccination. The results of the vaccination are of interest, however, for comparison with those of the preceding experiment in which the 2 hogs treated by the serum-simultaneous method with 10 c. c. of serum became distinctly sick. In Experiment XIV none of the 12 hogs which were given serum with disease producing blood became sick, although both of the hogs treated with the blood alone died of hog cholera. Owing to the lateness of the season and to the fact that our plans to hold vaccinated hogs for several months before exposing them had been interfered with considerably by the accidental outbreak of hog cholera in our quarantine pasture, it was decided to reserve all of the vaccinated hogs in Experiment XIV, together with their pen checks, for exposure during the summer of 1907.

TABLE 17.—*Secondary experiment with first drawing of serum from hog 1274 ("quick" immune—Syphax).*

No. of hog.	Weight.	Material injected.	Inoculation.		Remarks.
			Date.	Result.	
1938....	55	10 c. c. serum and 2 c. c. disease-producing blood of hog 1925.	Dec. 20, 1906	Remained well.....	Not exposed.
1939....	50do.....do.....do.....	Do.
1940....	70do.....do.....do.....	Do.
1941....	45do.....do.....do.....	Do.
1942....	40do.....do.....do.....	Do.
1943....	40do.....do.....do.....	Do.
1946....	40	20 c. c. serum and 2 c. c. disease-producing blood of hog 1925.do.....do.....	Do.
1947....	40do.....do.....do.....	Do.
1948....	50do.....do.....do.....	Do.
1949....	40do.....do.....do.....	Do.
1950....	40do.....do.....do.....	Do.
1951....	75do.....do.....do.....	Do.
1954....	75	10 c. c. serum alone.....do.....do.....	Do.
1955....	40do.....do.....do.....	Do.
1956....	50do.....do.....do.....	Do.
1957....	60do.....do.....do.....	Do.
1958....	50	20 c. c. serum alone.....do.....do.....	Do.
1959....	80do.....do.....do.....	Do.
1960....	75do.....do.....do.....	Do.
1961....	40do.....do.....do.....	Do.
1944....	40	Pen check with hogs 1938 to 1943.do.....do.....	Do.
1945....	40do.....do.....do.....	Do.
1952....	40	Pen check with hogs 1946 to 1951.do.....do.....	Do.
1953....	40do.....do.....do.....	Do.
1962....	30	2 c. c. disease-producing blood of hog 1925.	Dec. 20, 1906	Sick Dec. 27, 1906; died Jan. 3, 1907.	Hemorrhages.
1963....	70do.....do.....	Sick Dec. 27, 1906; died Jan. 7, 1907.	Do.
1964....	40	Pen check with hogs 1962 and 1963.do.....	Sick Jan. 8, 1907; died Jan. 12, 1907.	Hemorrhages and ulcers.

Summary of results obtained with first drawing of serum from immune 1274.

Eighteen hogs were vaccinated by the serum-simultaneous method, serum in doses of 5 c. c., 10 c. c., 15 c. c., and 20 c. c. being used in conjunction with 2 c. c. of disease-producing blood. Of these 18 hogs 2 became sick; none died.

Four hogs were injected with disease-producing blood alone; all became sick; 3 died, and 1 was killed when in a moribund condition.

Eight hogs were injected with serum alone and all remained well.

Only 6 of the vaccinated hogs were later exposed to infection; 1 of these died, but without exhibiting either before death or at autopsy positive signs of hog cholera. Of 3 pen checks which were exposed at the same time as the 6 vaccinated hogs, 1 died of hog cholera, the other became sick but recovered, while the third check did not become plainly ill.

As a result of the experiments with the first drawing of serum from hog 1274, we may conclude that—

(1) The serum when given in sufficient dose will protect non-immunes from a simultaneous injection of a fatal dose of disease-producing blood.

(2) The serum-simultaneous injections in which the largest dose of hyperimmune serum was used seemed to afford quite as complete protection from hog cholera when exposure took place three weeks after vaccination as was afforded by the injection of disease-producing blood with a smaller dose of serum.

(3) An insufficient dose of serum with disease-producing blood appeared to result in more or less permanent injury to the hogs treated in that manner.

SERUM FROM IMMUNE 1297 (SLOW METHOD).

EXPERIMENT XV.—*First drawing of serum.*

The preliminary experiment with the serum from immune 1297 shows that 5 c. c. of that serum was not sufficient to insure protection from a fatal dose of disease-producing blood administered with the serum, for, as may be seen from Table 18, hog 1742 died of hog cholera after vaccination. The other hog injected in the same manner as hog 1742, however, remained well, as did the pen check that associated with these two hogs. None of the hogs treated with doses of 10 c. c. and 15 c. c. of serum plus disease-producing blood showed any symptoms of illness, but both of the hogs that received disease-producing blood alone became sick, and one of them died. The pen check that was exposed to the hogs that were injected with disease-producing blood alone contracted hog cholera from them and died. With the exception of hogs 1743 and 1744, all of the surviving vaccinated hogs with their pen checks were placed in the pasture on October 17 to

TABLE 18.—*Preliminary experiment with first drawing of serum from hog 1297 ('slow' immune—Sylphax).*

No. of hog.	Weight. Pounds.	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
1742....	50	5 c. c. serum and 2 c. c. disease - producing blood of hog 1664.	Sept. 29, 1906	Sickened Oct. 6; died Oct. 9, 1906.					
1743....	40	do.	do.	Remained well.	Oct. 29, 1906	Remained well, but killed by hogs.			Hemorrhagic lesions.
1745....	40	10 c. c. serum and 2 c. c. disease-producing blood of hog 1664.	do.	do.	Oct. 17, 1906	Remained well.	Dec. 22, 1906	Remained well.	No pathological lesions.
1746....	35	do.	do.	do.	do.	do.	do.	do.	
1748....	45	15 c. c. serum and 2 c. c. disease-producing blood of hog 1664.	do.	do.	do.	do.	do.	do.	
1749....	45	do.	do.	do.	do.	do.	do.	do.	
1744....	40	Pen check with hogs 1742 and 1743.	do.	do.	Oct. 29, 1906	do.	do.	do.	
1747....	35	Pen check with hogs 1745 and 1746.	do.	do.	Oct. 17, 1906	Sickened and died Dec. 3, 1906.	Jan. 23, 1907	do.	Ulcers.
1750....	40	Pen check with hogs 1748 and 1749.	do.	do.	do.	Sickened but recovered.			
1751....	40	2 c. c. disease-producing blood of hog 1664.	Sept. 29, 1906	Sickened Oct. 8; died Oct. 13, 1906.					Hemorrhagic lesions.
1752....	40	do.	do.	Sick Oct. 8 to Nov. 5, 1906; recovered.	Dec. 4, 1906	Remained well.			
1753....	35	Pen check with hogs 1751 and 1752.	do.	Sickened Oct. 18; died Nov. 1, 1906.					Hemorrhages and ulcers.

await future exposure. The accidental outbreak of hog cholera in this pasture made its appearance about the time that the hogs were placed there, and they may therefore be regarded as having been exposed to disease on October 17, or twenty-two days after vaccination. Both pen checks that were exposed at the same time with the vaccinated hogs became sick; one died, but the other recovered. None of the 4 treated pigs became sick in the pasture, and during a subsequent exposure in the Scribner exposure pen they likewise remained well. Hogs 1743 and 1744, one a vaccinated hog and the other a pen check, were placed on October 29, 1906, in a separate pasture that was used for hogs that had been made sick by vaccination or that had been removed from the exposure pen. Although there was undoubtedly ample opportunity for these hogs to contract hog cholera in this pasture, neither of them did so, but hog 1743 was killed by other hogs. The pen check 1744 was placed in the Scribner exposure pen on January 23, 1907, to further test its immunity, with the result that it survived without showing disease.

EXPERIMENT XVI.—*Second experiment with first drawing of serum.*

In the second experiment with serum from immune 1297 the usual plan was followed. Twelve hogs were treated by the serum-simultaneous method, 6 of them being given 10 c. c. of serum, and 6, 20 c. c. of serum with 2 c. c. of disease-producing blood in each case; 4 hogs were injected with 10 c. c. of serum alone and 4 with 20 c. c. of serum alone; 2 hogs were injected with 2 c. c. of the disease-producing blood alone. Two uninoculated checks were placed in the pens with each of the lots treated by the serum-simultaneous method, and 1 was placed in the pen with the hogs that were injected with disease-producing blood alone. As may be seen from Table 19, all of the animals that were injected with serum alone or with serum and disease-producing blood combined, together with their pen checks, remained well after treatment. Both of the hogs that were injected with disease-producing blood alone contracted hog cholera, and their pen check contracted the disease from them and died of hog cholera.

In order to test further the immunity of the vaccinated hogs, half of those vaccinated by the serum-simultaneous method, together with half of those treated with serum alone, were placed in the Scribner exposure pen three weeks after vaccination. All 4 of the surviving pen checks were exposed at practically the same time. The serum-simultaneous vaccinated hogs all remained well as a result of this exposure, but 3 of the 4 hogs treated with serum alone died of hog cholera, and 3 of the 4 untreated check hogs also died.

The other treated hogs that were not exposed with the lot just described were exposed as follows: Five hogs treated by the serum-simultaneous method were exposed in the Scribner exposure pen fifty-one days after vaccination. Four hogs that were treated with

TABLE 19.—Secondary experiment with first drawing of serum from hog 1297 ("slow" immune—Sylphax).

No. of hog.	Weight.	Material injected.	Inoculation.		Pen exposure—Scribner.		Autopsy.
			Date.	Result.	Date.	Result.	
1836	<i>Pounds.</i> 60	10 c. c. serum and 2 c. c. disease-producing blood of hog 1631.	Nov. 1, 1906	Remained well	Nov. 22, 1906	Remained well.	
1837	65	do.	do.	do.	do.	do.	
1838	55	do.	do.	do.	do.	do.	
1839	45	do.	do.	do.	Dec. 22, 1906	do.	
1840	70	do.	do.	do.	do.	do.	
1841	70	do.	do.	do.	do.	do.	
1844	60	20 c. c. serum and 2 c. c. disease-producing blood of hog 1631.	do.	do.	Nov. 22, 1906	Not exposed.	
1845	60	do.	do.	do.	do.	Remained well.	
1846	60	do.	do.	do.	do.	do.	
1847	60	do.	do.	do.	Dec. 22, 1906	do.	
1848	60	do.	do.	do.	do.	do.	
1849	60	do.	do.	do.	do.	do.	
1852	55	10 c. c. serum alone.	do.	do.	do.	do.	
1853	75	do.	do.	do.	Jan. 31, 1907	Died Feb. 19, 1907.	Hemorrhages and ulcers.
1854	55	do.	do.	do.	Nov. 22, 1906	Died Dec. 6, 1906.	Hemorrhagic lesions.
1855	40	do.	do.	do.	Jan. 31, 1907	Remained well.	
1856	45	20 c. c. serum alone.	do.	do.	Nov. 22, 1906	Died Dec. 9, 1906.	Do.
1857	60	do.	do.	do.	Jan. 31, 1907	Died Feb. 22, 1907.	Do.
1858	45	do.	do.	do.	do.	Died Feb. 11, 1907.	Do.
1859	60	do.	do.	do.	Nov. 22, 1906	Remained well.	
1842	40	Pen check with hogs 1836 to 1841.	do.	do.	do.	Died Dec. 9, 1906.	Do.
1843	30	do.	do.	do.	Nov. 17, 1906	Died Dec. 1, 1906.	Do.
1850	50	Pen check with hogs 1844 to 1849.	do.	do.	Nov. 22, 1906	Died Dec. 2, 1906.	Do.
1851	50	do.	do.	do.	do.	Died Dec. 4, 1906.	Do.
1867	50	2 c. c. disease-producing blood of hog 1631.	Nov. 1, 1906	Sick Nov. 7; died Nov. 11, 1906.	do.	Remained well.	Do.
1868	50	do.	do.	Sick Nov. 7; killed Nov. 14, 1906.	do.	do.	Do.
1869	40	Pen check with hogs 1867 and 1868.	do.	Sick Nov. 15; died Nov. 20, 1906.	do.	do.	Do.
1902	50	do.	do.	do.	Dec. 27, 1906	Sickened; died Jan. 16, 1907.	Do.
1903	30	do.	do.	do.	do.	Sickened; died Jan. 11, 1907.	Do.
1904	40	do.	do.	do.	do.	Sickened; died Jan. 8, 1907.	Do.
1905	40	do.	do.	do.	do.	Sickened; died Jan. 11, 1907.	Do.
1906	35	do.	do.	do.	do.	Sickened; died Jan. 14, 1907.	Do.
1907	35	do.	do.	do.	do.	Sickened; died Jan. 16, 1907.	Do.
1908	40	do.	do.	do.	do.	Sickened; died Jan. 18, 1907.	Do.

