

STUDIES ON HOG CHOLERA.*

EXPERIMENTAL HYPERIMMUNIZATION.

WALTER E. KING AND ROBERT H. WILSON.

(From the Research Laboratory, Parke, Davis & Co., Detroit, Mich.)

During the period that *B. cholerae suis* was accepted as the causative factor of hog cholera, investigators attempted to attenuate the organism sufficiently to produce a practical vaccine. Chemicals, heat, and various other methods were employed for this purpose, but the results obtained with these vaccines were not entirely satisfactory. In some instances they protected hogs inoculated with cultures of *B. cholerae suis*, but in the field, as a rule, failed to protect against natural infection.

When it became known that the specific cause of cholera was involved in the so-called "filterable virus," attention was turned to its modification for preventive inoculation. Practically all of the experimental methods used in the attenuation of *B. cholerae suis* have been unsuccessfully applied to the virus. Numerous experiments have been conducted in attempting to attenuate the virus by heating. Dorset and Niles¹ have worked along this line with negative results. Graham² also reports unfavorable results with similar experiments. Peters³ claims to have had more or less success with serum virus heated at a temperature of 60° C. for one-half hour. He has inoculated over 16,000 hogs with this attenuated virus and reports good results in many herds. It is the consensus of opinion, however, that this method of attenuation is not reliable for field use, owing to the difficulty in obtaining a uniform product, and the fact that there is a great variation in the susceptibility of hogs to cholera.

In 1908, King⁴ published his results of the attempted attenuation of hog cholera virus by passage through animals of other species, particularly the horse. He concluded that the virus under-

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¹ U.S. Live Stock Sanitary Proceedings, 1911.

² Amer. Vet. Rev., 1912.

³ U. S. Live Stock Proceedings, 1911.

⁴ Bull. Kansas Exp. Sta., 1908, No. 157.

goes some unexplainable modification resulting in an attenuation after several hours' residence in the circulatory system of the horse. He noted that during the first two hours' residence the virus seemed to become activated in some manner. Typical cases of acute cholera were produced in normal hogs by the injection of two to four cubic centimeters of serum drawn from a horse which had received intravenously a quantity of hog cholera virus two hours previously. Serum drawn at a later period than this seemed to be less virulent, the virulence decreasing in proportion to the length of time elapsing after the horse had received the cholera virus.

In 1910, we¹ attempted to determine whether or not "horse serum virus" (serum drawn from a horse within two hours after the injection of hog cholera virus) represents a mere dilution of the infectious agent. Tests were made to determine this point by preparing corresponding dilutions of the same strains of virus in normal horse blood *in vitro* and also in sterile physiologic salt solution. The proportionate dilutions were made by weighing the horse and taking one-fifteenth of the body weight as representing the total amount of blood in the animal. These dilutions were then kept at body temperature for the same period of time as that of the residence of the cholera virus in the circulatory system of the horse. The virulence of each dilution was tested by the injection of normal hogs kept in separate, well isolated pens. As noted, the serum drawn from the horse within two hours after injection with virus produced cases of acute cholera with an average incubation period of seven days, and an average duration of the disease of 15 days. With the same lot of virus in proportionate dilution in normal horse blood and physiologic salt solution *in vitro*, fewer cases of cholera were produced and those which did develop had a longer average incubation period, and were more chronic in nature. These results indicated that horse serum virus does not represent a mere dilution of cholera virus.

¹ *Ibid.*, 1910, No. 171.

HYPERIMMUNIZATION WITH HORSE SERUM VIRUS.

I. EXPERIMENTAL.

The fact that serum drawn from a horse one or two hours after it receives an intravenous injection of approximately 150 c.c. of cholera virus, is capable of producing acute cholera when injected in small doses into normal hogs, suggested the possibility of substituting horse serum virus for hog serum virus in the production of Dorset-Niles hyperimmune serum. Assuming that a reliable, potent serum could be produced by this modified method certain advantages would follow:

The cost of production would be less than that involved in the present method. The serum from one horse could be substituted for the blood from a large number of hogs. This would result, not only in greater economy, but in greater convenience and in better regulated manipulations in serum production. Furthermore, more adequate precautions against contamination could be exercised.

We have published the results of some preliminary experiments relative to hyperimmunization with horse serum virus. These results were fairly uniform in character and indicated that the procedure warranted further experimentation. During the past two years the following data have been collected on this subject.

The virus used in the injection of the horses was in every instance secured from cases of acute cholera in which the symptoms and lesions were characteristic. The moribund hogs were bled from the carotid artery under aseptic conditions. The receptacle containing the blood was placed in a refrigerator for 10 or 12 hours to allow the coagulum to harden and contract. The serum was then poured into sterile flasks which were placed in the refrigerator pending the time of injection.

The horses used in the work, three in number, weighed approximately 1,100 pounds each. They were each about nine years old and constitutionally sound. Each was injected and bled four times. The amount of hog cholera serum injected into the horses at a given time varied from 100 to 150 c.c. The injections, in practically every case, were followed by severe reactions, beginning five or 10 minutes after the horse had received the virus. The

chief characteristics of these reactions were increased respiratory and cardiac movements, stimulation of nearly all the body secretions, and marked general depression. The first symptoms usually disappeared in one hour, leaving the horse more or less exhausted for a day or two. An important feature noted in connection with the injection was that the reactions were usually more pronounced after the first injection. A horse may be repeatedly treated with hog cholera virus intravenously for an indefinite period, as the three animals used in this work are at present alive and in good condition. The bleedings were made one-half to three-quarters of an hour after injection, experience showing that serum drawn from the horse at this time is more virulent than that drawn at an earlier

TABLE 1.
VIRULENCY TESTS OF HORSE SERUM.

