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STUDIES OF AGGLUTINATION REACTIONS IN HOG CHOLERA DURING THE PROCESS OF SERUM PRODUCTION

[Preliminary]

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FOREWORD.

About one year ago the Bureau of Animal Industry turned over to the Experiment Stations and other state institutions the Dorset-Niles *method, by demonstration, of manufacturing hog cholera serum, for the* purpose of preventing and fighting the disease. This was done to meet the demands of swine-breeders for this serum. Unfortunately, with the transmission of the method came a multitude of unanswered questions and unsettled problems which have materially interfered with the practical application of the treatment and the manufacture of the serum. Of this overwhelming burden of problems the one which has impressed itself upon us more than any other has been the lack of some method by *which the potency of the serum can be satisfactorily measured; for* prolonged manufacture emphasizes the fact that potency in serum is a most variable quantity. If this is true (our experiences seem to establish it) no serum is fit for use until the potency has been determined. While it is possible to use pigs for this work, they will never be satisfactory because of the great expense involved, the limited number that can be employed for each test, and the likelihood of errors creeping in from inability to control.

With these conditions in mind, the work of this bulletin has been undertaken, hoping to discover some clew to the *actual value* of serum produced for distribution.

The efficiency of the serum is not a matter of dispute, provided its potency can be definitely placed.

CHARLES E. MARSHALL.

STUDIES OF AGGLUTINATION REACTIONS IN HOG CHOLERA DURING THE PROCESS OF SERUM PRODUCTION.

GENERAL DISCUSSION.

It does not seem advisable to ignore the part played by B. cholerae suis in hog cholera infection, even though we assume that the primary etiological factor in the great swine epizoötics in this country is a filterable, ultramicroscopic organism. Whatever the relation may be between the filterable virus and B. cholerae suis and hog cholera, for the three appear to be almost, if not quite, constantly associated, we cannot fail to recognize the immense value that has accrued to serum therapy in hog cholera as a result of the partial abandonment of the formerly accepted etiological relation of B. cholerae suis to hog cholera.

The immunizing serum prepared according to the Turner-Kolle method is aimed at the protection of hogs from the ultramicroscopic virus. Conaway[1] believed that in the process of hyperimmunization he could also impart to the immunizing serum protective properties against B. cholerae suis by feeding the viscera of hog cholera or virus hogs. This is a tacit admission that the Dorset-Niles immunizing serum is not, at least, necessarily, potent to protect swine against B. cholerae suis infection. The process of hyperimmunization might appear to furnish ample proof of the immunity against B. cholerae suis in hogs immunized against the filterable virus. In this process, the immune hogs are injected with enormous quantities of virus blood either intravenously, or intramuscularly, and are frequently fed the diseased meat and viscera, so that they must receive into their system great numbers of B. cholerae suis and do not suffer the fatal results that would attend the introduction of a similar number of the same species of bacteria into the system of non-immune hogs.

On the other hand, it has been shown by Dorset, Bolton and McBryde[2] that resistance toward certain methods of exposure to B. cholerae suis in no way indicates immunity to the filterable virus. Notwithstanding this apparent demonstration of the non-identity of the two virus, there is still an unexplained intimate relation between hog cholera and B. cholerae suis.

If it can be demonstrated that the easily handled B. cholerae suis has a constant and intimate relation to hog cholera infection and to the immunizing serum, it has occurred to us that this fact, if a fact, might be used in some laboratory method by which the potency of the serum and perhaps of the virus could be estimated in vitro. At present, only biological tests, limited in their application to pigs, are available. These tests are expensive, not without objection as to accuracy, and require a long time for the development of results.

The object in the agglutination tests was to determine the relation, if any, between the phenomenon of agglutination of B. cholerae suis and the virulence of hog cholera virus or potency of immunizing serum. It is not to be inferred from the above statement that we interpret the agglutination reaction as a reliable means of measuring either the degree of immunity or infection in any or all infectious diseases where this test has been applied; but there are cases recorded[3] in which the agglutinative power of serum and its opsonizing and bactericidal power increase simultaneously. It is true, likewise, that there are records which fail to show this comparison. The technic involved in the agglutination test is simpler and the results are more easily interpreted than in any tests for determining the opsonizing or bactericidal power of serum. The work of Bolton[4] on the bacteriolytic power of hog serum does not encourage one to expect uniform results in this connection, though its repetition here would be interesting and perhaps of far-reaching significance. We are satisfied that the results of our experiments add to the sum of our knowledge of the subject covered in the title of this paper, even if we have failed in demonstrating a method wholly satisfactory at this moment for standardizing the potency of hog cholera virus and serum.

PREVIOUS WORK.

The very recent introduction of the Dorset-Niles hog cholera serum together with our present attitude toward B. cholerae suis as a factor in the production of hog cholera preclude the possibility of a very extensive literature on the agglutination of that organism in connection with serum production.

The agglutination of bacteria has long been recognized as a phenomenon having more or less significance in the diagnosis of specific infectious diseases and in the identification of specific pathogenic bacteria. The similarity of B. cholerae suis to B. typhosus and the similarity of the disease formerly supposed to be due to the former organism, and the disease at present attributed to the latter organism has stimulated a few workers with hog cholera to apply the Widal test or some modification of it to that disease.

McClintock, Boxmeyer and Siffer[5] have reviewed the literature on agglutination in hog cholera and note that Dinwiddie[6] is the only one they are able to find who has applied the test with the blood of diseased hogs. Dawson[7] and Smith[8] found that the blood of vaccinated rabbits often agglutinated in high dilutions. The treatment of animals with B. cholerae suis appears to result in the production of agglutinins for that organism. Dinwiddie's results were all negative but no hog cholera bacilli were isolated from the pigs furnishing the tested serum.

The work of McClintock, Boxmeyer, and Siffer is more exhaustive and fruitful in its results. They used suspensions of agar cultures in chloretone and the macroscopic method. The tubes were examined for the last time 20 hours after making the dilutions. The presence of "plain floccules" constituted a positive reaction. They state that bacterial suspensions remain fit for use for several weeks. The reaction varied in intensity with the strain of B. cholerae suis used. Some of their conclusions, based upon a rather limited number of tests, are as follows:

"1. The serum of normal hogs agglutinates strains of ordinary hog cholera bacilli in dilutions occasionally as high as 1 to 250.