serum alone were exposed in the same pen twelve weeks after vaccination. The virulence of the disease to which the serum-simultaneous treated hogs were subjected is shown by the fact that 7 check hogs (1992-1998, Table 19) placed in the exposure pen five days later all died of hog cholera, but notwithstanding this very severe test, all of the serum-simultaneous vaccinated hogs remained well. Of the 4 hogs vaccinated with serum alone and exposed twelve weeks after treatment 3 died, as was to be expected, since the hogs treated similarly and exposed only three weeks after treatment did not show any appreciable degree of resisting power.

Summary of results obtained with serum from immune 1297.

Eighteen hogs were vaccinated by the serum-simultaneous method, the serum being used in doses of from 5 c. c. to 20 c. c. Only 1 of these hogs died from the treatment, and this hog (1742) received but 5 c. c. of the serum. Four hogs were injected with the same dose of the same disease-producing blood as was given the serum-simultaneous treated hogs, and as a result all became sick and 3 died. Of 6 pen checks exposed to the serum-simultaneous treated hogs after vaccination all remained well, although both of the checks exposed to hogs treated with diseased blood alone contracted hog cholera and died.

In regard to immunity in the treated hogs subsequent to vaccination it is seen that of the 16 serum simultaneous treated hogs all remained well when exposed from three to seven weeks after vaccination. Of 8 hogs treated with serum alone and exposed to hog cholera from three to twelve weeks after vaccination we find that 6 died of hog cholera, while of 4 checks not inoculated and exposed to the same infection 3 died.

With regard to the experiments with the first drawing of serum from immune 1297, the following conclusions may be drawn:

(1) When administered in sufficient dose this serum will completely protect hogs from an otherwise fatal dose of disease-producing blood given simultaneously with the serum.

(2) The immunity produced by a serum-simultaneous vaccination remained quite firm for at least seven weeks.

(3) This immunity was quite as firm in the hogs that received 20 c. c. of serum with disease-producing blood as in those that received only 10 c. c.

(4) It is not necessary for hogs to show visible symptoms of illness after vaccination in order to secure immunity that will last at least seven weeks.

(5) The immunity in serum-simultaneous vaccinated hogs was not due to serum alone, as shown by the death of animals injected with serum alone when exposed to hog cholera.

TABLE 20.—Preliminary experiment with first drawing of serum from hog 1310 ("slow" immune—*Syphax*).

No. of hog.	Weight. <i>Pounds.</i>	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.	
			Date.	Result.	Date.	Result.	Date.	Result.		
1671	45	5 c. c. serum and 2 c. c. disease-producing blood of hog 1641.	Sept. 8, 1906	Sick Sept. 14; died Sept. 24, 1906.					Hemorrhages and ulcers.	
1672	45	do.	do.	Sick Sept. 15 to 26, 1906; recovered.						
1674	50	10 c. c. serum and 2 c. c. disease-producing blood of hog 1641.	do.	Sick Sept. 14; died Oct. 2, 1906.				Oct. 29, 1906	Remained well.	Do.
1675	50	do.	do.	Sick Sept. 15 to 21, 1906; recovered.						
1677	55	15 c. c. serum and 2 c. c. disease-producing blood of hog 1641.	do.	Remained well.				Oct. 15, 1906	Remained well.	
1678	50	do.	do.	do.				do.	do.	
1673	35	Pen cheek with hogs 1671 and 1672.	do.	Sick Oct. 9; died Oct. 13, 1906.				do.	do.	Intestinal ulcers.
1676	30	Pen cheek with hogs 1674 and 1675.	do.	Sick Sept. 21 to 25, 1906; recovered.				Oct. 15, 1906	Killed by other hogs.	No autopsy.
1679	45	Pen cheek with hogs 1677 and 1678.	do.	Remained well.				do.	Died Nov. 2, 1906.	Hemorrhagic lesions.
1680	50	2 c. c. disease-producing blood of hog 1641.	Sept. 8, 1906	Sick Sept. 15; died Sept. 23, 1906.						Hemorrhages and ulcers.
1681	50	do.	do.	Sick Sept. 15; died Sept. 22, 1906.						Do.
1682	40	Pen cheek with hogs 1680 and 1681.	do.	Sick Sept. 25; died Sept. 28, 1906.						Do.

(6) Hogs immunized with serum prepared from one strain of disease (Syphax) may be expected to resist infection of the same nature from an entirely different source (Scribner).

(7) Hogs vaccinated by the serum-simultaneous method, if not made sick by the treatment, will not communicate disease to other hogs.

SERUM FROM IMMUNE 1310 (SLOW METHOD).

EXPERIMENT XVII.—*Preliminary experiment with first drawing of serum.*

In this experiment only the serum-simultaneous method of vaccination was used, and Table 20 shows the results. Six hogs were vaccinated by the serum-simultaneous method, doses of 5 c. c., 10 c. c., and 15 c. c. of serum being used. Of the 4 hogs injected with less than 15 c. c. of serum all became sick and 2 died, and the 2 pen checks contracted hog cholera from them. The 2 hogs that were injected with 15 c. c. of serum in combination with the disease-producing blood remained well, while the 2 injected with disease-producing blood alone died and communicated disease to their pen check.

Three of the surviving vaccinated hogs in this experiment were exposed to hog cholera in the quarantine pasture and 1 in the Syphax exposure pen. It was to be expected that the hogs that had been sick would be subsequently immune, and this was found to be the case.

The chief interest in the exposure of these hogs lay in the behavior of the hogs that received 15 c. c. of serum with disease-producing blood and which did not become ill as a result of vaccination. These 2 hogs (1677 and 1678) were exposed to hog cholera in the pasture thirty-seven days after vaccination. They did not become sick as a result of this exposure, although their pen check which was exposed with them contracted hog cholera and died. A later exposure of these 2 hogs in the Scribner exposure pen three and one-half months after vaccination proved them to be still immune.

EXPERIMENT XVIII. *Second experiment with first drawing of serum.*

In this experiment, through an error, doses of 10 c. c. and 20 c. c. of serum were used with disease-producing blood, although the preliminary experiment had shown that 10 c. c. of this serum was not sufficient to protect hogs from a fatal dose of disease-producing blood. It appears, however, that even the dose of 10 c. c. of serum may have increased the resisting power of the vaccinated hogs somewhat. Six hogs were injected with 10 c. c. of serum plus disease-producing blood, 6 with 20 c. c. of serum plus disease-producing blood, 4 with 10 c. c. of serum alone, 4 with 20 c. c. of serum alone, and 2 with 2 c. c. of disease-producing blood alone. Two pen checks were placed with each lot of serum-simultaneous treated hogs and one with the hogs injected with disease-producing blood alone. Table 21 shows that 4 of the 6 hogs treated with 10 c. c. of serum plus disease-producing

TABLE 21.—Secondary experiment with first drawing of serum from hog 1310 ("slow" immune—Syphax).

No. of hog.	Weight. Pounds.	Material injected.	Inoculation.		Pen exposure—Scribner.		Autopsy.
			Date.	Result.	Date.	Result.	
1886	55	10 c. c. serum and 2 c. c. disease-producing blood of hog 1801.	Nov. 23, 1906	Sick Nov. 30; died Dec. 2, 1906.			Hemorrhagic lesions.
1887	50	do.	do.	Remained well.			
1888	50	do.	do.	Sick Nov. 30; died Dec. 4, 1906.	a Dec. 27, 1906	Remained well.	Lesions not marked.
1889	50	do.	do.	do.	do.	do.	do.
1900	50	do.	do.	Sick Nov. 30; died Dec. 5, 1906.			do. Hemorrhages and ulcers.
1901	50	do.	do.	Remained well.	a Dec. 27, 1906	Remained well.	
1904	80	20 c. c. serum and 2 c. c. disease-producing blood of hog 1801.	do.	do.	do. a	do.	
1905	80	do.	do.	do.	Feb. 4, 1907	do.	
1906	60	do.	do.	do.	do.	do.	
1907	60	do.	do.	do.	a Dec. 27, 1906	do.	
1908	40	do.	do.	do.	do.	do.	
1909	60	do.	do.	do.	do. a	do.	
1910	80	10 c. c. serum alone.	do.	do.	Feb. 4, 1907	do.	
1912	80	do.	do.	do.	Dec. 11, 1906	do.	Hemorrhagic lesions.
1913	80	do.	do.	do.	do.	do.	Hemorrhages and ulcers.
1914	80	do.	do.	do.	Jan. 31, 1907	do.	do.
1915	70	do.	do.	do.	do.	do.	do.
1916	80	20 c. c. serum alone.	do.	do.	Dec. 11, 1906	Remained well.	No autopsy.
1917	80	do.	do.	do.	do.	do.	
1918	80	do.	do.	do.	Jan. 31, 1907	Remained well.	
1919	80	do.	do.	do.	do.	do.	
1902	50	Pen check with hogs 1896 to 1901	do.	do.	Dec. 27, 1906	Died Feb. 15, 1907	Hemorrhagic lesions.
1903	40	do.	do.	do.	do.	Died Jan. 14, 1907	Hemorrhages and ulcers.
1910	40	Pen check with hogs 1904 to 1909	do.	do.	Dec. 27, 1906	Died Jan. 13, 1907	do.
1911	45	do.	do.	do.	do.	Died Dec. 21, 1906	do.
1929	45	2 c. c. disease-producing blood of hog 1801.	Nov. 23, 1906	Sick Nov. 29; died Dec. 2, 1906.	do.	Died Dec. 30, 1906.	Hemorrhagic lesions.
1930	45	do.	do.	do.	do.	do.	do.
1931	30	Pen check with hogs 1929 and 1930	do.	Sick Nov. 29; died Dec. 3, 1906.			Hemorrhages and ulcers.
2003	40	do.	do.	Sick Dec. 8; died Dec. 19, 1906.			do.
2004	40	do.	do.	do.	Feb. 4, 1907	Died Feb. 9, 1907.	do.
2005	40	do.	do.	do.	do.	Died Feb. 14, 1907.	do.
					do.	Died Feb. 16, 1907.	do.

a Compare records of check hogs 1932, 1933, etc., Table 19.

blood died from the treatment, but, strange to say, the checks in the same pen did not contract the disease from them; the hogs that received 20 c. c. of serum with disease-producing blood all remained well, as did those injected with serum alone. The 2 hogs injected with disease-producing blood alone died of hog cholera, as did the check exposed in the pen with them.