Hog	Weight	Date	Amount Serum	Horse	Results	
175.....	46	9/23	4 c.c.	1,313	Symptoms after 3 days; died after 15 days	Typical lesions
196.....	55	11/15	4 "	1,313	Symptoms after 5 days; died after 19 days	Typical lesions
250.....	55	2/28	4 "	1,422	Symptoms after 8 days; died after 30 days	Typical lesions
251.....	48	2/28	4 "	1,422	Symptoms after 10 days; died after 27 days	Typical lesions
278.....	60	4/22	4 "	1,423	Symptoms after 6 days; died after 20 days	Typical lesions
279.....	56	4/22	4 "	1,422	Symptoms after 3 days; died after 13 days	Typical lesions
292.....	80	5/17	4 "	1,423	Symptoms after 5 days; died after 14 days	Typical lesions
293.....	80	5/20	4 "	1,314	Symptoms after 7 days; died after 14 days	Typical lesions
294.....	75	5/22	4 "	1,314	Symptoms after 5 days; died after 15 days	Typical lesions
295.....	95	5/29	4 "	1,422	No reaction	(Natural immune?)
297.....	95	6/9	4 "	1,422	Symptoms after 5 days; died after 12 days	Typical lesions
301.....	48	6/26	4 "	1,423	Symptoms after 4 days; died after 22 days	Typical lesions
334.....	40	12/4	4 "	1,422	Symptoms after 12 days; died after 22 days	Typical lesions
348.....	50	11/8	4 "	1,422	Symptoms after 6 days; died after 27 days	Typical lesions

or later period. A specially devised apparatus which defibrinates the blood as it is drawn was used in bleeding. About six liters of defibrinated blood were obtained at each bleeding.

The horse serum virus was tested for virulence before being used for experimental hyperimmunization. One or more normal hogs were injected, subcutaneously, each with four cubic centimeters of the serum from the horse under test. These test hogs were kept in well isolated pens and every precaution was observed

to guard against extraneous infection. Table 1 shows the results of these tests. It will be noted that of the 14 cases reported in the table only one failed to contract cholera from the horse serum virus. Three or four of the animals developed chronic cases, but all succumbed to the disease, showing typical lesions. The average incubation period was 6.2 days and the average duration of the disease 20 days.

Thirteen immune hogs received injections of horse serum virus for the purpose of experimental hyperimmunization. The technic employed in the injecting, bleeding, and handling of the serum varied but little from that in use at most of the hog cholera serum laboratories. The intraperitoneal method of injection was used in practically all cases. One-half of one per cent carbolic acid was added to the defibrinated blood as a preservative. Table 2 gives the number of experimental hyperimmune hogs, their weight, method of injection, amount of defibrinated blood injected, and the number of bleedings.

TABLE 2.
EXPERIMENTAL HYPERIMMUNES.

Hog	Weight	First Injection	Injection Amount	Bleedings	Second Injection	Injection Amount	Bleedings
173.....	200	Intraperitoneally and intravenously	1,700 c.c.	8	Intraperitoneally	1,600 c.c.	4
174.....	175	Intraperitoneally	900 "	8
177.....	230	"	1,600 "	8	Intraperitoneally	1,800 c.c.	3
217.....	58	"	1,400 "	..	"	800 "	7
221.....	300	"	2,400 "	3	"	2,200 "	11
254.....	200	"	2,000 "	..	"	1,000 "	13
255.....	190	"	1,800 "	..	"	900 "	14
272A.....	140	"	1,400 "	10
288.....	190	"	1,800 "	900 c.c.	11
289.....	196	"	2,000 "	Died
290.....	185	"	1,800 "	Died
291.....	190	"	1,800 "	..	Intraperitoneally	1,200 c.c.	15
296.....	100	"	900 "	10

Hyperimmune 173.—A Poland China, weight 200 pounds, immunized by the injection of 70 c.c. hyperimmune serum (Mich. Agric. College) and 2 c.c. of virus on September 13, 1910. Fourteen days later it was injected with 1,700 c.c. of $\frac{3}{4}$ -hour horse serum from horse 1,314. Of this amount, 520 c.c. were given intravenously and the remainder intraperitoneally. The hog experienced considerable difficulty in breathing and was otherwise depressed for a few hours after receiving the serum. The first bleeding was made on October 20, when 400 c.c. of serum was obtained. The bleedings were continued at intervals of a week until eight bleedings had been made. As the serum from these various bleedings did not protect the test hogs against cholera, it was deemed advisable to give the hog another injection of the horse serum virus to determine whether this would increase the potency of its serum. This second injection consisted of 1,600 c.c. of horse serum virus given intraperitoneally on

February 16, 1911. The next tail bleeding was made on March 21, followed by three others at intervals of one week. The hog was bled to death on April 19, 1,800 c.c. of blood being obtained. Postmortem examination of the hog revealed no pathological conditions resulting from the injection of horse serum virus.

TABLE 3.
POTENCY TESTS OF SERUM FROM HOG 173.