. . . . . .

"3. Agglutination is of no value for the diagnosis of hog cholera, as the disease is at present defined.

"4. The presence of a positive reaction does, however, indicate an infection with the hog cholera bacilli.

"5. There are occasional instances of both natural and artificial infection in which no increase of the agglutinins for hog cholera over those normally present can be demonstrated.

"6. The maximum amount of agglutinin develops in a hog's blood within six or seven days after a single inoculation with hog cholera vaccine.

"7. Hogs react to intraperitoneal injections of hog cholera vaccines, usually with the production of large quantities of agglutinins, the amount of the vaccine bearing no relation to the amount of agglutinin produced."

Uhlenhuth[9] and his associates have made extensive researches into certain phases of agglutination with the purpose to designate the etiological factors at work in hog cholera, while it has been our aim to measure the potency of hyperimmune serum by means of agglutination.

METHODS AND REACTIONS.

I. The Agglutination Test.

In our agglutination tests we have used the macroscopic method developed during the study of the agglutination reactions in the diagnosis of glanders[10] and modified to meet the requirements of the present work. An emulsion was prepared by washing off the growth on agar slants with .5% carbolic acid in physiological salt solution. The growth on agar was 24 to 48 hours old at 37° C. and was killed by 15 minutes exposure to 60° C. in water bath. This emulsion was filtered several times through cotton and greatly diluted with carbol-salt solution. In each of a series of test tubes was placed 4 cc. of this faintly cloudy bacterial suspension to which was added blood serum in varying quantities to make the different dilutions. A tube of bacterial suspension without the serum served as a check in each test. The tubes were then incubated 48 hours at 37° C. A positive reaction, according to our interpretation, consisted in, first, the clumping of the dead germs in greater or smaller flocculent masses of macroscopic size, which later fell to the bottom of the tube forming a membrane of agglutinated germs spreading over the whole hemispherical bottom of the tube, leaving the supernatant fluid more or less clear. Of course, the reaction varied in intensity with the different sera and with the degree of dilutions.

In studying the reaction, then, we take into consideration two factors, viz,—(a), The clumping or agglutination of the bacteria and consequent clearing of the test fluid and (b), The formation of a characteristic membrane on the bottom of the tube.

There was never any reaction in the check tubes, although there was usually a slight, finely granular sediment consisting of non-agglutinated bacteria that naturally settled to the extreme bottom of the tube. The time limit was 48 hours, as a rule, for the reaction was not always com-

plete until this time in the higher dilutions and there was usually no tendency for the process to progress after this time. Frequently, in the lower dilutions or with a strongly agglutinating serum, the reaction would be complete in less than 24 hours. The agglutinated masses of bacteria were macroscopic in size and remained suspended in the fluid some time after formation. In some cases, while there was an abundant flocculent sediment of agglutinated bacteria, there were still many non-agglutinated bacteria in suspension as shown by both macroscopic and microscopic observations. This may be accounted for by the fact that the emulsion of bacteria was too dense, i. e., the test fluid contained too many bacteria in suspension as a consequence of which the agglutinin in the serum diluted above 1—250 was exhausted after having agglutinated a portion of the dead bacteria as great in amount as was ordinarily present in the bacterial suspension for the other tests. The matter of diluting the bacterial suspension to just the proper degree of cloudiness is not at all simple. A hanging drop of the test fluid gives some idea of the number of bacteria present, but an efficient method of determining the proper dilution has not yet been formulated.

II. Drawing and Preparing the Blood.

The blood from the normal and serum pigs was drawn from the tail after the methods devised by Niles[2]; that from the virus hogs, from the throat when these pigs were killed. In each case, except where otherwise stated, the blood was defibrinated by whipping and allowed to stand at a low temperature (about 10° C.) until the corpuscles settled leaving a clear, amber-colored or haemoglobin-stained serum above. The presence of erythrocytes or excess of haemoglobin in the serum did not prevent the agglutination, but masked the appearance so that it was difficult to judge accurately the degree of reaction.

NON-VIRULENT CULTURE.

The Manistee Culture.

Our initial experiments with B. cholerae suis proved interesting in many respects, but were possibly of less value, because of the absence of virulence in the culture used. The "Manistee culture" had been used several months before and had produced quite uniform results, killing rabbits in very small doses; pigs injected intravenously and frequently intramuscularly, died with extensive hemorrhagic lesions.

The following inoculation experiments show the former virulence and the lack of virulence of the "Manistee culture" at this time:

1. VIRULENCE.*

Nov. 27, 1907, inoculated six 25 lb. pigs into femoral artery with a 42 hr. bouillon culture (Manistee) grown at 37° C. as follows:
Two with 2 cc. each, Series 50, Nos. 11 and 12.
Two with 1.5 cc. each, Series 50, Nos. 13 and 14.
Two with 1 cc. each, Series 50, Nos. 15 and 16.

Series 50, No. 11, showed a rise in temperature to 105.2°.
Died about 85 hours after inoculation.

* Only a few typical cases are cited here from the large number of inoculations with the "Manistee culture" at the time of its virulence.

Autopsy: Skin over ventral aspect and on inside of legs, hyperaemic; lungs hyperaemic, a few hepatized lobules and hemorrhagic areas; heart cavities filled with clotted blood; liver shows coagulation necrosis over surface; spleen, enlarged, dark red; kidneys, congested and swollen; stomach shows a patch of mucous membrane very highly inflamed and diphtheritic with an ulcerous patch and many small ulcers about its border; walls of small intestine, thickened and covered with catarrhal exudate; large intestine shows highly inflamed patches, purplish *red in color; lymphatic glands, hemorrhagic and engorged.*

Series 50, No. 12, showed a rise of temperature to 105°.

Died about 80 hours after inoculation.