The surviving hogs from the protective inoculations, including 4 pen checks (1902, 1903, 1910, and 1911) which had not been sick, were exposed to hog cholera at different lengths of time after vaccination.

Of the hogs treated with serum alone, 2 that received 10 c. c. and 2 that received 20 c. c. of serum were placed in the Scribner exposure pen eighteen days after vaccination with 2 of the pen checks; both of the checks and 3 of the 4 serum-treated pigs died of hog cholera. The surviving treated hog was one that had received 20 c. c. of serum. The 4 remaining hogs treated with serum alone were exposed first sixty-eight days after vaccination, and 3 out of 4 died of hog cholera, the surviving hog being again one of those that received 20 c. c. of serum, though it is very doubtful whether the resistance of this hog can be properly attributed to the previous serum injection. The behavior of the serum-simultaneous hogs after exposure to hog cholera was quite different from that of the hogs treated with serum alone.

It will be remembered that 12 hogs were vaccinated by the serum-simultaneous method, and of these 4 died as a result of vaccination, these having received only 10 c. c. of serum with the disease-producing blood. The 2 surviving hogs that received 10 c. c. of serum plus disease-producing blood were placed in the Scribner exposure pen with 3 of those treated with 20 c. c. of serum plus disease-producing blood thirty-four days after vaccination; all of these hogs remained well, although the 2 checks (1902 and 1903) exposed at the same time died of hog cholera. The 3 remaining hogs treated with 20 c. c. of serum plus disease-producing blood were first exposed to hog cholera by being placed in the Scribner exposure pen seventy-two days after vaccination. All of them remained well, although unvaccinated check hogs 2003, 2004, and 2005, subjected to the same exposure died of hog cholera.

Summary of experiments with first drawing of serum from immune 1310.

Results of vaccination. Eighteen hogs were vaccinated by the serum-simultaneous method; of these 10 received 10 c. c. or less of serum, while the remaining 8 received 15 c. c. or more of serum. Eight out of the first 10 became sick, and 6 died, as a result of vaccination, while none of the 8 hogs that received larger doses of serum became sick. Four hogs that were injected with disease-producing blood alone died of hog cholera as a result of the injection. The 8 hogs that were injected with serum alone as usual remained well.

All pen checks with vaccinated hogs remained well, notwithstanding the fact that some were exposed to hogs that died as a result of vaccination.

Results of exposure to hog cholera.—Twelve hogs treated by the serum-simultaneous method were exposed to hog cholera after vaccination, some being exposed after an interval of twenty-four days while others were not exposed until approximately two and one-half months after vaccination. Of these hogs only 2 had shown symptoms of sickness after vaccination. None of the 12 became sick as a result of exposure. Eight hogs that had been treated with serum alone were exposed to hog cholera, 4 being exposed eighteen days after vaccination, and 4 two months and eighteen days after vaccination; 6 of these died of hog cholera. Five unvaccinated hogs were exposed with the treated hogs as checks, and all of these died of hog cholera.

From these experiments we may conclude that—

(1) The serum from hog 1310, first drawing, will, when given in sufficient dose, protect hogs from a fatal dose of disease-producing blood administered simultaneously with the serum.

(2) This simultaneous vaccination confers on the treated hogs an immunity which lasts for at least two and one-half months.

(3) The immunity in hogs which showed no symptoms after vaccination seems to be quite as firm as in those that were made visibly ill.

(4) Serum alone did not confer a satisfactory immunity for even three weeks in the dose used.

(5) The immunity in serum-simultaneous hogs was not due to the action of the serum alone.

(6) Check hogs exposed to hogs which became sick as a result of vaccination contracted disease from them in some cases.

PRODUCTION OF A PROTECTIVE SERUM BY USING A REDUCED DOSE OF DISEASED BLOOD.

The hogs in the preceding experiments that were hyperimmunized by the "quick method" all received a dose of disease-producing blood in the proportion of 1,000 c. c. of blood to 100 pounds of body. We have carried out only one experiment with serum from an immune hyperimmunized with a smaller dose of disease-producing blood. This hog (1401) received only 500 c. c. of disease-producing blood for hyperimmunization. The history of hog 1401, as well as the experiments carried out with serum from that hog, are described below.

EXPERIMENT XIX.—*Serum from immune 1401.*

Hog 1401 is one of those that was vaccinated by the serum-simultaneous method in the experiments of 1905, and its detailed history is as follows:

Hog 1401 weighed 60 pounds December 2, 1905, and on this date, together with other hogs, was injected simultaneously with disease-

producing blood and 10 c. c. of serum from immune 1234 (see p. 11). No symptoms of sickness followed this injection, and the hog was exposed to disease on December 27, 1905. Hog 1401 again remained well and was not further exposed until September 3, 1906, when it was injected subcutaneously with 500 c. c. of Scribner disease-producing blood. The weight of the hog at this time was approximately 100 pounds. No visible disturbance of health followed this injection. On September 24, 1906, just three weeks after the injection of disease-producing blood, hog 1401 was bled in order to secure serum for experimental purposes. This hog may be classed with the Scribner quick immunes, though the dose of serum used in proportion to the body weight of the immune is only half of that used on the other Scribner quick immunes.

Unfortunately, we were not able to carry out as extensive experiments with this serum as was desirable, but the number of animals available for experimental purposes and the requirements of other experiments in the way of pens and hogs made it impossible to do more than make a preliminary test of the potency of the serum from immune 1401.

The tests of this serum were made in the usual manner, the serum being used in doses of 5 c. c., 10 c. c., and 15 c. c., given simultaneously with a fatal dose of disease-producing blood. Table 22 shows the results of these injections. Both of the hogs injected with only 5 c. c. of serum became sick and 1 died, and they also communicated disease to their pen check, hog 1814. One of the 2 hogs that were injected with 10 c. c. of serum plus disease-producing blood became slightly sick but recovered, while the other remained well; the pen check in this pen contracted hog cholera. Neither of the hogs that received 15 c. c. of serum with the disease-producing blood showed any symptoms of illness following vaccination, and their pen check likewise remained well. Both of the hogs that were injected with disease-producing blood alone died of hog cholera and communicated the disease to their pen check, hog 1823.

It will thus be seen that of 6 hogs inoculated simultaneously with serum and diseased blood, 3 became sick but only 1 died, and this one received the smallest dose (5 c. c.) of serum. Four of the 5 surviving vaccinated hogs were later exposed to hog cholera to test the duration of the immunity conferred by vaccination. Two of these hogs were placed in the Scribner exposure pen on November 17, 1906, thirty days after vaccination; the surviving pen check, hog 1820, was exposed at the same time. The check died, but both of the treated hogs remained well. The 2 other vaccinated hogs were first exposed to hog cholera three and one-half months after vaccination. They both remained well, although unprotected hogs exposed at the same time died of hog cholera.

Although we have hyperimmunized only 1 hog by the quick method with a dose of disease-producing blood equal to 500 c. c. per 100 pounds of body weight, the results obtained with this serum are

such that there is good reason to believe that this reduced dose might be sufficient to produce a satisfactory serum. Owing to reasons already stated, we have not as yet been able to decide this point, but from what has been done we may conclude that (1) the serum of hog 1401 is as potent as that of some other immunes injected with larger doses of disease-producing blood, but that it is not equal in potency to that from the majority of immunes which received twice as much disease-producing blood; (2) when given in sufficient dose this serum will protect perfectly from a fatal dose of disease-producing blood administered simultaneously with the serum; (3) the immunity produced by the serum-simultaneous injections lasted at least three and one-half months, even though the treated hogs showed no signs of reaction after injection; (4) it appears possible that the injection of serum did not lower the infectiousness of the disease to as great an extent as other sera from immunes that were given larger doses of diseased blood,

TABLE 22.—*Preliminary experiment with first drawing of serum from hog 1401 (500 c. c. "quick" immune—Scribner).*

No of hog.	Weight, Pounds.	Material injected.	Inoculation.		Pen exposure—Scribner.		Autopsy.
			Date.	Result.	Date.	Result.	
1812	40	5 c. c. serum and 2 c. c. disease-producing blood of hog 1774.	Oct. 18, 1906	Sick Oct. 27; died Oct. 29, 1906.	Pleuritis; no other marked lesions.
1813	50	do.	do.	Sick Oct. 10, 1906; recovered	Not exposed
1815	45	10 c. c. serum and 2 c. c. disease-producing blood of hog 1774.	do.	Slightly sick Nov. 3, 1906; recovered	Nov. 17, 1906	Remained well.
1816	50	do.	do.	Remained well.	do.	do.
1818	40	15 c. c. serum and 2 c. c. disease-producing blood of hog 1774.	do.	do.	do.	do.
1819	50	do.	do.	do.	do.	do.
1814	35	Pen check with hogs 1812 and 1813.	do.	Sick Nov. 3; died Nov. 14, 1906.	do.	do.	Hemorrhages.
1817	45	Pen check with hogs 1815 and 1816.	do.	Sick Nov. 12; died Nov. 21, 1906.	do.	do.	Do.
1820	35	Pen check with hogs 1818 and 1819.	do.	Remained well.	do.	do.	Hemorrhages and ulcers.
1821	40	2 c. c. disease-producing blood of hog 1774.	Oct. 18, 1906	Sick Oct. 27; died Nov. 1, 1906.	Nov. 17, 1906	Died Dec. 6, 1906.	Hemorrhages.
1822	50	do.	do.	Sick Oct. 27; died Nov. 7, 1906.	do.	do.	Do.
1823	40	Pen check with hogs 1821 and 1822.	do.	Sick Nov. 3; died Nov. 23, 1906.	do.	do.	Do.

a See Table 21 for record of hogs 2063, 2004, 2005, checks exposed in same pen on same date.

for pen check 1817 contracted hog cholera from exposure to hogs 1815 and 1816, although only one of these became slightly sick. It will be remembered that as a rule the pen checks with serum-simultaneous vaccinated hogs remained well, and that when the vaccinated hogs were made desperately sick by the treatment the pen checks occasionally contracted disease.

EXPERIMENTS WITH SERUM FROM NONHYPERIMMUNIZED IMMUNES.

In view of the very marked protective power possessed by the serum of hog 1401, as shown in the experiment just described, it was considered advisable to test the serum from at least 2 immunes that had not been given any injections of diseased blood for the purpose of hyperimmunization. Previous experiments of this character had not revealed any noticeable protective power in the blood of normal immunes, but nevertheless for the sake of comparison it was deemed best to repeat those tests. The history of the 2 immunes selected for this work is given in brief in connection with the tests of serum from each of them described below.

EXPERIMENT XX.—*Serum from nonhyperimmunized immune 1424.*

This hog was vaccinated by the serum-simultaneous method in the 1905 experiments, as noted in Table 3 of this paper. The detailed history is as follows: On December 16, 1905, hog 1424, which weighed at that time 45 pounds, was injected simultaneously with 2 c. c. of disease-producing blood and 10 c. c. of hyperimmune serum from hog 1234. No visible symptoms of illness followed this injection, although the same diseased blood caused the death of all unprotected hogs that were injected at the same time. (See Table 3.) On January 9, 1906, this hog was exposed to virulent disease by being placed in the exposure pen. This exposure was without effect.