Bleeding	Hog	Weight	Date	Material Injected	Results	
1st.....	183	56	10/20	25 c.c. serum + 2 c.c. virus	Symptoms after 11 days; died after 32 days	Cholera lesions not pronounced
	184	59	10/20	35 c.c. serum + 2 c.c. virus	Symptoms after 11 days; died after 23 days	Fair cholera lesions
	185	51	10/20	2 c.c. virus	Symptoms after 5 days; died after 13 days	Typical lesions
2d.....	191	52	10/27	25 c.c. serum + 2 c.c. virus	Symptoms after 18 days; died after 21 days	Typical lesions
3d.....	191	58	10/27	35 c.c. serum + 2 c.c. virus	Symptoms after 7 days; died after 17 days	Typical lesions
	192	53	10/27	35 c.c. serum + 2 c.c. virus	Symptoms after 10 days; died after 18 days	Typical lesions
	193	65	10/27	2 c.c. virus	Symptoms after 4 days; died after 8 days	Typical lesions
4th.....	195	53	11/14	25 c.c. serum + 2 c.c. virus	Remained well	
	210	50	11/14	4.6 gm. dried serum + 2 c.c. virus	Symptoms after 7 days; died after 19 days	Typical lesions
	211	55	11/14	6.4 gm. dried serum + 2 c.c. virus	Symptoms after 7 days; died after 21 days	Typical lesions
	212	46	11/14	2 c.c. virus	Symptoms after 6 days; died after 8 days	Typical lesions
6th.....	228	31	1/25	25 c.c. serum + 1.5 c.c. virus	Symptoms after 12 days; died after 18 days	Typical lesions
	229	40	1/25	35 c.c. serum + 1.5 c.c. virus	Symptoms after 11 days; died after 16 days	Typical lesions
	230	33	1/25	1.5 c.c. virus	Symptoms after 6 days; died after 11 days	Typical lesions
7th.....	235	50	2/3	35 c.c. serum + 2 c.c. virus	Symptoms after 9 days; died after 18 days	Typical lesions
	236	34	2/3	25 c.c. serum + 2 c.c. virus	Symptoms after 6 days; died after 12 days	Typical lesions
	233	38	2/3	2 c.c. virus	Symptoms after 5 days; died after 18 days	Typical lesions
8th.....	247	40	2/22	25 c.c. serum + 2 c.c. virus	Symptoms after 4 days; died after 23 days	Fair lesions
1st after 2d in- jection horse serum	256	50	3/21	50 c.c. serum + 2 c.c. virus	Remained well	
	257	50	3/21	40 c.c. serum + 2 c.c. virus	Died after 19 days	Not cholera
	259	48	3/21	2 c.c. virus	Symptoms after 5 days; died after 9 days	Fair lesions
3d.....	271	100	4/10	40 c.c. serum + 2 c.c. virus	Remained well	
	273	40	4/10	2 c.c. virus	Symptoms after 6 days; died after 15 days	Typical lesions
Slaughter..	283	90	4/28	40 c.c. serum + 2 c.c. virus	Remained well	
	284	88	4/28	30 c.c. serum + 2 c.c. virus	Symptoms after 12 days; died after 30 days	Fair lesions
	286	67	4/28	2 c.c. virus	Symptoms after 7 days; died after 11 days	Typical lesions

The serum from hog 173 in most instances failed to protect the test hogs from cholera. It will be noted, however, that the incubation period and duration of the disease were considerably longer in the serum test hogs than in the controls, indicating that a slight resistance to cholera resulted from the injection of the serum.

Hyperimmune 174.—Yorkshire sow, weight 175 pounds. Immunized by the injection of 70 c.c. hyperimmune serum (Mich. Agric. College) and 2 c.c. of cholera virus. Two weeks later injected intraperitoneally with 850 c.c. of $\frac{3}{4}$ -hour horse serum virus. A slight reaction followed the injection as evidenced by anorexia and listlessness lasting a day. First bleeding on October 19, 22 days after receiving the horse serum. Eight bleedings made at intervals of a week.

TABLE 4.
POTENCY TESTS OF SERUM FROM 174.

Bleeding	Hog	Weight	Date	Material Injected	Results	
1st.....	180	58	10/19	25 c.c. serum + 2 c.c. virus	Symptoms after 7 days; died after 17 days	Typical lesions
	181	65	10/19	35 c.c. serum + 2 c.c. virus	Remained well	
	182	62	10/19	2 c.c. virus	Symptoms after 10 days; died after 20 days	
2d.....	187	85	10/26	40 c.c. serum + 2 c.c. virus	Symptoms after 20 days; died after 25 days	Typical lesions
	188	62	10/26	30 c.c. serum + 2 c.c. virus	Symptoms after 19 days; died after 24 days	Fair lesions
	189	65	10/26	2 c.c. virus	Symptoms after 5 days; died after 5 days	Typical lesions
3d.....	204	45	12/19	25 c.c. serum + 2 c.c. virus	Remained well	
4th.....	205	58	12/19	25 c.c. serum + 2 c.c. virus	Remained well	
5th.....	214	50	1/12	25 c.c. serum + 2 c.c. virus	Remained well	
	215	54	1/12	35 c.c. serum + 2 c.c. virus	Remained well	
	216	49	1/12	2 c.c. virus	Symptoms after 6 days; died after 14 days	Typical lesions
6th.....	222	75	1/24	40 c.c. serum + 2 c.c. virus	Remained well	
	223	58	1/24	25 c.c. serum + 2 c.c. virus	Remained well	
	224	56	1/24	2 c.c. virus	Symptoms after 7 days; died after 12 days	Typical lesions
7th.....	237	45	2/8	25 c.c. serum + 2 c.c. virus	Remained well	
	238	59	2/8	30 c.c. serum + 2 c.c. virus	Remained well	
	239	48	2/8	2 c.c. virus	Symptoms after 6 days; died after 13 days	Typical lesions
	288	190	5/6	75 c.c. serum + 2 c.c. virus	Remained well	
	289	196	5/6	75 c.c. serum + 2 c.c. virus	Remained well	
Slaughter ..	290	185	5/12	75 c.c. serum + 2 c.c. virus	Remained well	
	291	190	5/12	75 c.c. serum + 2 c.c. virus	Remained well	
	286	68	4/28	2 c.c. virus	Symptoms after 7 days; died after 11 days	Typical lesions

With the exception of the first two bleedings, the serum from hog 174 afforded ample protection to the test hogs.