Autopsy: Hyperaemic condition of skin over ventral aspect and inside of legs; *lymph glands, generally hemorrhagic* in cortex; lungs, hyperaemic, with scattered hemorrhagic and hepatized areas; heart, full of clotted blood; few sub-epicardial petechiae; liver shows coagulation necrosis; spleen, engorged, dark red; stomach shows ecchymoses and ulceration in mucosa; small and large intestines show hemorrhages in mucosa.

Series 50, No. 13, showed rise in temperature to 105.3°.

Died about 94 hours after inoculation.

Autopsy: Skin hyperaemic over ventral aspect and on inside of legs; lymph glands, generally hemorrhagic in cortex; lungs, generally hyperaemic with scattered hemorrhagic and hepatized areas; heart, filled with clotted blood, with few sub-epicardial petechiae; liver shows coagulation necrosis; spleen, enlarged *and dark, purplish red: kidneys show hemorrhages in cortex; small patch of* gastric mucosa, inflamed; small intestine shows inflammation and catarrhal exudate with hemorrhagic patches; large intestine shows scattered hemorrhagic patches.

Series 50, No. 14, showed rise of temperature to 106.4°.

Died 6 days after inoculation.

Autopsy: Skin, hyperaemic over ventral aspect and on inside of legs; lymph glands, generally hemorrhagic in the cortex; lungs, hyperaemic, right lung hemorrhagic in large areas, left, petechiated; heart, filled with dark, clotted blood, shows sub-epicardial petechiae; liver shows coagulation necrosis; spleen, enlarged, engorged and purplish red in color; fundus of stomach, greatly reddened, *numerous petechiae; large intestine shows catarrhal inflammation,* and numerous petechiae; large intestine shows various sized hemorrhages throughout and ulcers in colon near rectum; kidneys show numerous petechiae in cortex.

Series 50, No. 15, showed a rise of temperature to 107° on third day with fluctuating temperatures thereafter from normal to 105.6° until death.

Died 38 days after inoculation.

Autopsy: Lymph glands, generally hemorrhagic in cortex; lungs, hyperaemic, extensive hemorrhagic patches and few hepatized lobules; heart contains clotted blood and numerous sub-epicardial petechiae; liver shows extensive coagulation necrosis; spleen, enlarged, engorged and dark red in color; kidneys, show petechiae in cortex; fundus of stomach, hemorrhagic and ulcerated; few petechiae and ecchymoses in small intestine; button ulcers throughout large intestine.

Series 50, No. 16, showed rise of temperature to 105.8°.

Died 9 days after inoculation.

Autopsy: Skin over ventral aspect and inside of legs, greatly reddened, due to *subcutaneous hemorrhage; lymph glands, generally hemorrhagic on cortex; lungs,* hyperaemic and greatly hemorrhagic, with few patches of hepatization; clotted blood in heart with numerous sub-epicardial hemorrhages; liver shows coagulation necrosis; spleen enlarged, engorged, and dark purple in color; kidneys show numerous petechiae in cortex; fundus of stomach, hemorrhagic and ulcerated; catarrhal inflammation of small intestine and numerous petechiae throughout; large intestine shows very numerous petechiae and small ulcers.

NOTE.—For the notes on pigs in Series 50, showing the virulence of the "Manistee culture," I have to thank Dr. Marshall to whom I am also greatly indebted for many helpful suggestions in the planning and execution of this work.

Dr. Marshall suspects that the loss of virulence in the "Manistee culture" may be due to the fact that it was transferred from bouillon to bouillon at too prolonged intervals and thus propagated instead of on some more favorable medium with shorter intervals of transfer.

II. NON-VIRULENCE.

(a) *Untreated Animals*—

Our inoculations were made about fourteen months after those described above.

Rabbit 20. Full grown; Feb. 4, 1909, a. m., injected intraperitoneally 0.1 cc. 40 hr. bouillon culture grown at 37° C. Remained well.

Rabbit 21. Full grown: Feb. 4, 1909, a. m., injected intraperitoneally 0.5 cc. 40 hr. bouillon culture grown at 37° C. Remained well.

CLASSIFICATION OF PIGS.*

Expt. pig 147, Chester White: weight, 54 lbs.; no previous treatment. Feb. 24, 1909, injected 2 cc. 40 hr. bouillon culture grown at 37° C. in ear vein. Remained well.

Expt. pig 148, Chester White; weight, 68 lbs.; no previous treatment. Feb. 24, 1909, injected 3 cc. 40 hr. bouillon culture grown at 37° C. in ear vein. Remained well.

Expt. pig 149, Chester White; weight, 48 lbs.; no previous treatment. Feb. 24, 1909, injected 5 cc. 40 hr. bouillon culture grown at 37° C. in ear vein. Remained well.

(b) *Treated Animals*—

Expt. pig 24, weight, 50 lbs.; immunized with 10 cc. mixed serum 15 and 1 cc. virus 59, Nov. 25, 1908. On Feb. 12, 1909, injected 1.8 cc. of a 48 hr. culture in ear vein. Remained well.

Expt. pig 34, weight, 50 lbs.; immunized with 10 cc. mixed serum 16 and 1 cc. virus, Expt. 26, Dec. 3, 1908. On Feb. 12, 1909, injected intraperitoneally 4 cc. of a 48 hr. bouillon culture. Remained well.

Expt. pig 97, weight, 50 lbs.; immunized with 15 cc. mixed serum 15 on Dec. 19, 1908. Injected 1 cc. virus, Expt. 69 on Dec. 28, 1908; 2.5 cc. virus, Expt. 120 on Jan. 18, 1909. On Feb. 12, 1909, injected 2 cc. of a 48 hr. bouillon culture in ear vein. Remained well.

Expt. pig 112, weight, 50 lbs.; immunized with 15 cc. mixed serum 17 and 1 cc. virus 62 on Jan. 5, 1909. Injected 1 cc. virus, Expt. 123 on Jan. 26, 1909 and 2.5 cc. virus, Expt. 141 on Feb. 11, 1909. On Feb. 18, 1909, injected 3 cc. of a 24 hr. bouillon culture in the ear vein and 1 cc. in the perivascular tissue. Remained well.