On October 8, 1906, blood was drawn from hog 1424, and after being defibrinated and mixed with the carbolic acid solution in the usual manner it was used in the following experiment.

Two hogs were injected with 10 c. c. each of serum from hog 1424 plus disease-producing blood, and 2 with 20 c. c. each of serum plus disease-producing blood. One untreated healthy hog was placed in the pen with each lot in the usual manner, and 2 hogs were inoculated with disease-producing blood alone, a check being placed in the pen with these also. The manner of carrying out this experiment and the results obtained are very well shown in Table 23.

From Table 23 it may be seen that the 4 hogs that were injected with disease-producing blood and serum became sick. It will be seen, however, that only 2 of these died, while both of those that received disease-producing blood alone died. It may also be of significance, though not necessarily so, that both of the vaccinated hogs that died showed symptoms of disease somewhat later than the hogs

that received no serum, and they also lived a longer time. From the small number of tests carried out it can not be stated with complete assurance that this greater resisting power on the part of the vaccinated hogs was due to the influence of the immune serum.

EXPERIMENTS WITH SERUM FROM NON-HYPERIMMUNIZED IMMUNE 1295.

In order to test further the value of nonhyperimmunized immune serum, the blood of another immune was used in the following-described experiment:

Immune hog 1295 was selected for comparison with hog 1424 because the latter had never shown any symptoms of illness after exposure to hog cholera, while the former had recovered from a mild attack of that disease. It might be supposed, therefore, that hog 1424 possessed natural immunity, while hog 1295 probably acquired immunity through a mild attack of hog cholera. This supposition in regard to hog 1424 is not of necessity true, however, because our experiments have shown that susceptible hogs rarely become sick after vaccination, and it is quite probable that hog 1424 acquired immunity from vaccination. This hog 1295 was first exposed to hog cholera on September 12, 1905. At that time the hog weighed 100 pounds. The exposure to disease was made by the subcutaneous injection of disease-producing blood. Following this injection hog 1295 became

TABLE 23.—Experiment with serum from hog 1424 (nonhyperimmunized immune).

No. of hog.	Weight.	Material injected.	Inoculation.		Pen exposure—Scribner.		Autopsy.
			Date.	Result.	Date.	Result.	
1801.....	Pounds 40	10 e. c. serum and 2 e. c. disease-producing blood of hog 1711.	Oct. 10, 1906	Sick Oct. 17; died Nov. 4, 1906.	Hog cholera.
1802.....	45	do	do	Sick Oct. 18, 1906; recovered.	Nov. 17, 1906	Remained well.
1804.....	60	20 e. c. serum and 2 e. c. disease-producing blood of hog 1711.	do	Slightly sick; recovered.	do	do
1805.....	35	do	do	Sick Oct. 19; died Dec. 6, 1906.	Do.
1803.....	35	Pen check with hogs 1801 and 1802.	Sick Nov. 5, 1906; died Jan. 8, 1907.	No marked lesions.
1806.....	35	Pen check with hogs 1804 and 1805.	Sick Nov. 1; died Dec. 1, 1906.	Hog cholera.
1807.....	50	2 e. c. disease-producing blood of hog 1711.	Oct. 10, 1906	Sick Oct. 15; died Oct. 28, 1906.	Do.
1808.....	35	do	do	Sick Oct. 16; died Oct. 29, 1906.	Do.
1809.....	30	Pen check with hogs 1807 and 1808.	Sick Oct. 26; died Oct. 31, 1906.	Do.

distinctly though not desperately sick, showing the usual symptoms of a rather mild attack of hog cholera. After recovery from this illness hog 1295 was placed in the exposure pen, but did not become sick. This hog was finally removed from the exposure pen, but was not again exposed to hog cholera. Blood was drawn from hog 1295 on November 22, 1906, and after being prepared in the usual manner was used in the experiment next described.

EXPERIMENT XXI.—*Serum from nonhyperimmunized immune 1295.*

This experiment was modeled directly after the preliminary experiments with the hyperimmune serum, the serum-simultaneous method only being used, as previous experiments had shown that all of the hyperimmunized hogs furnished serum that would protect, when given in sufficient dose, from a simultaneous injection of disease-producing blood, and it was evident that a failure to protect from a simultaneous injection of such blood would certainly render unnecessary an experiment to determine the duration of immunity conferred by serum alone.

TABLE 24.—*Experiment with serum from hog 1295 (nonhyperimmunized immune).*

No. of hog.	Weight.	Material injected.	Inoculation.		Autopsy.
			Date.	Result.	
1920....	<i>Pounds.</i> 45	5 c. c. serum and 2 c. c. disease-producing blood of hog 1891.	Nov. 23, 1906	Sick Nov. 29; died Dec. 1, 1906.	Hemorrhagic lesions.
1921....	45	..do.....do.....	Sick Nov. 29; died Dec. 2, 1906.	Do.
1922....	30	Pen check with hogs 1920 and 1921.do.....	Sick Dec. 8; died Dec. 16, 1906.	Hemorrhages and ulcers.
1923....	45	10 c. c. serum and 2 c. c. disease-producing blood of hog 1891.	Nov. 23, 1906	Sick Nov. 29; died Dec. 2, 1906.	Hemorrhagic lesions.
1924....	45	..do.....do.....	Sick Nov. 29, 1906; died Dec. 2, 1906.	Do.
1925....	35	Pen check with hogs 1923 and 1924.do.....	Sick Dec. 13; killed Dec. 29, 1906.	No marked lesions.
1926....	40	15 c. c. serum and 2 c. c. diseased blood of hog 1891.	Nov. 23, 1906	Sick Nov. 29; died Dec. 1, 1906.	Hemorrhagic lesions.
1927....	40	..do.....do.....	Sick Nov. 29; died Dec. 3, 1906.	Do.
1928....	40	Pen check with hogs 1926 and 1927.do.....	Sick Dec. 5; died Dec. 9, 1906.	Hemorrhages and ulcers.
1929....	50	2 c. c. disease-producing blood of hog 1891.	Nov. 23, 1906	Sick Nov. 29; died Dec. 2, 1906.	Hemorrhagic lesions.
1930....	40	..do.....do.....	Sick Nov. 29; died Dec. 3, 1906.	Hemorrhages and ulcers.
1931....	35	Pen check with hogs 1929 and 1930.do.....	Sick Dec. 8; died Dec. 19, 1906.	Do.

Table 24 shows without question the lack of potency of the serum from hog 1295. Doses of 15 c. c. of serum did not prove to be any more potent than doses of 5 c. c., and every hog in this experiment died of hog cholera, the disease being communicated to the pen

checks in all cases. It does not appear that the serum, even in the largest doses, exercised any retarding influence upon the incubation period or upon the course of the disease once it had appeared.

Summary of results obtained with serum from nonhyperimmunized immunes.

Serum from 2 immunes was used in doses of from 5 c. c. to 20 c. c., and this serum was injected into nonimmunes simultaneously with a fatal dose of disease-producing blood. Ten were treated by this process; all contracted hog cholera, and 8 died of that disease. Of 4 hogs injected with disease-producing blood alone, all died of hog cholera. Five checks were placed in the pens with the vaccinated hogs, and all contracted disease from them and died. The 2 pen checks exposed in the pen with the hogs that received disease-producing blood only also died of hog cholera.

From the results stated above it will be seen that (1) the protection afforded to nonimmune hogs by the injection of serum from nonhyperimmunized immunes is very slight as compared with that afforded by hyperimmune serum; (2) even in doses of 20 c. c. the protection afforded was not sufficient to prevent the death of 1 of the 2 animals that received that dose; (3) the contagiousness of the disease is not noticeably lessened by the injection of serum from a nonhyperimmunized immune; and (4) the serum from nonhyperimmunized immunes is not potent enough to render its use practicable in combating hog cholera.

EXPERIMENTS WITH SUCKLING PIGS.

The following experiments with young pigs were carried out for the purpose of determining whether very young pigs would react to vaccination with immune serum in the same manner as the larger shoats. It was desirable to determine also whether very much smaller doses of serum might not be used on the small pigs, thus effecting a considerable saving in the cost of vaccination. The plan adopted was to vaccinate part of a litter of pigs and leave part unvaccinated, so that when the time came for testing the immunity of the vaccinated pigs we would have a sufficient number of checks for exposure along with the vaccinated pigs, and additional information regarding the danger of hog cholera being transmitted through the agency of vaccinated hogs would also be secured.

TABLE 25.—First experiment with suckling pigs—first drawing of serum from hog 1383.

No. of hog.	Weight. Pounds.	Maternal injected.	Inoculation.		Exposure—Seribner.		Autopsy.
			Date.	Result.	Date.	Result.	
1724		Sow—40 c. c. serum.	Oct. 5, 1906	Remained well.			
1725	3	2 c. c. serum and 0.25 c. c. disease-producing blood of hog 1681.	Sept. 21, 1906	Sickened Oct. 2, 1906; recovered.		Not exposed.	
1726	5	do.	do.	Sick Oct. 2; died Oct. 7, 1906.			Hemorrhagic lesions.
1727	3	do.	do.	Remained well.	Feb. 20, 1907	Remained well.	
1728	43	Uninoculated pen check.	do.	Remained well.	Feb. 20, 1907	Not exposed.	
1729	4	do.	do.	do.	Feb. 20, 1907	Died Mar. 10, 1907.	Hemorrhages and ulcers. Hemorrhages.
1730	40	2 c. c. disease-producing blood of hog 1681.	Oct. 5, 1906	Sick Sept. 27; died Oct. 2, 1906.			Do.
1731	50	do.	do.	Sick Sept. 27; killed Sept. 29, 1906.			
1732	40	Pen check with hogs 1730 and 1731.	do.	Sick Oct. 4; died Oct. 13, 1906.			Hemorrhages and ulcers.

EXPERIMENT XXII.—*Serum-simultaneous vaccination of suckling pigs, using serum from immune 1383.*

The 5 suckling pigs used in this experiment were 10 days old at the time of vaccination and all were vigorous and healthy. Two older pigs (1730 and 1731) were injected at the same time as the suckling pigs with disease-producing blood alone, in order to test the virulence of the blood. By referring to Table 25 the method of carrying out the experiment may be best understood.

Three of the suckling pigs were given 2 c. c. each of serum with 0.25 c. c. of disease-producing blood subcutaneously; the remaining 2 were not treated, but the entire litter was left together, the sow being given 40 c. c. of serum as a protection against possible infection from the vaccinated pigs. No pigs were injected with 0.25 c. c. of disease-producing blood alone, but the death of pig 1726 and the sickness of 1725, in spite of the injection of the serum, indicate that 0.25 c. c. of the disease-producing blood used in this experiment would probably have been sufficient to destroy all of the injected pigs if the serum had not been used. The virulence of the disease-producing blood in the usual dose, 2 c. c., is shown by the records of hogs 1730 and 1731, and the contagiousness of the disease is proven by the sickness and death of uninoculated hog 1732, exposed in the pen with hogs 1730 and 1731. These 3 hogs—1730, 1731, and 1732—were of course placed in a pen to themselves and did not at any time associate with the other hogs in this experiment. The low degree of contagiousness of the disease following vaccina-

tion, which has been remarked upon in connection with other experiments already described, is again well shown in this experiment, where the uninoculated suckling pigs remained well even though they associated with pig 1726, which died from vaccination.