Hyperimmune 177.—A Poland China, weight 230 pounds, received immunizing treatment October 4, consisting of 55 c.c. of hyperimmune serum (Mich. Agric. College) and 2 c.c. of virus. Twenty-seven days later was given intraperitoneally 1,600 c.c. of $\frac{3}{4}$ -hour horse serum virus. First bleeding made November 8. The serum from the first eight bleedings did not prove to be sufficiently potent for practical purposes, and the hog was given a second injection of horse serum February 21, consisting of 1,600 c.c. of serum. Bleedings resumed one month later. Slaughter bleeding April 26.

TABLE 5.
POTENCY TESTS OF SERUM FROM 177.

Bleeding	Hog	Weight	Date	Material Injected	Results	
1st.....	194	55	11/9	25 c.c. serum + 2 c.c. virus	Symptoms after 7 days; died after 21 days	Slight lesions
2d.....	197	49	11/16	25 c.c. serum + 2 c.c. virus	Symptoms after 7 days; died after 19 days	Typical lesions
3d.....	207	46	12/23	25 c.c. serum + 2 c.c. virus	Remained well	
	208	51	12/23	35 c.c. serum + 2 c.c. virus	Symptoms after 8 days; died after 22 days	Fair lesions
	209	43	12/23	2 c.c. virus	Symptoms after 7 days; died after 17 days	Typical lesions
5th.....	217	58	1/16	35 c.c. serum + 2 c.c. virus	Remained well	
	218	50	1/16	25 c.c. serum + 2 c.c. virus	Remained well	
	219	51	1/16	2 c.c. virus	Symptoms after 5 days; died after 10 days	Typical lesions
6th.....	225	35	1/24	25 c.c. serum + 2 c.c. virus	Symptoms after 11 days; died after 23 days	Typical lesions
	226	55	1/24	35 c.c. serum + 2 c.c. virus	Remained well	
	227	38	1/24	15 c.c. virus	Symptoms after 5 days; died after 9 days	Typical lesions
7th.....	232	50	2/3	35 c.c. serum + 2 c.c. virus	Symptoms after 13 days; died after 21 days	Lesions not pronounced
	233	44	2/3	25 c.c. serum + 2 c.c. virus	Symptoms after 16 days; died after 21 days	Lesions not pronounced
	234	45	2/3	2 c.c. virus	Symptoms after 5 days; died after 18 days	Typical lesions
1st after 2d in- jection horse serum	258	45	3/21	25 c.c. serum + 2 c.c. virus	Symptoms after 24 days; died after 34 days	Typical lesions
	259	38	3/21	2 c.c. virus	Symptoms after 5 days; died after 9 days	Typical lesions
2d.....	272	64	4/10	30 c.c. serum + 2 c.c. virus	Symptoms after 10 days; died after 28 days	Typical lesions
Slaughter..	285	52	4/28	30 c.c. serum + 2 c.c. virus	Remained well	
	286	85	4/28	40 c.c. serum + 2 c.c. virus	Remained well	
	287	67	4/28	2 c.c. virus	Symptoms after 8 days; died after 12 days	Typical lesions

These experiments indicate that the serum from 177 was lacking in protective properties. The second injection of horse serum apparently failed to increase the potency to any extent.

Hyperimmune 217.—Yorkshire, weight 58 pounds, immunized January 16, with 35 c.c. of hyperimmune serum (175, 5th bleeding) and 2 c.c. virus. On May 3, received 1,400 c.c. of $\frac{3}{4}$ -hour horse serum and 800 c.c. one month later, no bleedings

intervening. The first bleeding was made July 3, and bleedings continued weekly until September 1, when the hog was killed.

TABLE 6.
POTENCY TESTS OF SERUM FROM 217.

Bleeding	Time After Injection	Hog	Weight	Material Injected	Results	
3, 4, 5, 6, 7, slaughter..	40, 47, 54, 61, 68 days..	321	55	35 c.c. serum + 2 c.c. virus	Remained well	Typical lesions
		322	45	25 c.c. serum + 2 c.c. virus	Remained well	
		323	42	2 c.c. virus	Symptoms after 5 days; died after 18 days	

The blood from all the bleedings of 217 was combined and tested collectively, with the result that both hogs which received treatment were sufficiently protected, while the control succumbed to the disease.

Hyperimmune 221.—Poland China, weight 250 pounds. Immunized January 23, with 75 c.c. serum (174, 3d bleeding) and 2 c.c. virus. On February 4, was injected intraperitoneally with 2,600 c.c. of $\frac{3}{4}$ -hour horse serum. The first bleeding was made a month later, followed by two others at intervals of a week. A second injection of $\frac{3}{4}$ -hour horse serum was given April 22, consisting of 2,200 c.c. Bleedings were resumed one month later and continued weekly for 11 weeks.