The lack of virulence or even slight pathogenic properties in this "Manistee culture" is worthy of comment, as it illustrates how cultures of B. cholerae suis lose their virulence when removed from the body of pigs. The tests made with a non-virulent culture will be valuable for comparison with later tests with a virulent culture from one of our virus hogs.

In the table under each dilution are the signs arranged in two columns to indicate the degree of reaction having reference to the two factors considered above. (See page 7.)

* To avoid confusion, it is well to bear in mind the features which distinguish the several classes of pigs referred to in the autopsy notes and tables and in the discussions.

A virus pig is one that has received some treatment, usually intravenous or intramuscular injection of blood from a sick hog for the purpose of producing in that pig hog cholera.

An immune pig is one that has passed through the disease and is at the time resistant to further treatment sufficient to produce the disease in susceptible animals, or one that has resisted exposure to the disease without becoming sick—natural immunity, or one that has been artificially immunized either by the so-called "Serum-simultaneous" or "Serum-alone" methods.

A serum pig, or hyperimmune pig, is an immune pig that has been treated with intramuscular or intravenous injections of virus, i. e., blood from a virus hog, until there is produced in the blood of the treated pig protective substances,—immunizing serum, against hog cholera.

The virus and serum pigs have been used in the routine work of producing immunizing serum for use in combating hog cholera throughout the state.

An experimental pig is one that has been used for any experimental work in connection with hog cholera. The experimental pigs may have been virus, immune or hyperimmune pigs or they may have been used in tests with cultures of B. cholerae suis.

TABLE I.

Showing agglutination tests with "Manistee Culture" and blood from normal, virus and serum hogs.

In the table, the sign (+) indicates a complete reaction, the sign (−) indicates that the reaction has progressed considerably, but not completely, and the sign (0) indicates no change.

Source of blood serum tested.	Classification of pigs tested.	Method of securing blood.	Dilution of blood serum. 1-10		1-25		1-50		1-100		1-125		1-200		1-250		1-333		1-500		1-700		1-1000	
			Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
Expt. pig 144	Normal	From tail	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0
Expt. pig 145	Normal	From tail	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0
Expt. pig 146	Normal	From tail	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0
Serum pig 70	Hyperimmune	1st tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0
Serum pig 80	Hyperimmune	3rd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0
Serum pig 81	Hyperimmune	2nd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0
Serum pig 81	Hyperimmune	3rd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serum pig 83	Hyperimmune	2nd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serum pig 83	Hyperimmune	3rd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serum pig 84	Hyperimmune	3rd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serum pig 85	Hyperimmune	1st tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serum pig 87	Hyperimmune	2nd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serum pig 87	Hyperimmune	3rd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Virus pig 63*	Sick, hog cholera	Pig killed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	−	+	−	+
Expt. pig 137	Sick, hog cholera	Pig killed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Expt. pig 138	Sick, hog cholera	Pig killed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	−	+	−	+
Expt. pig 139	Sick, hog cholera	Pig killed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Expt. pig 140	Sick, hog cholera	Pig killed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Expt. pig 141	Sick, hog cholera	Pig killed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Expt. pig 144*	Sick, hog cholera	Pig killed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Expt. pig 145*	Sick, hog cholera	Pig killed	+	+	+	+	+	+	+	+	−	+	−	+	−	+	−	+	0	+	0	+	0	+
Expt. pig 146*	Sick, hog cholera	Pig killed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

* These three pigs are the three normal pigs included in the table after being inoculated with hog cholera virus.

SUMMARY OF TABLE 1.

A total of 22 tests were made as follows:

Kind of blood tested.	No. of tests.	No. of pigs.
Normal pig's blood	3	3
Immunizing blood from serum or hyperimmune hogs	10	7
Virus blood from inoculated pigs	9	9
Totals	22	19

NORMAL BLOOD.

Of the normal blood the highest and lowest maximum dilutions at which agglutination occurred were the same in all cases:

Three cases or 100% at 1—125.

IMMUNIZING BLOOD.

Of the immunizing blood, the highest maximum dilution at which agglutinations occurred was:

Six cases or 60% at 1—1000.*

The lowest maximum dilution at which agglutination occurred was:

Two cases or 20% at 1—250.

One case or 10% agglutinated at 1—333.

One case or 10% agglutinated at 1—500.

VIRUS BLOOD.

Of the virus blood the highest maximum dilution at which agglutination occurred was:

Three cases or 33 1/3%, 1—1000.

The lowest maximum dilution at which agglutination occurred was:

One case or 11 1/9%, 1—333.

Three cases or 33 1/3% agglutinated at 1—500.

Two cases or 22 2/9% agglutinated at 1—700.

DISCUSSION OF TABLE 1.

These tests, therefore, indicate that there is a considerable increase in the agglutinative power for B. cholerae suis in the blood of serum and virus hogs treated according to the Turner-Kolle method, and that in serum hogs there is usually a greater production of agglutinin than in virus hogs.

VIRULENT CULTURE.

Culture "No. 2, Rab. 25."

Our preliminary tests proved very valuable in suggesting details for more extended investigations along similar lines. Whatever conclusions we deduce from the foregoing experiments, must be looked upon as

* Unfortunately, dilutions higher than 1-1000 were not made until later in the work. On account of this fact, it is not possible in some cases to state exactly what is the maximum dilution at which agglutination would have occurred.

tentative or only as indications of what may be applicable in a general way.

In continuing our experiments, we attempted at the outset to secure a culture of B. cholerae suis from one of our virus hogs that would prove highly virulent.

The plan of our work was to establish the susceptibility of normal and immunized hogs and rabbits to different methods of administering a culture of B. cholerae suis from a virus hog and then to demonstrate the agglutinative power of normal, immune and virus blood for this germ.

SOURCE OF CULTURE.