The 4 surviving pigs—1725, 1727, 1728, and 1729—were with the mother transferred to a pasture that was free from hog cholera. All remained well, and approximately four months after vaccination they were weaned. Shortly after this, pigs 1725 and 1728 became sick and died. Neither showed lesions of hog cholera, and as there is nothing to show that disease had ever been introduced into this pasture, we must consider the death of these pigs to have been due to some other cause, the exact nature of which we were not able to determine. For this reason Table 25 does not show the history of pigs 1725 and 1728 subsequent to vaccination. The remaining pigs of this litter, 1727 and 1729, one a vaccinated pig and the other a check, were placed in the Scribner exposure pen on February 20, 1907, just five months after pig 1727 had been vaccinated. As a result of this exposure the check (1729) sickened and died on March 10, 1907, while the treated pig (1727) remained well. The results of this exposure furnish additional proof that the death of pigs 1725 and 1728 in the pasture before exposure was not due to hog cholera, for, upon undoubted exposure to that disease, vaccinated pig 1727, treated in the same manner as pig 1725, remained well, while the check (1729) died, thus indicating that had the disease in the pasture been hog cholera the vaccinated pig (1725) would not have succumbed, while pig 1729, which was later found to be susceptible, would have contracted disease at that time.

Owing to the loss of 1 of the pigs of this litter from vaccination and of 2 others through an intercurrent disease, as just explained, this experiment does not throw very much light upon the feasibility of vaccinating suckling pigs. It does indicate, however, that the serum of hog 1383, in doses of 2 c. c., is sufficient in most cases to protect pigs weighing 5 pounds from a fatal dose of disease-producing blood administered simultaneously with the serum. It appears also that the serum exerted decided influence in lessening the contagiousness of the disease. The weight that should be attached to the death of check pig 1729 and the survival of vaccinated pig 1727 when exposed together to hog cholera can only be properly considered in connection with the behavior of other pigs under similar conditions and treated in a similar manner.

TABLE 26.—Second experiment with suckling pigs—first drawing of serum from hog 1313.

No of hog.	Weight.	Material injected.	Inoculation.		Exposure—Scribner.		Autopsy.
			Date.	Result.	Date.	Result.	
	Pounds.						
1821	9	Sow—not treated.					
1825	9	3 c. c. serum and 0.25 c. c. disease-producing blood of hog 1774.	Oct. 18, 1906	Slightly sick Nov. 1, 1906, recovered.	Feb. 20, 1907	Remained well.	
1827	9	do.	do.	do.	do.	do.	
1828	9	4 c. c. serum and 0.25 c. c. disease-producing blood of hog 1774.	do.	do.	do.	do.	
1829	9	do.	do.	do.	do.	do.	
1830	9	Not injected.		Remained well.	do.	do.	
1831	8	do.		do.	do.	do.	
1831	5	do.		do.	do.	do.	
1831	40	2 c. c. disease-producing blood of hog 1774.	Oct. 18, 1906	Sick Oct. 27; died Nov. 1, 1906.	do.	do.	Hemorrhages. Hemorrhages and ulcers. Hemorrhages. Do.
1832	50	do.	do.	Sick Oct. 27; died Nov. 7, 1906.	do.	do.	Do.
1823	40	Pen cheek with hogs 1821 and 1822.		Sick Nov. 3; died Nov. 23, 1906.			Do.

EXPERIMENT XXIII.—Serum-simultaneous vaccination of suckling pigs, using serum from immune 1313. ^a

Seven suckling pigs, all of the same litter and about 2 weeks old, were used in this experiment. The weight of these pigs was approximately 9 pounds each, with the exception of 2 of those reserved as checks, which were a very little lighter in weight. Of the vaccinated pigs, 2 were given 3 c. c. of serum from hog 1313 and 2 were given 4 c. c. of the same serum, each receiving at the same time 0.25 c. c. of Scribner disease-producing blood. The remaining 3 pigs were left untreated to serve as controls. Unfortunately, as in Experiment XXII, we did not have a sufficient number of pigs to permit the injection of some with 0.25 c. c. of disease-producing blood alone and thus determine the protection afforded by the serum at the time of vaccination. For this reason we were forced to be content with the injection of larger pigs with a larger dose, 2 c. c., of disease-producing blood. The vaccinated and the unvaccinated pigs of this litter were left together with the sow after the vaccination; the larger pigs, 1821 and 1822, that were injected with disease-producing blood only, and their pen check, 1823, were placed in a pen to themselves.

^a For history of this immune see p. 21.

The death of the 2 hogs (1821 and 1822) from the diseased blood injection and the sickness and death of hog 1823 from association with these two show the virulence and the contagiousness of the disease produced by the blood used in the vaccination of the suckling pigs. All of the vaccinated suckling pigs became slightly sick about twelve days after vaccination. The symptoms exhibited were droopiness and conjunctivitis, and slight diarrhea in some cases; this had all passed away after four or five days. None of the untreated pigs became sick. After the recovery of the vaccinated pigs the entire litter with the mother was placed in a pasture free from disease. They all thrived, and after being weaned were placed in the Scribner exposure pen on February 20, 1907, approximately four months after vaccination. As a result of this exposure all of the checks died and showed at autopsy the typical lesions of hog cholera. In marked contrast is the behavior of the vaccinated pigs, none of which became sick in the exposure pen.

The results of this experiment indicate (1) that suckling pigs may be vaccinated by using smaller doses of serum and disease-producing blood than is feasible in the case of larger hogs; (2) that the immunity conferred by the injection of these small doses of serum and disease-producing blood is quite durable, and (3) that the vaccinated pigs are not likely to transmit hog cholera to unprotected pigs that associate with them.

EXPERIMENT XXIV.—*Serum-simultaneous vaccination of suckling pigs, using serum from immune 1274.*

The pigs used in this experiment were 10 days old and weighed about 6 pounds each. On November 1, 1906, 3 of them were injected simultaneously with 3 c. c. of serum and 0.25 c. c. of disease-producing blood and 3 were left untreated. Two larger hogs (1867 and 1868) were given 2 c. c. of diseased blood alone, and an untreated hog was placed in the pen as a control on the contagiousness of the disease. Table 27 shows very clearly that this experiment was not a success, for 2 of the 3 vaccinated pigs died as a result of the treatment they received and 1 of the checks contracted disease from these and died, thus leaving only 1 treated pig with 2 checks for subsequent exposure. This exposure, which took place three months and twenty days after vaccination, caused the death of 1 of the checks, while the 2 other pigs remained well. Incidentally these results show that 0.25 c. c. of the disease-producing blood used in this experiment was sufficient to cause the death of suckling pigs.

EXPERIMENT XXV.—*Immunization of suckling pigs with serum alone from immune 1274.*

This experiment was designed to determine the protective power of the serum when used alone. A litter of 9 healthy pigs, 1 week old and weighing about 6 pounds each, was used. Five of these pigs were given 5 c. c. each of serum from hog 1274 subcutaneously on November 14, 1906, and the remaining 4 pigs were left untreated. As was to be expected, no sickness followed the injection of the serum, and the mother, with the entire litter of pigs, was placed in a lot supposed to be free from disease. They all remained well until some time during the month of January, when the checks were found to be sick. Pig 1988 was the first of these to die, but the other 3 checks did not live much longer. The vaccinated pigs all remained well. From the lesions found at the autopsy on the checks there seems to be no reason to doubt that these pigs died of hog cholera. We are unable to determine just when they were first exposed to infection, but it seems probable that this exposure took place about ten or twelve days prior to the death of the first pigs. This would place the date of exposure about January 10, approximately two months after the vaccination.

It is fair to assume, therefore, that the serum alone conferred upon the treated pigs immunity which lasted for two months at least.

TABLE 27.—*Third experiment with suckling pigs—first drawing of serum from hog 1274.*

No. of hog.	Weight, Pounds.	Material injected.	Inoculation.		Date.	Exposure.		Autopsy.
			Date.	Result.		Result.		
1870	6	Sow, not treated.	Nov. 1, 1906	Sick Nov. 17; died Nov. 21, 1906.				Hemorrhagic lesions.
1871	6	3 c. c. serum and 0.25 c. c. disease-producing blood of hog 1831.	do.	do.				Do.
1872	6	do.	do.	Sick Nov. 29; died Nov. 30, 1906.	Feb. 20, 1907	Remained well.		Ulcers.
1873	6	do.	do.	Sick, but recovered.				Do.
1874	6	Not injected.	do.	Sick Dec. 1; died Dec. 11, 1906.	Feb. 20, 1907	Remained well.		Hemorrhages and ulcers.
1875	6	do.	do.	Remained well.	do.			Do.
1876	6	2 c. c. disease-producing blood of hog 1831.	Nov. 1, 1906	Sick Nov. 7; died Nov. 11, 1906.				Hemorrhages.
1877	6	do.	do.	Sick Nov. 7; killed Nov. 14, 1906.				Hemorrhages.
1878	6	do.	do.	Sick Nov. 15; died Nov. 20, 1906.				Do.
1879	6	Pritchek with hogs 187 and 1808.						

The five treated pigs were given another exposure by being placed in the Scribner exposure pen on February 20, 1907. They did not thrive in this pen, but none of them contracted hog cholera.

Summary of results with suckling pigs.

In looking over the four experiments which have just been described and which were carried out with very young pigs it will be seen that serum from three different immunes was used, and this was given in varying doses. For this reason it is not possible by merely averaging the losses among vaccinated and unvaccinated pigs to determine the value of the serum for practical use, for it is probable that if we had used doses of 5 c. c. in all cases there would have been no deaths from vaccination, and in the light of the experiments with older pigs it also seems probable that this increased dose of serum would not have lessened the duration of immunity in the vaccinated pigs.

In spite of the facts just stated, it is at least interesting to consider all together the results of the experiments with suckling pigs. With 3 of the litters the serum-simultaneous method was used, while serum alone was used for vaccinating the fourth litter. In all, 10 suckling pigs were treated by the serum-simultaneous method, and all showed more or less marked symptoms of illness after vaccination, 3 of them finally dying of hog cholera. The remaining 7 recovered, and 6 of these were exposed to hog cholera in an infected pen from three and one-half to five months after vaccination; all remained well. In the same litters with these 10 vaccinated pigs there were 8 untreated pigs reserved as checks. Only 1 of these checks, all of which associated with the vaccinated pigs, con-

TABLE 28.—Fourth experiment with suckling pigs—serum alone (first drawing) from hog 1274.

No. of hog.	Weight. Pounds.	Material injected.	Inoculation.		Exposure.		Autopsy.
			Date.	Result.	Date.	Result.	
1982	6	Sow—not treated.					
1983	6	5 c. c. 1274 serum alone.	Nov. 14, 1906	Remained well.	Jan. 10, 1907	Not exposed.	
1984	6	do.	do.	do.	do.	Remained well.	
1985	6	do.	do.	do.	do.	do.	
1986	6	do.	do.	do.	do.	do.	
1987	6	do.	do.	do.	do.	do.	
1988	6	Not injected.	do.	do.	do.	do.	
1989	6	do.	do.	do.	do.	Died Jan. 22, 1907.	Hemorrhagic lesions.
1990	6	do.	do.	do.	do.	Died Jan. 24, 1907.	Hemorrhages and ulcers.
1991	6	do.	do.	do.	do.	Died Jan. 25, 1907.	No autopsy.
						Died Jan. 29, 1907.	Ulcers.

tracted hog cholera from them. Of the 7 remaining checks, 6 were exposed to hog cholera along with the 6 vaccinated pigs described above. Of the 6 checks, 5 died of hog cholera, thus showing a marked difference from the 6 vaccinated pigs, all of which remained well.