TABLE 7.
POTENCY TESTS OF SERUM FROM 221.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
1st.....	33 days....	253	35	25 c.c. serum + 2 c.c. virus	Remained well	
2d.....	43 days....	259	58	40 c.c. serum + 2 c.c. virus	Symptoms after 12 days; died after 23 days	Fair lesions
		260	40	30 c.c. serum + 2 c.c. virus	Symptoms after 11 days; died after 20 days	Typical lesions
		261	36	2 c.c. virus	Symptoms after 5 days; died after 9 days	Fair lesions
3d.....		266	75	40 c.c. serum + 2 c.c. virus	Symptoms after 14 days; died after 35 days	Typical lesions
		267	55	30 c.c. serum + 2 c.c. virus	Symptoms after 10 days; died after 30 days	Fair lesions
		268	70	2 c.c. virus	Symptoms after 6 days; died after 13 days	Typical lesions
1st after 2d injection..	30 days	296	99	40 c.c. serum + 2 c.c. virus	Remained well	
5th.....	62 days	305	42	40 c.c. serum + 2 c.c. virus	Remained well	
		306	39	30 c.c. serum + 2 c.c. virus	Symptoms after 7 days; died after 26 days	Fair lesions
		307	39	2 c.c. virus	Symptoms after 6 days; died after 19 days	Typical lesions
6th.....	67 days	308	135	40 c.c. serum + 2 c.c. virus	Remained well	
7, 8, 9, 10, slaughter..	74, 81, 88, 95	314	125	40 c.c. serum + 2 c.c. virus	Remained well	
		315	50	30 c.c. serum + 2 c.c. virus	Remained well	
		317	125	2 c.c. virus	Severe reaction, recovered	

The potency tests showed that the serum from the first three bleedings from 221 was lacking in protective properties. That the test hogs received a degree of immunity is evidenced by the fact that the incubation period and duration of the disease were more prolonged than was the case with the controls in the same tests. The second injection of horse serum stimulated the formation of additional antisubstances. It will be noted that the blood from the seventh to slaughter bleedings was mixed and a composite test made, owing to the shortage of hogs at the time.

TABLE 8.
POTENCY TESTS OF SERUM FROM 254.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
2d.	30 days	298	77	40 c.c. serum + 2 c.c. virus	Symptoms after 12 days; died after 22 days	Typical lesions
		299	64	25 c.c. serum + 2 c.c. virus	Symptoms after 13 days; died after 22 days	Typical lesions
		300	60	2 c.c. virus	Symptoms after 8 days; died after 22 days	Typical lesions
3d.	44 days	309	56	40 c.c. serum + 2 c.c. virus	Symptoms after 8 days; died after 17 days	Typical lesions
		310	58	30 c.c. serum + 2 c.c. virus	Symptoms after 8 days; died after 14 days	Lesions not pron'ced
		313	46	2 c.c. virus	Symptoms after 5 days; died after 12 days	Less pronounced
4, 5, 6, 7, 8, 9	51, 58, 65, 72, 77, 84	319	47	35 c.c. serum + 2 c.c. virus	Remained well	
		320	37	25 c.c. serum + 2 c.c. virus	Remained well	
		322	45	2 c.c. virus	Symptoms after 5 days; died after 12 days	Lesions not pron'ced
10, 11, 12, } 13, 14, } slaughter }	91, 98, 105, 112, 119	329	65	40 c.c. serum + 2 c.c. virus	Remained well	
		330	62	30 c.c. serum + 2 c.c. virus	Remained well	
		333	65	2 c.c. virus	Symptoms after 6 days; died after 9 days	Typical lesions

Hyperimmune 254.—Yorkshire sow, weight 200 pounds. Received immunizing treatment March 17, consisting of 75 c.c. serum (hog 174, 5th bleeding) and 2 c.c. virus. On April 12 received intraperitoneally 1,800 c.c. of $\frac{3}{4}$ -hour horse serum and 900 c.c. May 16. No ill effects followed these treatments other than a slight soreness, which soon passed off. The first bleeding was made one month after the second injection of horse serum. The hog was bled 15 times, and slaughtered September 22.

The first three bleedings yielded a serum which did not afford protection against cholera. Serum from the later bleedings was sufficiently potent to immunize the test hogs.

Hyperimmune 255.—Yorkshire, weight 190 pounds. Immunized May 17, with 75 c.c. serum (hog 174, 5th bleeding) and 2 c.c. virus. On April 12 and May 16, injected with 1,800 c.c. and 900 c.c., respectively, of $\frac{3}{4}$ -hour horse serum. No pronounced reactions were caused by these injections. Bleeding commenced one month after second injection.

TABLE 9.
POTENCY TESTS OF SERUM FROM 255.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
2d.	30 days	302	75	40 c.c. serum + 2 c.c. virus	Remained well	
		303	30	30 c.c. serum + 2 c.c. virus	Symptoms after 8 days; died after 28 days	Lesions not pronounced
		304	64	2 c.c. virus	Symptoms after 8 days; died after 18 days	Typical lesions
3d.	46 days	311	62	40 c.c. serum + 2 c.c. virus	Symptoms after 10 days; died after 21 days	Typical lesions
		312	58	30 c.c. serum + 2 c.c. virus	Symptoms after 10 days; died after 19 days	Typical lesions
		313	46	2 c.c. virus	Symptoms after 6 days; died after 13 days	Lesions not pronounced
4, 6, 7.	53, 67, 74	316	120	45 c.c. serum + 2 c.c. virus	Remained well	
		317	130	45 c.c. serum + 2 c.c. virus	Remained well	
		318	125	2 c.c. virus	Severe reaction, recovered	
8 to 15.	60, 67, 74, 82, 89, 96, 103	331	62	40 c.c. serum + 2 c.c. virus	Remained well	
		332	55	30 c.c. serum + 2 c.c. virus	Remained well	
		333	65	2 c.c. virus	Symptoms after 6 days; died after 9 days	Typical lesions

Hyperimmune 272A.—A Poland China stag, weight 146 pounds. Vaccinated April 10, with 40 c.c. hyperimmune serum and 2 c.c. virus (hog 177, 3d bleeding). Received but one injection of horse serum (1,500 c.c.), this being May 29. Was not bled until three months later.