The culture used in this work was obtained from the spleen of Expt. pig 146, brief clinical and autopsy notes on which are as follows: Susceptible, Chester White pig; wt. 63 lbs.; Feb. 22, 1909, bled from tail about 50 cc. for agglutination test with "Manistee culture." March 1, 1909, injected 1 cc. virus 63 in ear vein and 1 cc. in perivascular tissue. Quite sick by seventh day; weak, no appetite. Killed on the eighth day after injection. Autopsy: Slight blush of skin over ventral aspect; inguinal, iliac, and renal glands hemorrhagic in cortex, portal glands oedematous; spleen, slightly enlarged; kidneys, greatly enlarged, soft and flabby; pale in cortex with few faint petechiae, papillae greatly reddened, small cyst in left kidney; liver engorged with blood, with greatly congested areas under capsule; lungs congested, hemorrhagic in ventral and cephalic lobes.

Pieces of spleen, the size of a pea, produced vigorous growth in bouillon. Cultural tests showed pure culture of B. cholerae suis. Rabbit 25, inoculated with 1 cc. of a 20-hour bouillon culture intraperitoneally, March 13, 1909, died March 16, 1909.

Autopsy: Lungs, greatly congested and slightly hemorrhagic in dependent anterior lobules; spleen greatly enlarged, dark and friable; liver enlarged and gorged with blood; kidneys enlarged and congested. Smears from spleen stained with carbol-fuchsin showed few irregular staining rod-shaped organisms which proved to be pure culture of B. cholerae suis upon cultural tests.

Cultures 1, 2, and 3 were taken from three different colonies on agar plates. Cultures 1 and 2 killed rabbits in four days and culture 3 killed a rabbit in 5 days and grew less vigorously in all culture media. Culture 2, designated "No. 2, Rab. 25," was used in the following experiments:

INOCULATIONS.

(a) *Untreated animals*—

Expt. pig 169, wt., 81 lbs., not exposed to any previous infection or subjected to any other treatment. On March 31, 1909, fed 100 cc. of a 24 hr. bouillon culture "No. 2 Rab. 25," after 24 hrs. fasting. Apr. 4, 1909, or four days after feeding, pig died. Had diarrhoea, no appetite and appeared sick day following ingestion of culture.

Autopsy: All lymph glands very slightly hemorrhagic in cortex; spleen enlarged, dark and friable; liver very friable, parboiled appearance, necrotic spots show on surface; intestines congested throughout; large intestine nearly empty, except for dry, yellowish ingesta firmly adherent to mucous ridges, mucosa shows superficial necrosis; fibrinous exudate on serosa; lungs greatly congested,

Expt. pig 165, wt., 45 lbs.; not exposed to any previous infection or subjected to any other treatment. On March 23, 1909, injected 3 cc. 24 hr. bouillon culture "No. 2 Rab. 25" in ear vein. On March 25, 1909, or in less than 48 hrs., dead.

Autopsy: Skin over ventral aspect, purple; external inguinal, iliac, mediastinal, and portal glands hemorrhagic throughout; spleen greatly enlarged, dark and friable; kidneys firm and normal in color and size with few petechiae showing on surface; liver gorged with blood, bluish in color; lungs greatly congested; left ventricle in systole, right filled with fluid blood.

(b) *Treated animals—*

Experiments with pigs immunized with hog cholera serum or virus:

Expt. pigs 112 and 118 were immunized by the serum-simultaneous method Jan. 5, 1909, the former receiving 15 cc., and the latter, 30 cc. mixed serum 17, and each 1 cc. of virus 62. Jan. 26, 1909, each received 1 cc. virus Expt. 123, and again on Feb. 11, 1909, each received 2.5 cc. virus Expt. 141. No. 112 gained from 24 lbs. to 52 lbs. and No 118 from 21 lbs to 47 lbs. by March, when each pig received 3 cc. of a 24 hr. bouillon culture "No. 2 Rab. 25," in the ear vein. They both remained perfectly well and vigorous, although the distal portion of the injected ear of No. 112 became hemorrhagic, showed dry gangrene in about ten days and eventually sloughed off, leaving a healthy line of demarcation with the unaffected portion of the ear. This probably indicates a general immunity with local susceptibility. On Apr. 8, 1909, each pig was fed 50 cc. of a 24 hr. bouillon culture "No. 2 Rab. 25." Each ate all of it and remained well. On May 1, 1909, in company with four other smaller pigs, one of which did not eat, these two pigs were fed after a 24 hr. fast, 400 cc. of culture "No. 2 Rab. 25" in Dunham's peptone solution grown 48 hrs. at 37° C. and 24 hrs. at room temperature. It is safe to say that each of these two pigs ate at least 100 cc. of the culture.

There was no indication of bad results.

Expt. pigs 107, 113 and 115, weighing about 20 lbs. each, were immunized by the serum-simultaneous method Jan. 5, 1909, Expt. 107 receiving 5 cc. mixed serum 17 and the two latter, 15 cc. each, and each 1 cc. virus 62. On Jan. 26, 1909, each pig received 1 cc. virus Expt. 123 and on Feb. 11, 1909, each received 2.5 cc. virus Expt. 141 in the muscles on inside of thigh. At this time, Expt. 107 was suffering from dyspnoea and ate very irregularly. April 6, 1909, Expt. 107; wt., 39 lbs., and Expt. 115, wt., 35 lbs., were fed 100 cc. each of a 24 hr. bouillon culture No. 2 Rab. 25." The former ate part immediately and the remainder during the night; the latter ate all of it immediately. No evidences of sickness were noted. On Apr. 17, 1909, after fasting 24 hrs., Expt. pigs 107 and 115 were fed each 50 cc. of a 24 hr. bouillon culture "No. 2 Rab. 25." The former ate none; the latter, all of it without producing any signs of sickness. May 1, 1909, all three pigs were fed, in connection with Expt. pigs 112, 118 and 24, 400 cc. of culture "No. 2 Rab. 25," as indicated in notes on Expt. pigs 112 and 118 above. Expt. 107 ate none, the others ate greedily. None of these pigs was affected by feeding cultures under the above conditions.

Before the first feeding experiment, as mentioned above, Expt. 107 was not in a perfectly healthy condition, dyspnoea being the probable cause of unthriftiness. In order to determine the cause of the dyspnoea and ascertain what effect all previous treatment had produced on the various organs of the body, Expt. 107 was bled to death May 7, 1909.