The remaining litter of pigs, it will be remembered, was treated with serum alone, 5 being given 5 c. c. of serum while 4 were reserved as checks and not treated in any way. Of these, all 4 of the checks died of hog cholera when exposed two months later, while the 5 vaccinated pigs all remained well.

The experiments with suckling pigs are not sufficiently extensive to warrant definite conclusions being drawn, and, especially in view of the success of the vaccination with serum alone, more experiments designed to test the duration of immunity resulting from such injections should be carried out, together with additional experiments with the serum-simultaneous method. The latter experiments should be carried out with somewhat larger doses of serum, and the immunity of the vaccinated pigs should first be tested at the end of six or eight months. We regard such experiments as being of great practical importance owing to the lessened cost resulting from the reduced dose of serum and the longer period of protection that might be secured by vaccinating suckling pigs.

CURATIVE VALUE OF HYPERIMMUNE SERUM.

In previous pages a large number of experiments dealing with immunization, through the use of serum alone, as well as through the combined action of serum and disease-producing blood, have been described, but in those experiments no attempt was made to determine the curative value of the immune serum.

Two experiments which were designed to throw some light upon this question have been carried out, but before proceeding to a description of these a few words should be said concerning the way in which the hogs used in these experiments were exposed to infection.

In vaccinating hogs by the serum-simultaneous method, it will be remembered, the method of introducing the infection consisted in the injection of the hogs subcutaneously with a fatal dose of disease-producing blood. The same end might have been attained by allowing the hogs which were to be vaccinated to associate with sick hogs, but owing to the uncertainty as to the exact time when infection would take place by mere association, and also because such a method would not be suitable for practical use, the blood-injection method of infection was used exclusively in the immunization work.

It will be understood, however, that in testing the curative power of a serum it is very desirable to carry out the experiments with animals under natural conditions as nearly as possible. For this reason, and also in order that we might compare results, one of the experiments to test the curative value of hyperimmune serum was carried out with hogs that had been exposed to infection by association simply, while in the other experiments hogs that had been infected through disease-producing blood injections were used.

In this connection it may be of interest, before considering the experiments designed to test the curative value of the serum, to note the results of a single experiment which was designed to test the effect of immediate exposure to hog cholera by association with sick animals upon hogs that had been injected with varying sized doses of immune serum.

EXPERIMENT XXVI.—*Immediate exposure of serum-treated hogs to disease by association.*

Six hogs were injected subcutaneously with serum from immune 1383; two received 5 c. c., two 10 c. c., and two 15 c. c. of the serum. Immediately after injection of the serum all were placed in the Scribner exposure pen. Two checks, not treated, were placed in the same pen several days later, as shown in Table 29.

TABLE 29.—*Experiment to test protective value of 1383 serum where the treated animals were given immediate exposure by association.*

No. of hog.	Weight.	Injected Oct. 3, 1906, with—	Exposure.		Autopsy.
			Date.	Result.	
1767....	<i>Pounds.</i> 50	5 c. c. serum.....	Oct. 3, 1906	Remained well.....	Hemorrhages and ulcers.
1768....	50do.....do.....	Sickened Oct. 10; died Oct. 15, 1906.	
1769....	40	10 c. c. serum.....do.....	Remained well.....	
1770....	40do.....do.....do.....	
1771....	45	15 c. c. serum.....do.....do.....	
1772....	50do.....do.....do.....	
1773....	50	Unvaccinated check.....	Oct. 8, 1906	Sickened and died Nov. 4, 1906.	Hemorrhagic lesions. Do.
1774....	50do.....do.....	Sickened and was killed Oct. 18, 1906.	

With one exception (hog 1768, injected with 5 c. c. of serum) the hogs that received serum remained well, while both of the checks contracted hog cholera, one of them being killed to secure blood for other experiments. This experiment should be considered in connection with Experiment XXVIII, in which the same serum, instead of being injected simultaneously with the exposure to disease, was injected at varying intervals of time after exposure had taken place.

TABLE 30.—*Experiment to test curative value of 1383 serum.*
 [Injected Sept. 29, 1906, with 2 c. c. of disease-producing blood of hog 1731.]

No. of hog.	Weight.	Injection with serum.		Interval between injection of disease-producing blood and injection of serum.	Result of injections.	Date.	Exposure.	Autopsy.
		Date.	Amount.					
1734	50	Oct. 1, 1906	15 c. c.	2 days	Remained well.	Oct. 25, 1906	Remained well.	
1735	50	do.	do.	do.	do.	do.	do.	
1736	50	Oct. 3, 1906	do.	4 days	Slightly sick Oct. 5 to 10, 1906; recovered.	do.	do.	
1737	50	do.	do.	do.	do.	do.	do.	
1738	50	Oct. 5, 1906	30 c. c.	6 days	Sickened Oct. 4; died Oct. 10, 1906.			Hemorrhagic lesions.
1739	50	do.	do.	do.	Sickened Oct. 4; died Oct. 12, 1906.			Hemorrhages and ulcers.
1740	50	do.	do.	do.	Sickened Oct. 4; died Oct. 10, 1906.			Hemorrhagic lesions.
1741	50	do.	35 c. c.	do.	do.			Hemorrhages and ulcers.
1743	50	Oct. 7, 1906	50 c. c.	8 days	Sickened Oct. 4; died Oct. 10, 1906.			Hemorrhagic lesions.
1744	50	do.	do.	do.	do.			Do.
1742	50	do.	do.	do.	Sickened from injection of diseased blood and died Oct. 4, 1906.			Do.
1763	50	do.	do.	do.	Sickened from injection of diseased blood and died Oct. 10, 1906.			Do.
1766a	50	do.	do.	do.	Sickened Oct. 14; died Oct. 29, 1906.			Hemorrhages and ulcers.

of unincubated pen check, exposed to hogs 1734 to 1763.
 NOTE.—Hogs 1734 to 1761, inclusive, received first drawing of serum from hog 1383. Hogs 1763 and 1764 received second drawing of serum from hog 1383. All hogs in this experiment were kept together in one pen.

It is also interesting to compare these results with those of experiments in which the same serum was used in approximately the same doses, but in which the simultaneous exposure to disease was made by injecting disease-producing blood. (See Experiments V, VI, VII, and VIII.)

EXPERIMENT XXVII.—
Curative value of serum from hog 1383; exposure through blood injections.

Twelve hogs weighing 50 pounds each were injected subcutaneously with 2 c. c. of blood from a sick hog on September 29, 1906. Of these 12 hogs, 2 were not given any serum, but were held as controls on the virulence of the diseased blood. One hog (1766) was not injected, but was placed in the pen with the others to test the contagiousness of the disease.

Two days after infection with disease-producing blood 2 of the infected hogs were

injected with 15 c. c. of serum each; four days after infection 2 more hogs were given a similar dose of serum; six days after infection 4 more of the infected hogs were given serum, two being given 30 c. c. each, one 35 c. c., and one 50 c. c.; eight days after the disease-producing blood was administered the 2 remaining hogs of the 10 reserved for treatment were given 50 c. c. each of serum. The accompanying table (Table 30) illustrates very well the method of carrying out the experiment and the results obtained. The 2 hogs that were given the serum two days after injection remained perfectly well; those that were first given serum four days after infection became slightly sick, but recovered. The hogs that were given serum on the sixth and eighth days after infection were all sick when treated, but the course of the disease was apparently not affected by the treatment, although comparatively large doses of serum were used. The same serum, however, when administered simultaneously with disease-producing blood proved effective in quite small doses (5 c. c.—See Experiments V and VI). The 2 hogs (1762 and 1765) that were not treated with serum died promptly, as was to be expected, and the pen check also contracted disease and died. The 4 surviving hogs in this experiment were placed in the Syphax exposure pen a few days less than one month after the serum injection; all survived this exposure.

If conclusions may be drawn from this one experiment, we would infer that even very large doses of serum will not save hogs when it is injected more than four days after infection, but that prior to that time much smaller doses will suffice.

EXPERIMENT XXVIII.—*Curative value of serum from hog 1403; exposure by association.*

Twelve healthy hogs, weighing approximately 60 pounds each, were placed in the Scribner exposure pen on November 5, 1906. One day later 3 of these were removed from the exposure pen and 2 of the 3 were given 20 c. c. each of serum, the third hog being left untreated as a control. This procedure was repeated three times, 3 hogs being removed from the exposure pen on the second, the fourth, and the sixth days after exposure. In each instance 2 of the 3 hogs were injected subcutaneously with 20 c. c. of serum from hog 1403.

Table 31 shows that none of the hogs that were removed from the exposure pen within forty-eight hours after being placed there showed any signs of illness as a result of that exposure; even the controls which received no serum remained well. Of the 3 hogs that were taken out of the exposure pen four days after exposure all became sick, but the 2 that received serum recovered, while the control died. The results were almost precisely similar in the case of the 3 hogs that were removed from the exposure pen six days after having been placed

TABLE 31. Experiment to determine the curative value of serum of hog 1193 upon animals which had been exposed to virulent disease for varying lengths of time.

No. of hog.	Weight Pounds.	Pen exposure.		Number of days in exposure pen.	Serum injected.		Pen exposure.		Autopsy.
		Date.	Result.		Amount.	Date.	Result.		
170	50	Nov. 5 to 6, 1906.	Remained well.	1	Nov. 6, 1906	20 c. c.	Dec. 4, 1906	Remained well.	
171	50	do.	do.	1	do.	do.	do.	do.	
172	50	do.	do.	1	do.	Not injected.	do.	do.	Hemorrhagic lesions.
173	50	Nov. 5 to 7, 1906.	do.	2	Nov. 7, 1906	20 c. c.	do.	do.	
174	50	do.	do.	2	do.	do.	Nov. 17, 1906	do.	
175	50	do.	do.	2	do.	Not injected.	Dec. 4, 1906	Died Dec. 18, 1906.	Hemorrhages and ulcers.
176	50	Nov. 5 to 9, 1906.	do.	4	Nov. 9, 1906	20 c. c.	Slightly sick Nov. 16; recovered.	do.	
177	50	do.	do.	4	do.	do.	do.	do.	
178	50	do.	do.	4	do.	Not injected.	Sick Nov. 15; died Nov. 24.	do.	Do.
179	50	Nov. 5 to 11, 1906.	Slightly sick.	6	Nov. 11, 1906	20 c. c.	Slightly sick; recovered.	do.	
180	50	do.	Remained well.	6	do.	do.	Sick Nov. 13; died Nov. 24, 1906.	do.	Hemorrhagic lesions.
181	50	do.	do.	6	do.	Not injected.	do.	do.	

there, for in this case also the 2 serum-treated hogs recovered after having been somewhat sick, while the untreated control died of hog cholera.

In summing up the results of this experiment, the behavior of hogs 1870 to 1875, inclusive, when later exposed to infection, must be taken into consideration, for the deaths of control hogs 1872 and 1875 as a result of this second exposure shows that they were all along susceptible to the disease. This points strongly to the likelihood that none of the first 6 animals in this experiment became infected from the first exposure of one and two days, respectively, in the exposure pen, and the immune serum can not, therefore, be considered as having exerted any curative influence in these cases, although its immunizing action is well shown by the exposure of December 4, 1906, just referred to.