TABLE 10.
POTENCY TESTS OF SERUM FROM 272A.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
1 to 10.	1st, 63 days, others weekly	339	52	40 c.c. serum + 2 c.c. virus	Remained well	
		340	51	30 c.c. serum + 2 c.c. virus	Remained well	
		341	58	2 c.c. virus	Symptoms after 6 days; died after 16 days	Typical lesions

This experiment shows that the various lots of serum from 272A, tested collectively, protected hogs from the virus. This animal received but one treatment of horse serum and was not bled until three months later, suggesting that considerable time must elapse between the injection of horse serum and the first bleeding in order to obtain a more potent serum.

Hyperimmune 288.—Yorkshire sow, weight 190 pounds. Immunized to cholera May 6, with 75 c.c. serum (hog 174, 6th bleeding) and 2 c.c. virus. Injected twice with $\frac{3}{4}$ -hour horse serum May 20, 1,800 c.c., and June 22, 900 c.c. Bleedings started one month later.

TABLE 11.
POTENCY TESTS OF SERUM FROM 288.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
1 to 11	1st, 34 days	337	72	40 c.c. serum + 2 c.c. virus	Remained well	
	Others weekly	338	56	30 c.c. serum + 2 c.c. virus	Symptoms after 6 days; died after 16 days	Typical lesions
		341	58	2 c.c. virus	Symptoms after 6 days; died after 16 days	Typical lesions

Hyperimmune 291.—Poland China, weight 190 pounds. Immunized May 12, with 75 c.c. serum (hog 221, 4th bleeding) and 2 c.c. virus. Injected May 31, with 1,800 c.c. of $\frac{3}{4}$ -hour horse serum and again June 30 with 1,200 c.c. No reaction followed these injections. Bleedings started July 29, and continued at intervals of a week.

TABLE 12.
POTENCY TESTS OF SERUM FROM 291.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
1 to 7	30 days	345	70	40 c.c. serum + 2 c.c. virus	Symptoms after 5 days; died after 17 days	Lesions of chronic cholera
	Weekly intervals	346	67	30 c.c. serum + 2 c.c. virus	Symptoms after 6 days; died after 21 days	Typical lesions
		347	60	2 c.c. virus	Symptoms after 7 days; died after 22 days	Typical lesions

Only the first seven bleedings were tested in this case. As the table indicates, the serum was not potent.

Hyperimmune 296.—Chester white, stag, weight 100 pounds. Injected May 29, with 40 c.c. serum (hog 221, 4th bleeding) and 2 c.c. cholera virus. One month later received 900 c.c. of $\frac{3}{4}$ -hour horse serum. Was not bled until August 1, practically two months after receiving the horse serum.

TABLE 13.
POTENCY TESTS OF SERUM FROM 296.

Bleeding	Time after Injection	Hog	Weight	Material Injected	Results	
1 to 9	1-32 days	343	64	40 c.c. serum + 2 c.c. virus	Remained well	
	Weekly intervals	344	77	30 c.c. serum + 2 c.c. virus	Remained well	
		347	60	2 c.c. virus	Symptoms after 7 days; died after 22 days	Typical lesions

The serum from the various lots of bleedings proved to be sufficiently potent to protect the test pigs against cholera.

TABLE 14.
TABULATED RESULTS OF EXPERIMENTAL HYPERIMMUNIZATION.

Hog	Results	Potency Established
173.....	Serum not potent
174.....	Serum potent	60 days
177.....	Serum potent	80 days
217.....	Serum potent	47 days
221.....	Serum potent	65 days
254.....	Serum potent	60 days
255.....	Serum potent	68 days
272A.....	Serum potent	80 days
288.....	Potency of serum below standard	34 days
291.....	First three bleedings not potent. Others not tested	Not determined
296.....	Serum potent	62 days

SUMMARY.

The results of these various tests relative to experimental hyperimmunization with horse serum virus suggest the following conclusions:

1. Cholera immune hogs can withstand intraperitoneal injections of large quantities of horse serum virus. Ten cubic centimeters per pound body weight can be injected without anaphylactic effects.
2. Horse serum virus when injected into immune hogs is capable of stimulating the formation of antibodies in the blood of the treated hogs.
3. Blood drawn from a hog at least one month after it has received an injection of horse serum virus is more potent than blood drawn at any earlier period.
4. Two injections (intraperitoneal) of horse serum virus, one month intervening, consisting of 10 c.c. and 5 c.c. per pound weight respectively, appear to produce a more potent blood than that produced by one injection.
5. It requires a larger dose of hyperimmune serum, prepared by this modified method, to protect against the test dose of virus, than is necessary with serum prepared after the original method.

II. FIELD TESTS WITH HYPERIMMUNE SERUM.

In order to determine the efficiency of the serum in immunizing against cholera under natural conditions, a number of field tests were conducted. The serum used in these tests had proven potent

at the laboratory and included that from hogs 174, 177, 217, 221, 254, and 255.