Autopsy: Oedema of glottis producing partial occlusion of larynx; few necrotic foci in hepatized cephalic and ventral lobes of both lungs; liver, very light in color and showed considerable increase in interlobular connective tissue; cholecyst distended with semi-solid, greenish bile; ascarides numerous. Nothing found that could be directly attributed to any portion of the treatment.

In the table under each dilution are the signs arranged in two columns to indicate the degree of reaction having reference to the two factors sidered above. (See page 7.)

TABLE II.

Showing agglutination tests with Culture "No. 2 Rab. 25" on immune, hyperimmune and virus hogs.

In the table, the sign (+) indicates a complete reaction, the sign (−) indicates that the reaction has progressed considerably, but not completely, and the sign (0) indicates no change.

Dilution of blood serum.			1–10		1–25		1–50		1–100		1–250		1–500		1–1000		1–2000	
Source of blood serum tested.	Classification of pigs tested.	Method of securing blood.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.		
Expt. pig 107	Immune	Killed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	.....	.....
Virus pig 79	Sick, hog cholera	Killed	+	+	+	+	+	+	+	+	+	+	+	+	0	0	.....	.....
Virus pig 80	*Sick, hog cholera*	*Killed*	+	+	+	+	+	+	+	+	+	+	0	+	0	0	.....	.....
Virus pig 83	Sick, hog cholera	Killed	+	+	+	+	+	+	+	+	+	+	−	+	0	0	.....	.....
Virus pig 84	Sick, hog cholera	Killed	+	+	+	+	+	+	+	+	+	+	0	+	0	0	.....	.....
Virus pig 85	Sick, hog cholera	Killed	+	+	+	+	+	+	0	+	+	+	0	−	0	0	.....	.....
Virus pig 86	Sick, hog cholera	Killed	+	+	+	+	+	+	+	+	0	+	0	0	0	0	.....	.....
Virus pig 87	Sick, hog cholera	Killed	+	+	+	+	+	+	0	+	0	+	0	0	0	0	.....	.....
Expt. pig 197	Sick, hog cholera	Killed	+	+	+	+	+	+	+	−	+	+	−	−	0	0	.....	.....
Expt. pig 199	*Sick, hog cholera*	*Killed*	+	+	+	+	+	+	+	+	0	+	0	0	0	0	.....	.....
Virus pig 88	Sick, hog cholera	Killed	+	+	+	+	+	+	+	+	+	+	0	0	0	0	.....	.....
Virus pig 90	Sick, hog cholera	Killed	+	+	+	+	+	+	+	+	0	+	0	0	0	0	.....	.....
Serum pig 89	Hyperimmune	1st tail bleeding	+	+	+	+	+	+	+	+	+	+	0	0	+	+	.....	.....
Serum pig 89	*Hyperimmune*	*2nd tail bleeding*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	.....	.....
Serum pig 89	Hyperimmune	3rd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serum pig 90	Hyperimmune	1st tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	.....	.....
Serum pig 90	Hyperimmune	2nd tail bleeding	+	+	+	+	+	+	+	+	+	+	−	−	−	−	.....	.....
Serum pig 90	Hyperimmune	3rd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	−	−	0	0
Serum pig 93	Hyperimmune	1st tail bleeding	+	+	+	+	+	+	+	+	+	+	−	−	0	0	.....	.....
Serum pig 93	Hyperimmune	*2nd tail bleeding*	+	+	+	+	+	+	+	+	+	+	−	−	0	0	.....	.....
Serum pig 93	Hyperimmune	3rd tail bleeding	+	+	+	+	+	+	+	+	+	+	−	−	0	0	0	0
Serum pig 95	Hyperimmune	1st tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	.....	.....
Serum pig 95	Hyperimmune	Killed	+	+	+	+	+	+	+	+	+	+	+	+	0	0	.....	.....
Serum pig 96	Hyperimmune	2nd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	0	0	.....	.....
Serum pig 96	Hyperimmune	3rd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0
Serum pig 97	Hyperimmune	3rd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0
Serum pig 97	Hyperimmune	Killed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	.....	.....
Serum pig 109	Hyperimmune	Killed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0
Serum pig 117	Hyperimmune	2nd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	.....	.....
Serum pig 118	Hyperimmune	2nd tail bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serum pig 119	*Hyperimmune*	*1st tail bleeding*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Having secured a culture of B. cholerae suis that promised considerable pathogenic power for untreated pigs and little or no pathogenicity for serum-immunized pigs, we continued our agglutination experiments along the same line as before. Table II shows the results of the tests with culture "No. 2, Rab. 25," and serum from immune, virus, and hyperimmune hogs.

SUMMARY OF TABLE II.

A total of 31 tests were made as follows:

Kind of blood tested.	No. of tests.	No. of pigs.
Blood from immune pigs	1	1
Virus blood from inoculated pigs	11	11
Immunizing blood from serum or hyperimmune pigs.	19	10
Total	31	22

BLOOD FROM IMMUNE PIG.

Expt. pig 107 is classed as an immune, but the treatment of this pig up to the date of drawing the blood for the agglutination test was unusual and undoubtedly influenced the degree of reaction which was very pronounced at 1—1000 and probably would have shown had much higher dilutions and tests been made. The clinical and autopsy records on Expt. 107 have been recorded above. (See page 14.) We can only assume that the feeding of the same culture used in the agglutination tests augmented the agglutinin in the serum of Expt. 107. The only additional tests made with the blood serum of immune pigs not hyperimmunized are recorded in Table III; but, while these tests indicate that the production of agglutinins is increased over those normally present and less than are present in hyperimmunes, they are not comparable with the case of Expt. 107.

VIRUS BLOOD.

Of the virus blood the highest maximum dilution at which agglutination occurred was:

Four cases or 36+% at 1—500.

The lowest maximum dilution at which agglutination occurred was:

One case or 9+% at 1—50.

Three cases or 27+% agglutinated at 1—100.