The hogs that were treated with serum four days and six days, respectively, after being placed in the exposure pen can not be regarded as having become infected at once after being placed in the exposure pen, for, as shown by the behavior of the controls in the lots that were removed on the

first and second days, infection probably did not take place in any of the hogs during the first forty-eight hours in the exposure pen. On the other hand, it is quite evident from the behavior of hogs 1876, 1877, and 1878 that infection did take place some time prior to the end of the fourth day in the exposure pen. While it is impossible, as already stated, to fix the time of infection with exactness, we know that it took place on the third or fourth day. It is plain, therefore, that the serum in doses of 20 c. c. saved the life of hogs that had been infected from two to four days previously.

DISCUSSION OF CURATIVE ACTION OF IMMUNE SERUM.

Earlier experiments have shown that the serum from hyperimmunized immunes, when given in sufficient dose, may be expected to protect nonimmune hogs from a simultaneous infection with hog cholera, and the two experiments just described show that this serum may also be expected to prevent the death of hogs when it is administered in moderate doses within four days after infection actually takes place. Later than this it is probable that the serum in virulent attacks of hog cholera will do little good, even though comparatively large doses are given. For this reason it seems possible that serum from immunes prepared in the manner described in this paper may not prove suitable for general use as a curative agent for hog cholera, although in cases where the disease is recognized soon after its appearance in a herd the serum would probably save many hogs that had been exposed only a few days. In stating that the serum should be administered within four days after infection to insure success, we wish to make it plain that we refer to the date of actual infection and not to the time of first exposure, for, as is shown by Experiment XXVIII, several days may elapse before infection takes place after exposure to disease by association. (See records of hogs 1872 and 1875, Experiment XXVIII.)

DURATION OF PROTECTIVE POWER IN BLOOD OF IMMUNES, AND KEEPING QUALITY OF HYPERIMMUNE SERUM.

The original plan of our experiments included the testing of serum drawn from the immunes at different periods of time after the last injection of disease-producing blood, and we were able in some instances to test the second and third drawings of immune serum, but as these later drawings of serum were apparently quite as potent as the first drawing, the question of the duration of protective power in the blood of the immune had not been decided by the experiments already described. For the purpose of testing this matter still further a special experiment was carried out with a drawing of serum from immune 1234. This hog furnished part of the serum used in the 1305 experiments. (See p. 11.)

TABLE 32.—Potency of serum from hyperimmunized immune 1234, seven and one-half months after last injection with disease-producing blood. [Experiment with serum from hog 1234 ("slow" immune—1905).]

No. of hog.	Weight, Pounds.	Material injected.	Inoculation.		Pasture exposure.		Pen exposure.		Autopsy.
			Date.	Result.	Date.	Result.	Date.	Result.	
157	45	10 c. c. serum and 2 c. c. disease-producing blood of hogs 1508 and 1614.	Sept. 3, 1906	Sick Sept. 11 to 16, 1906; recovered.	Oct. 15, 1906	Remained well	Dec. 22, 1906	Remained well.	
158	45	do.	do.	Sick Sept. 12 to 16, 1906; recovered.	do.	do.	do.	do.	
159	40	20 c. c. serum and 2 c. c. disease-producing blood of hogs 1508 and 1614.	do.	Sick Sept. 11 to 23, 1906; recovered.	do.	do.	do.	do.	
160	45	do.	do.	do.	do.	do.	do.	do.	
161	45	2 c. c. disease-producing blood of hogs 1508 and 1614.	do.	Sickened Sept. 9; died Sept. 14, 1906.	do.	do.	do.	do.	Hemorrhages and ulcers.
162	45	do.	do.	Sickened Sept. 9; died Sept. 17, 1906.	do.	do.	do.	do.	Hemorrhagic lesions.
163	45	do.	do.	Sickened Sept. 9; died Oct. 6, 1906.	do.	do.	do.	do.	Hemorrhages and ulcers.
164	40	Pen check with hogs 1601, 1602, and 1603.	do.	Sickened Sept. 19; killed Sept. 26, 1906.	do.	do.	do.	do.	do.

EXPERIMENT XXIX.—Potency of serum drawn seven and one-half months after last treatment of immune.

This experiment practically repeats the test of serum from immune 1234 that was carried out in 1905. Two hogs were injected with 10 c. c. of serum and 2 c. c. of disease-producing blood each, and 2 with 20 c. c. of serum plus the usual dose of disease-producing blood; at the same time 3 hogs were injected with 2 c. c. each of disease-producing blood alone, and 1 healthy hog was placed in the pen with these in order to make sure of the infectiousness of the disease.

Table 32 shows the manner of carrying out this experiment as well as the results obtained. The significance of the experiment may perhaps be best appreciated by a comparison of the action of serum from the same hog drawn nearly a year before, shortly after an injection of disease-producing blood. The action of blood from nonhyperimmunized immunes, as shown in Experiments XX and XXI, should also be kept in mind.

Table 32 shows that all of the vaccinated hogs became sick as a result of the treatment, but they all recovered. The 3 hogs (1661,

1662, and 1663) that received the same dose of the same disease-producing blood alone likewise became sick and all died, and they communicated the disease to their pen check.

The vaccinated hogs were exposed to hog cholera during the pasture outbreak about six weeks after vaccination. They all remained well and when placed in the Scribner exposure pen three months and nineteen days after vaccination were still completely immune to the disease.

Inasmuch as 10 c. c. and 20 c. c. of serum drawn from immune 1234 in 1905 gave complete protection from a fatal dose of disease-producing blood, and as the last drawing, made seven and one-half months after the last treatment with disease-producing blood, gave only partial protection (the serum in this case prevented death, but did not prevent illness), we may conclude that the protective power of the blood of immune 1234 had diminished somewhat seven and one-half months after the last disease-producing blood injection. But although the potency was diminished, it was still present in a marked degree, and although, owing to the limited number of tests that have been made, we can not be absolutely certain, it is at least reasonable to believe that this power was due to the previous injections of disease-producing blood and not to a natural protective power existing normally in the blood of the immune. (See experiments with blood from nonhyperimmunized immunes, p. 71.) This question is one of practical importance, and further experiments along this line will be carried out.

EXPERIMENT XXX.—*The keeping quality of immune serum.*

A quantity of hyperimmune serum prepared in 1905 remained unused in the experiments of that year, and as it was very desirable to know how long the serum, prepared with carbolic acid, would retain its potency, we have utilized some of this 1905 serum in experiments designed to test this point.

The serum used was from 1905 immunes Q No. 2 and 1234, and was drawn from these hogs on December 1, 1905, and November 21, 1905, respectively. Immediately after being drawn these sera were mixed with a 5 per cent solution of carbolic acid in such proportion that the serum contained 0.5 per cent carbolic acid. After the addition of this preservative the serum was placed in sterilized bottles and kept in a cool place until June 6, 1906, when it was used in the following experiment.

Two hogs, W 1925 and W 1926, were injected subcutaneously with 5 c. c. of Scribner disease-producing blood, and at the same time hog W 1925 was given 20 c. c. and hog W 1926 was given 17 c. c. of hyperimmune serum from hog 1234. Hogs W 1928 and W 1929 were given 5 c. c. each of the same disease-producing blood, but

TABLE 33.—Experiment to test keeping quality of immune sera.

No. of Hog.	Material injected.	Inoculation.		Pen exposure—Scribner.		Autopsy.
		Date.	Result.	Date.	Result.	
W 1925.....	20 c. c. serum of 1234 and 5 c. c. disease-producing blood of hog 1910.	June 7, 1906	Remained well	July 27, 1906	Slightly sick Aug. 6 to 8, 1906; recovered.	
W 1926.....	17 c. c. serum of 1234 and 5 c. c. disease-producing blood of hog 1910.	do.	do.	do.	Remained well.	
W 1927.....	Pen check with hogs 1925 and 1926	do.	do.	do.	Sickened Aug. 6; died Aug. 12, 1906.	Hemorrhagic lesions.
W 1928.....	20 c. c. serum of Q No. 2 and 5 c. c. disease-producing blood of hog 1910.	June 7, 1906	do.	do.	Remained well.	
W 1929.....	do.	do.	do.	do.	do.	Do.
W 1930.....	Pen check with hogs 1928 and 1929.	do.	do.	do.	Sickened Aug. 4; died Aug. 11, 1906.	Hemorrhages and ulcers.
W 1931.....	5 c. c. disease-producing blood of hog 1910	June 7, 1906	Sickened June 28; died July 9, 1906.	do.	do.	Ulceration of esecum.
W 1932.....	do.	do.	Sickened June 28; died July 19, 1906.	do.	do.	

NOTE: This experiment was carried out at Bethesda, Md., with sera from immune hogs 1234 and Q No. 2; these hogs were immunized at Ames, Iowa, in the fall of 1905, hog 1234 by the slow method and hog Q No. 2 by the quick method. The sera used in the above experiment were prepared from second drawings of blood.

received 20 c. c. each of hyper-immune serum from immune Q No. 2. An untreated hog was placed in each of the two pens with the vaccinated hogs. As controls, 2 hogs (W 1931 and W 1932) were each given 5 c. c. of the same disease-producing blood as was used for injecting the 4 serum-treated hogs. These 2 hogs were placed in a separate pen.

As may be seen from Table 33, none of the 4 hogs that received the simultaneous injections of serum and disease-producing blood showed any symptoms of illness after injection, while both of the hogs that were given the disease-producing blood alone sickened and died of hog cholera. The 4 vaccinated hogs, with their 2 pen checks, were exposed to hog cholera about seven weeks after vaccination. As a result of this exposure, both check animals died of hog cholera, while 1 of the vaccinated hogs became slightly sick, but soon recovered. The 3 other vaccinated hogs did not show any signs of illness.

This experiment shows that hyperimmune serum preserved with carbolic acid may retain its potency for at least six months.

GENERAL SUMMARY OF RESULTS.

In the experiments of 1905 and 1906, 13 immune hogs (including hog 1401) were used for the production of serum, and all of these, with one exception

(immune Q No. 1, 1905), after hyperimmunization have furnished serum that in doses of 15 c. c. or more gave protection from a simultaneous injection of disease-producing blood. When smaller doses of serum were given, differences in the potency of the various sera could be observed. The doses of immune serum given with disease-producing blood varied from $2\frac{1}{2}$ to 20 c. c., and, as was to be expected, some of the hogs that received the smaller doses were not sufficiently protected. In summarizing the results of these experiments, therefore, the percentage of loss which resulted from vaccination is not to be regarded as representing what would occur in the practical application of the method, for, as already stated, when a sufficient dose of serum is used no deaths are caused by the immunizing treatment. Even though, for the reasons just stated, the average results of our experiments are not to be considered as representing what should be obtained in practice, a summary of these results is not without interest as showing in a general way the behavior of serum-treated hogs as compared with unprotected hogs when both are exposed to disease through injections of disease-producing blood.

In all, 168 hogs were given a simultaneous injection of serum and disease-producing blood, the dose of serum varying from $2\frac{1}{2}$ c. c. to 20 c. c. Of these, 35, or 21 per cent, showed visible symptoms of illness after injection, and 15, or approximately 9 per cent of the total number, died. As a contrast to this we find that 54 hogs were injected with the same dose of the same disease-producing blood alone. Of these, 54, or 100 per cent, became sick, and 50, or 92.5 per cent, died of hog cholera. These figures, we believe, show beyond doubt that by hyperimmunizing immunes in the manner described in this paper a highly potent serum may be secured. We are confident also, as a result of the experiments we have described, that had we used the larger doses of serum in all cases or else reduced the dose of disease-producing blood the percentage of deaths following vaccination would have been so small as to be without practical significance.

TRANSMISSION OF DISEASE BY HOGS TREATED BY SERUM-SIMULTANEOUS METHOD.