Twenty-nine herds, located in three states, received treatment with the experimental serum. The animals in these several herds afforded conditions which are usually encountered in dealing with an epizootic of hog cholera. The "serum alone" method of treatment was employed in every case.

The following is a description of each herd, together with the treatment and results.

Herd 1.—This herd, an infected one, was located near Flint, Mich., and at the time of treatment consisted of 270 hogs of various sizes and breeds. The disease had been present in the herd for two weeks, apparently having been introduced by a shipment of hogs received from the Detroit stockyards. Thirty hogs had died and 20 were sick, showing symptoms of cholera at the time of treatment (August 26). Three of the sick hogs were killed and postmortem examination revealed characteristic lesions of the disease. One hundred hogs were treated with the experimental serum (221). Of this number 22 were suckling pigs three weeks old; 52 weighed about 50 pounds each; 15 weighed 150 pounds; and 12 weighed 350 pounds each. The doses were 5, 25, 50, and 75 c.c., respectively. Thirty-three hogs were left untreated to serve as controls.

The final result of this experiment shows that 29 per cent of the treated hogs and 84.8 per cent of the controls died. Considering the fact that the herd was badly infected at the time of treatment the outcome of the experiment was quite satisfactory. No attempt was made to isolate the animals in any way during the experiment.

Herd 2.—This herd was located a few miles from Detroit, Mich. It consisted of 15 hogs at the time that the disease appeared. Two weeks later the number was reduced to seven. The disease was prevalent on adjoining farms. Six of the hogs weighing 50 pounds each were treated with 40 c.c. of serum from experimental hyper-immune 221. The remaining shoat was left untreated as a control.

The control and one of the treated hogs died about a week after the treatment. The other five treated hogs remained well.

Herd 3.—This herd was located in northeastern Kansas, in a vicinity where cholera had been prevalent for several months. It consisted originally of 38 hogs, but six of them had already contracted the disease and died. The remaining number had every chance for exposure, as they were in same pen with those which died. Fifteen 30-pound shoats and one 400-pound sow received treatment, consisting of 40 c.c. and 75 c.c., respectively. Three or four of the treated hogs showed slight symptoms of cholera when injected. Sixteen hogs were not treated. Serum 217 was used on this herd. The last report of the experiment shows that 93.7 per cent of the treated hogs and 100 per cent of the controls succumbed. In this experiment the serum had little effect in checking the disease.

Herd 4.—This herd was located one mile from herd 3. Cholera had been present in this herd for two weeks during which time 40 hogs died of the disease. Of the 27 remaining hogs, 19 weighing approximately 70 pounds each were given injections of

the serum (217), the dose administered being 40 c.c. One of these hogs as well as one of the eight controls showed evidence of infection on the day of injection. The final report of this test showed that 42.2 per cent of the treated hogs and 87.5 per cent of the controls died. The serum evidently gave some protection.

Herd 5.—This herd was located one-half mile from herd 4. It consisted of 61 pure-bred Poland Chinas of various ages. The herd had escaped cholera, which was present on adjoining farms. Thirty-five hogs, 16 weighing 75 pounds; three weighing 400 pounds; and 16 pigs three weeks old received treatment (serum 254), the remainder of the herd (26) serving as controls. Cholera entered the herd soon after the treatment, causing the death of 11.4 per cent of the treated hogs and 34.6 per cent of the controls.

Herd 6.—This herd was situated in the same neighborhood as the three preceding herds and though free from disease was in close proximity to it. Fifty-seven shoats were injected, each with 40 c.c. serum (255). Forty-one were left untreated as controls. Reports show that this herd escaped cholera, both treated and untreated hogs remaining in normal condition.

Herd 7.—Non-infected. Herd consisted of 42 shoats, average weight 70 pounds. Owner had visited infected herd on nearby farm and did not exercise any precaution against carrying the disease to his herd. Twenty-eight of the 42 hogs received each 40 c.c. of serum (255). Two weeks later one of the controls died, showing lesions of cholera. Three others were sick at the time, and were destroyed. The treated hogs were in a healthy condition, but owner sold them before completion of the experiment.

Herd 8.—This herd consisted of 76 hogs, mostly spring shoats, weighing 60 pounds, which were apparently in healthy condition. Disease present on farm two miles distant. Thirty-eight were treated with serum (225) receiving 40 c.c. each. The owner reports that the herd is still free from cholera.

Herd 9.—This herd consisted of 85 hogs. They had access to water that flowed through badly infected farms, but at the time of treatment they were free from cholera. Forty-two 50-pound shoats received 40 c.c. serum (217); 43 serving as controls. When the farm was visited one month later, all hogs were in good condition. It was reported later that this herd escaped the disease.

Herd 10.—This was an infected herd consisting of 125, 60-pound shoats, three of which were showing symptoms of cholera. Serum (254) was administered to 63. Two weeks later 15.8 per cent of the treated hogs and 19.3 per cent of the controls had died. Owner sold the remainder before definite results could be obtained.

Herd 11.—This was a non-infected herd located one-half mile from cholera. Of the 48 hogs in the herd 29 received each 40 c.c. serum (217 and 234). When hogs reached marketable age, they were sold, the entire herd having escaped cholera.

Herd 12.—This herd consisted of 32 head, 5 sows and 27 shoats. They seemed to be in good health, although across the road, 40 hogs had died from cholera during the previous month. Three sows were treated each with 60 c.c. serum (217 and 255) and 20 shoats each with 35 c.c. of the same lot. Three weeks later 39.1 per cent of the treated hogs and 88.8 per cent of the untreated had died. The disease appeared in the herd a few days after treatment, six of the controls dying before any of the treated ones showed evidence of cholera.