Three cases or 27+% agglutinated at 1—250.

IMMUNIZING BLOOD.

Of the immunizing blood, the highest maximum dilution at which agglutination occurred was, in four cases or 21+%, 1—2000.

The lowest maximum dilution at which agglutination occurred was:

Six cases 31+% at 1—500.

Nine cases or 47+% agglutinated at 1—1000.

DISCUSSION OF TABLE II.

Suspecting that a reaction would appear at much higher dilutions in many cases, we tested eight samples of blood at a dilution of 1—2000 and recorded a typical and complete reaction in four of these cases. It is apparent that our work is materially weakened by failure to determine the maximum agglutinative power in each case, especially where

a comparison is to be made with the results of tests on different drawings from the same serum hog.

An opportunity was offered to test the agglutinative power of the blood from three different serum hogs at each of three successive bleedings. Serum pig 89 shows a high agglutinative power at the first bleeding, slightly less at the second and apparently greatly increased at the third. Since tests were not made of the first two drawings at dilutions above 1—1000 a careful comparison is impossible. This pig weighed about 110 lbs. at the beginning of the treatment and received over 1200 cc. of virus intramuscularly during a period of about three weeks ending eight days before the first bleeding. After the first bleeding, 350 cc. of virus was injected and after the second bleeding, 200 cc. of virus was injected.

Serum pig 90 showed a slight agglutinative power at the first bleeding, slightly less at the second, and practically the same at the third as at the first. This pig weighed about 110 lbs. at the beginning of the experiment and received about 900 cc. of virus into the muscles, and 100 cc. intravenously during the three weeks ending eight days before the first bleeding. After the first bleeding, 300 cc. of virus was injected and after the second bleeding, 145 cc.

Serum pig 93 showed a weak reaction at 1—500 at each of the first three bleedings. This pig weighed about 100 lbs. at the beginning of the treatment and received 1060 cc. of virus intramuscularly and 340 cc. intravenously during the three weeks preceding the eighth day before the first bleeding. After the first bleeding, 250 cc. of virus was injected and after the second bleeding, 150 cc.

Serum pig 95 could not be bled well from the tail for some undetermined cause, and, after the first attempt, was killed on the following week. A considerable decrease in agglutinative power is noticed from the first to the second bleeding. Only slight differences in agglutinative power are noticed in the two successive bleedings from serum pigs 96 and 97. Serum pigs 117, 118 and 119, show a very great agglutinative power in the one test made in each case.

We find nothing in the treatment of these pigs to suggest an explanation for the difference in agglutinative power of the serum secured at different stages in their treatment. There is, necessarily, an individual variation in pigs that causes them to respond differently to similar or nearly identical treatment, explanations for which fact are not at hand.

For a comparison of the agglutinative power of normal pig's blood and the blood of the pigs tested in Table II we must anticipate the results recorded in Table III, where we find that agglutination does not occur at a dilution even as low as 1—50 when normal defibrinated blood is used in connection with culture "No. 2, Rab. 25."

We find the following comparative results:

Blood of normal pigs, Table III	less than 1—50
Blood of immune pigs, Table II	1—1000
Blood of virus pigs, Table II	1—50 to 1—250
Blood of hyperimmune pigs, Table II	1—500 to 1—2000

In the table under each dilution are the signs arranged in two columns to indicate the degree of reaction having reference to the two factors considered above. (See page 7.)

TABLE III.

Showing agglutination tests with Culture "No. 2 Rab. 25" on pigs before and after treatment.

In the table, the sign (+) indicates a complete reaction, the sign (−) indicates that the reaction has progressed considerably, but not completely, and the sign (0) indicates no change.

Source of blood serum tested.	Classification of pigs tested.	Method of securing blood.	1–50		1–100		1–250		1–500		1–1000		Date blood was drawn.	Date pig died.	Treatment of blood or pig following first bleeding.
			Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.			
Expt. 211	Normal	Tail	0	0	0	0	0	0	0	0	0	0	5–18–09		Blood defibrinated.
Expt. 212*	Normal	Tail	0	0	0	0	0	0	0	0	0	0	5–18–09		Blood defibrinated.
Expt. 213	Normal	Tail	0	0	0	0	0	0	0	0	0	0	5–18–09		Blood defibrinated.
Expt. 214	Normal	Tail	0	0	0	0	0	0	0	0	0	0	5–18–09		Blood defibrinated.
Expt. 215	Normal	Tail	0	0	0	0	0	0	0	0	0	0	5–18–09		Blood defibrinated.
Expt. 216	Normal	Tail	0	0	0	0	0	0	0	0	0	0	5–18–09		Blood defibrinated.
Expt. 211	Normal	Tail	−	−	0	0	0	0	0	0	0	0	5–18–09		Blood coagulated.
Expt. 212	Normal	Tail	−	−	0	0	0	0	0	0	0	0	5–18–09		Blood coagulated.
Expt. 213	Normal	Tail	−	−	0	0	0	0	0	0	0	0	5–18–09		Blood coagulated.
Expt. 214	Normal	Tail	−	−	0	0	0	0	0	0	0	0	5–18–09		Blood coagulated.
Expt. 215	Normal	Tail	−	−	0	0	0	0	0	0	0	0	5–18–09		Blood coagulated.
Expt. 216	Normal	Tail	−	−	0	0	0	0	0	0	0	0	5–18–09		Blood coagulated.
Expt. 211	*Immunized	Tail	−	−	−	−	0	0	0	0	0	0	5–26–09	5–27–09	5 cc. mixed serum 21; 1 cc. virus.
Expt. 212	*Immunized	Tail	+	+	+	+	+	+	+	+	−	−	5–26–09	5–27–09	10 cc. mixed serum 21; 1 cc. virus.
Expt. 213	*Immunized	Tail	+	+	+	+	−	−	−	−	0	0	5–26–09	5–28–09	10 cc. mixed serum 21; 1 cc. virus.
Expt. 214	*Immunized	Tail	+	+	−	−	0	0	0	0	0	0	5–26–09	Alive	15 cc. mixed serum 21; 1 cc. virus.
Expt. 215	*Immunized	Tail	+	+	−	−	0	0	0	0	0	0	5–26–09	Alive	15 cc. mixed serum 21; 1 cc. virus.
Expt. 216	Virus	At post mortem	+	+	+	+	+	+	+	+	0	0	5–27–09	5–27–09	1 cc. virus.
Expt. 213	Immunized	At post mortem	+	+	+	+	+	+	−	−	0	0	5–28–09	5–28–09	

*These pigs are designated as "immunized" in the sense that they were treated with immunizing serum which was used in insufficient doses in three cases.