To test the possibility of disease being conveyed to normal hogs by hogs that had been vaccinated, 58 shoats were placed in the pens with the vaccinated hogs. Of these pen checks none became sick unless some of the vaccinated hogs with which they associated became severely ill; indeed in a number of instances the pen checks remained well, even though they associated with vaccinated hogs exhibiting undoubted symptoms of hog cholera. From this it appears that the immune serum not only possesses the power to protect hogs

from a simultaneous injection of disease-producing blood, but that even in cases where too little serum is given it yet has a marked tendency to lessen the infectiousness of the disease. Of the 58 pen cheeks exposed to vaccinated hogs, 6 contracted hog cholera and 5 died.

As a control experiment, 19 shoats were placed in the pens with the hogs that were injected with disease-producing blood alone. Of these, all became sick, 17 died, 1 was killed to secure blood for other experiments, and 1 recovered. In other words, of the pen cheeks exposed to hogs injected with disease-producing blood alone, 94 per cent died, whereas only 8 per cent of the cheeks exposed to vaccinated hogs succumbed.

IMMUNITY IN HOGS AFTER SERUM-SIMULTANEOUS VACCINATION.

In describing the individual experiments attention has been called to the accidental exposure to hog cholera of those vaccinated hogs that were being reserved for a late exposure in order to test the duration of the immunity conferred by the serum-simultaneous and the serum-alone methods. The pasture in which this accidental outbreak of disease occurred contained a large number of hogs which had been treated at different times, and as a consequence this exposure took place only a few weeks after vaccination in the case of some of the hogs, while in the case of others the interval between vaccination and exposure was as great as three months. Aside from the hogs that were accidentally exposed in this way, a considerable number were placed in the exposure pen within three weeks after vaccination, the object being to compare the immunity at this time with that exhibited by others treated in the same way but exposed very much later. For the reasons given, this comparison could not be made in all cases, and all results of exposure to hog cholera are therefore considered together in this summary, it being understood that exposure took place from three weeks to three months after vaccination.

Of the hogs treated by the serum-simultaneous method, 136 were exposed to hog cholera, and 4, or nearly 3 per cent, died as a result, while of 68 unvaccinated hogs exposed under the same conditions, 56, or approximately 82 per cent, died.

In regard to the 4 vaccinated hogs that are stated to have died from exposure after vaccination, it should be noted that there is room for considerable doubt as to the cause of death. In only one of these hogs (1780) were typical lesions of hog cholera found at autopsy; and as this hog remained unthrifty after vaccination, and as its mate (1781) treated in the same manner died from vaccination, it is not unlikely that the intestinal lesions found at the autopsy of hog 1780 resulted from vaccination and not from exposure. Neither of the other three hogs exhibited lesions of hog cholera at autopsy, and two of them (1552 and 1556) passed through exposure in the pasture and only suc-

cumbed when placed in the exposure pen. The conditions in this exposure pen were not conducive to thriftiness, for no attempt was made to disinfect or clean the pen, as this might have defeated its object, which was to furnish a certain and severe exposure to hog cholera. Before leaving this subject, we wish to state our conviction that as small as was the loss among vaccinated hogs exposed to hog cholera, this would have been even less had the exposure taken place under the conditions usually met with in practice.

IMMUNITY IN HOGS TREATED WITH SERUM ALONE.

In all, 58 hogs were treated with serum alone in the experiments of 1906. None of these showed any ill effects from the treatment, and 49 were subsequently exposed to hog cholera. Of these, 23, or 47 per cent, survived exposure, whereas of 68 untreated hogs which served as controls only 18 per cent survived. It is thus evident that the protection afforded by the hyperimmune serum when given alone, while it did not equal that afforded by the serum-simultaneous method, was nevertheless sufficient to protect nearly 50 per cent of the treated animals against subsequent exposure.

As regards the duration of the protection afforded by the serum when given alone, we find that of 49 animals treated with serum alone, 26 were exposed within three weeks after receiving the serum, and 23 were exposed after one to three and one-half months. Of the first lot, exposed within three weeks, 9, or 34.6 per cent, died as a result of the exposure, while of the 23 exposed after one to three and one-half months, 17, or 73 per cent, succumbed. From these results it would seem that the protection afforded by the serum when administered alone is not as lasting as when administered in combination with diseased blood.

CURATIVE VALUE OF HYPERIMMUNE SERUM.

The exact methods of carrying out the experiments has been described elsewhere. Twelve hogs that were certainly infected with hog cholera were treated with serum after the elapse of different lengths of time, and the results showed that doses of 15 or 20 c. c. were sufficient to save the life of the treated hogs, provided the serum was given within four days after the date of infection. If given later than this, even much larger doses (35 to 50 c. c.) were not sufficient to save the infected hogs from the severe exposure to which they were subjected.

RESULTS WITH SERUM FROM IMMUNES TREATED BY DIFFERENT METHODS WITH DIFFERENT DISEASE-PRODUCING BLOODS.

In the previous summaries simply the general results have been considered, without reference to any differences that may have been found in the action of serum prepared by the different methods or by the use of different strains of disease-producing blood, and also without regard to differences in the immunes themselves. In discussing these features we wish to call attention again to the fact that the figures given are not to be taken as representing what may be expected in actual practice, for it is probable that all of the serum would have given better results if the doses had been somewhat larger in certain cases.

COMPARISON OF THE QUICK AND SLOW METHODS OF PRODUCING HYPERIMMUNE SERUM.

The comparative potency of the sera prepared by the quick and slow methods may no doubt be best shown by comparing the results obtained by injecting nonimmune hogs simultaneously with serum and a fatal dose of disease-producing blood. In the experiments of 1905 and 1906, 86 hogs^a were treated in this way with serum prepared by the quick method. Of these 86 hogs, 7, or approximately 8 per cent, died from the injection. There were treated with serum prepared by the slow method 82 hogs, all of which were injected at the same time with disease-producing blood. Of these, nearly 10 per cent died as a result of the injection. The slight difference in favor of the quick method we are inclined to attribute to other causes than to the method used for producing the serum, and it seems probable that one method will be found as suitable as the other for producing a potent serum, though of course in either case the potency might be heightened by increasing the dose of disease-producing blood given the immune. The potency of the serum secured being equal, the quick method would probably be found more desirable for practical use.

THE COMPARATIVE POTENCY OF SERA SECURED BY HYPERIMMUNIZATION WITH THE SCRIBNER AND SYPHAX STRAINS OF DISEASE.

Serum from 8 hogs was used, 4 having been hyperimmunized with Scribner disease-producing blood, and 4 with Syphax blood. In all, 74 hogs were treated with serum from the Scribner immunes at the same time that they were injected with disease-producing blood; as a result, 4, or approximately 5½ per cent, died. In the case of the serum from the Syphax immunes, 72 hogs were given disease-producing blood simultaneously with serum, and, as a result, 8, or 11

^a This includes the hogs treated with serum from immune 1401.

per cent, died. The apparent lower potency of the Syphax hyperimmune serum is caused by the distinctly weaker power of the serum from hog 1310, a dose of 10 c. c. of this serum being too small to protect nonimmunes, although larger doses were entirely satisfactory. Inasmuch as the average potency of the serum from the other 3 immunes hyperimmunized with the Syphax blood was quite as great as that of those treated with the Scribner blood, we are inclined to believe that the lower potency of serum from hog 1310 was due to some peculiarity of this hog and not to a lower hyperimmunizing power of the Syphax blood.

COMPARATIVE POTENCY OF SERA FROM HOGS POSSESSING NATURAL IMMUNITY AND FROM THOSE POSSESSING ACQUIRED IMMUNITY.

Attention has already been directed to the difficulty experienced in deciding whether a particular animal should be classed as one possessing acquired immunity or natural immunity. In fact the whole question seems to be simply one of degree of resisting power on the part of the hog. In any outbreak of hog cholera it is probable that at least a few hogs will survive. Some of these may have been markedly sick and have recovered only after a long siege of the disease, while others may have been only slightly indisposed, and still others may not have exhibited visible symptoms of disease. It seems hardly correct to speak thereafter of one of these hogs as a natural immune, and to regard another as having acquired immunity through the attack of disease. We are rather inclined, in the light of our present knowledge of the disease, to regard all hogs as being naturally susceptible but varying greatly in the degree of resisting power which they possess. If this assumption be correct, it would be expected that a protective serum might be obtained by the hyperimmunization of any immune hog, the differences in the serum from different hogs being simply in degree of potency and depending probably upon the intensity of the reaction produced by the injection of diseased blood.

In actual practice we have been able to secure but one immune that had actually been sick of hog cholera that was vigorous and suitable for hyperimmunization. The serum from this immune (1392), as the records show, is not more potent than that obtained by similar methods of hyperimmunization from other hogs that had never shown visible symptoms of illness when exposed to hog cholera. In fact the sera from the different immunes, with the exception of hogs Q No. 2 and No. 1310, were quite uniform in potency. The lesser potency of the sera from the two hogs just mentioned can not be explained upon the ground of natural immunity, but probably was caused by some individual peculiarity which we do not understand.

The remaining experiments, not mentioned in this general summary, have already been discussed at some length and it seems unnecessary to refer to them further at this time. Before closing this discussion, however, we wish to suggest to anyone who may have occasion to repeat these experiments that it is essential to make sure that the outbreak from which blood is secured for hyperimmunization exhibits all of the characteristics of hog cholera^a and likewise that the blood used in conjunction with the serum for serum-simultaneous vaccination is taken from a hog suffering from undoubted hog cholera. In addition, more than one hog should be hyperimmunized in order that individual peculiarity on the part of one hog may not lead to erroneous conclusions. As the methods herein described involve the use of a disease-producing virus we believe that only those trained in bacteriological methods should attempt to use them, and in all cases before the serum is employed in a practical way the amount required to give protection from a fatal dose of disease-producing blood should be determined experimentally.

CONCLUSIONS.

(1) When hogs immune from hog cholera are injected with suitable amounts of virulent blood taken from hogs sick of hog cholera the blood serum of the immunes acquires the power to protect nonimmune hogs from an otherwise fatal dose of disease-producing blood administered simultaneously with the serum.

(2) Immunes which have never exhibited symptoms of disease after exposure to hog cholera (natural immunity) may furnish equally as potent serum as those which have recovered from an attack of the disease (acquired immunity).

(3) Hyperimmunization may be accomplished equally as well by the administration of one large dose of disease-producing blood as by repeated injections of smaller doses.

(4) Hyperimmunization may be accomplished with blood from any virulent strain of hog cholera.

(5) Hyperimmunized hogs probably retain for several months the power to furnish a potent serum.

(6) Serum from properly hyperimmunized hogs in doses of 20 c. c. should protect nonimmunes weighing from 25 to 50 pounds from a fatal dose of disease-producing blood administered simultaneously.

(7) Immunity in hogs treated by the serum-simultaneous method lasts at least three and one-half months and probably longer.

(8) In serum-simultaneous vaccination it is not necessary to produce illness in the vaccinated hog in order to secure an immunity lasting for at least three months.

^aSee Bulletin 72, Bureau of Animal Industry, p. 12.

(9) If a sufficient dose of serum is given, hogs are not injured by the serum-simultaneous vaccination.

(10) Hogs vaccinated by the serum-simultaneous method do not communicate disease to others unless they themselves become sick.

(11) Serum administered alone may not furnish complete protection for a longer period than three weeks.

(12) The serum probably can be used successfully as a curative agent if administered in the early stages of the disease.

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