SUMMARY OF TABLE III.

A total of 19 tests was made as follows:

Kind of blood tested.	No. of tests.	No. of pigs.
Blood from normal pigs	12	6
Blood from immunized pigs	6	5
Blood from virus pigs	1	1
Total	19	6*

NORMAL BLOOD.

Defibrinated blood serum from six normal pigs or 100% did not agglutinate at 1—50.

Coagulated blood serum from six normal pigs or 100% showed a faint reaction at 1—50.

There is apparently a slightly greater amount of agglutinin in coagulated blood serum than in defibrinated blood serum.

Definite conclusions cannot be drawn from the data at hand.

IMMUNIZED BLOOD.

In order to show the effect of the treatment both as to the amount and nature of the injected material on the production of agglutinin, the immunized pigs are divided into three groups depending upon the dose of serum injected as follows:

(a) The blood of one pig, wt. 15 lbs., receiving 5 cc. of serum, agglutinated at 1—100.

(b) The blood of one pig, wt. 21 lbs., receiving 10 cc. of serum, agglutinated at 1—1000.

The blood of one pig, wt. 26 lbs., receiving 10 cc. of serum, agglutinated at 1—500 upon two different tests.

(c) The blood of two pigs, wt. 24 and 26 lbs., receiving 15 cc. of serum, agglutinated at 1—100.

These results cannot, by themselves, furnish the basis for positive deductions. Until a greater number of tests are made under similar conditions, it is useless to conjecture as to the possible explanations for these results. For the time being, it is sufficient to state that the treatment of the pig both quantitatively and qualitatively influences the production of agglutinin in the blood.

VIRUS BLOOD.

The blood of one virus pig, wt. 26 lbs., agglutinated at a dilution of 1—500. The result in this case is comparable with the reactions noted on virus blood in Table II.

* The six normal pigs furnished the immune and virus blood after being treated.

DISCUSSION OF TABLE III.

That the agglutinative power of pig serum is dependent upon the treatment administered is made manifest in Table III. Six normal pigs from the same litter and subjected to identical environmental conditions showed no agglutinative power for culture "No. 2, Rab. 25" at dilutions of 1—50 when the serum was drawn off from defibrinated blood. Serum secured at the same time from these six pigs but drawn from clotted blood showed a very faint agglutinative power at dilutions 1—50. We have not made a careful or extended study of the influence upon agglutination exerted by different methods of securing and handling the serum.

Within a few hours after drawing the blood from the tails of Expt. pigs 211—216, they were treated as indicated in the table by the serum-simultaneous method, using 1 cc. of virus in each case and from 5 cc to 15 cc. of serum except in the case of Expt. 216, which was left as a check with virus only.

The effect of the treatment upon the agglutinative power of the blood in these six cases is interesting and indicates strongly that there is a close relation between the nature and amounts of the materials used in the treatment to the variations in agglutinative power. Only two of the pigs remained alive and well at the time of this writing. These two, Nos. 214 and 215, received the dose of serum that we have found as a rule necessary to protect pigs of this weight against 1 cc. of virus and subsequent exposure. These two pigs showed an equal increase in agglutinative power toward B. cholerae suis. No. 211, with only one-third the necessary dose of serum to immunize, showed a slight increase in agglutinative power. Nos. 212 and 213, with two-thirds the dose of serum necessary to immunize, showed considerable increase in agglutinative power. The check, No. 216, or virus pig, showed a marked increase in agglutinative power.

We have by no means arrived at that point in our work where we can offer a safe prediction as to what the exact effects will be upon the production of agglutinin as a result of any particular treatment.

GENERAL SUMMARY.

AGGLUTINATION.

The blood serum of normal hogs may agglutinate B. cholerae suis in dilutions as high as 1—125.

The blood serum of virus hogs may agglutinate B. cholerae suis in dilutions as high as 1—700.

The blood serum of immune pigs may agglutinate B. cholerae suis in dilutions as high as 1—1000.

The blood serum of hyperimmune pigs may agglutinate B. cholerae suis in dilutions as high as 1—2000 and possibly higher.

SUSCEPTIBILITY AND IMMUNITY.

Pigs may be killed by injection intravenously or by feeding culture of B. cholerae suis isolated from virus hogs made sick by intramuscular injections of hog cholera blood.

Pigs immunized according to the Turner-Kolle method may withstand intravenous injections of virulent cultures of B. cholerae suis.

CONCLUSION.

It does not seem necessary for us to record the many possible or probable explanations that have occurred to us for the more or less constant results secured in our experiments. To those interested in these problems, there will readily occur equally or more plausible explanations.

We have demonstrated that the hyperimmunization process of Dorset-Niles results in the production of agglutinins and immune bodies for B. cholerae suis. We know neither the nature of these immune bodies nor the constancy with which they occur. It is not determined by our experience whether the production of immune bodies for B. cholerae suis is the only result, i. e., the primary result, of the hyperimmunization process or merely a secondary reaction, incidental to the primary, production of immunizing substances against the filterable virus. Our experiments are valuable in paving the way for a clearer understanding of the etiology of hog cholera. Until the etiology of hog cholera is determined by uncontrovertible experimental and clinical evidences, the production of immunity toward hog cholera must remain a matter of uncertainty, constantly subjected to the interrupting influences of unknown factors. If B. cholerae suis is a species distinct from the filterable virus of Dorset and others, then there is truly opened a wonderful field in bacteriology as related to animal pathology, involving the associative action, the symbiotic relations of several pathogens and the common host.

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