Herd 13.—This herd located one mile from herd 12 was free from cholera at the time of treatment. Hogs on an adjoining farm were dying of the disease at the rate

of 10 per day. Sixty hogs in herd 13 were injected with the experimental serum (255), leaving 77 as controls. Neither the treated hogs nor the controls became infected while they were under observation.

Herd 14.—This was a non-infected herd consisting of twenty-two 60-pound shoats. There was no cholera within a radius of two miles. Sixteen of the 22 hogs received an injection of the serum (255). The entire herd remained well.

Herd 15.—This herd was located two miles from herd 14. Cholera had been present in this herd for three weeks, and of the original number of hogs (165) only 70 survived and practically all of these showed evidence of infection. Seventeen hogs, four sows, and 13 shoats received treatment, with a dosage of 60 c.c. for the former and 40 c.c. for the latter (serum 173). Twenty-eight animals which showed no symptoms were reserved as controls. One month later 5.9 per cent of the treated hogs and all of the controls had died. The serum proved of considerable value in this herd.

Herd 16.—In this herd 17 remained, out of a total number of 80. Infection had been on the premises for one month. Thirteen of the 17 were given 40 c.c. each of serum from experimental hyperimmune 174. The latest report from this herd shows that both treated and untreated hogs are well.

Herd 17.—This herd consisted of 30 hogs, three of which were sick at the time. The well hogs were placed in new pens and 19 of them (shoats weighing from 30 to 75 pounds) were injected with serum (217). Five weeks later 47.3 per cent of the treated hogs were dead and 45.4 per cent of the controls. Most of these hogs died during the week following the injection, indicating that they were developing the disease when treated.

Herd 18.—This herd was located four miles from cholera, but the owner intended to transfer the hogs to an infected farm four weeks later. Twenty-four were treated with serum (217), 45 c.c. being administered to each, and six were kept as controls. A report from this herd six weeks later shows that all of the controls and one of the treated hogs succumbed to cholera.

Herd 19.—This was a healthy herd of 65 hogs. Proximity of disease, one mile. Fifty-one were treated with serum (173 and 177). Of this number 48 were 60-pound shoats and three were sows weighing about 300 pounds each. Serum injected in amounts of 40 c.c. and 75 c.c. Both treated and untreated hogs remained well.

Herd 20.—These hogs were the property of a cattle feeder and had been purchased from neighborhoods where cholera was prevalent. At the time of treatment they appeared to be healthy. Twenty-nine of the 38 hogs received treatment, which consisted of an injection of 40 c.c. of serum (177 and 217). The final results of this experiment show that 88 per cent of the controls and 51 per cent of the treated hogs had died.

Herd 21.—This herd consisted of 16 shoats, averaging in weight 50 pounds. Twelve of these were treated each with 40 c.c. serum (173). These hogs as well as the controls withstood an infection which was present on adjoining farm.

Herd 22.—In this herd of eight hogs only three were treated, two of which had a temperature of 104.5 at the time of treatment. Both of these hogs died. The other treated hog and two controls survived. Serum used, 254. Dosage 40 c.c.

Herd 23.—Infection not present on farm. Treated 24 hogs, leaving three as controls. Serum 254 was used. This entire herd remained well as long as observation continued.

Herd 24.—Five hogs were treated with serum 253 and 255 in doses of 40 c.c. Two controls. Latest report indicates that cholera had not appeared in the herd.

Herd 25.—In this healthy herd of 14 head, 13 received treatment with serum 255. The disease was present on neighboring farm. A report from this farm six weeks later showed that all hogs were healthy.

Herd 26.—This herd consisted of three 50-pound shoats. Two were injected with serum (254) in a dose of 40 c.c. each. Final result, control dead and both treated hogs well.

Herd 27.—Fifteen hogs in herd. Two died a few days previous to the beginning of the experiment. Ten were injected with 35 c.c. of serum (254). Five weeks later the disease had run its course, with the result that two of the control hogs and none of the treated died.

Herd 28.—One hog treated, three controls. Treated hog received 60 c.c. of serum (254). The result was that the treated hog remained well and two of the controls died.

Herd 29.—There was an infection one-half mile from this herd. Ten of the hogs were given the serum treatment, a dose of 70 c.c. being administered (221). The latest report shows that cholera has not entered the herd.

TABLE 15.
SUMMARIZED RESULTS OF FIELD WORK.

Condition of Herds	Number of Hogs Treated	Number Died	Percentage	Number Controls	Number Controls Died	Percentage
Not exposed before or after treatment.	371	0	0	267	0	0
Exposed before treatment and infection evident.	308	99	32.1	191	122	63.8
Exposed, no symptoms before treatment.	87	5	5.7	46	19	41.3
Total.	766	104	504	141

A general survey of the field tests, as well as of those conducted at the laboratory, indicates that the experimental serum used in this work possessed protective properties. Of the 29 herds treated, 13 were infected at the beginning of treatment. The disease was of an extremely virulent type in four of these herds, namely numbers 1, 3, 4, and 12. In the infected herds a total of 308 hogs received treatment and 191 served as controls. The final results show that 67.9 per cent of the treated hogs and 36.2 per cent of the controls survived, which points to the fact that the serum had both a prophylactic and curative effect. In the case of the non-infected herds only three of the 16 became exposed after treatment. In the three herds which became exposed to hog cholera after experimental treatment (herds 5, 7, and 18) the average final result

is expressed by a survival of 94.3 per cent of treated hogs and of 58.7 per cent of the controls.

GENERAL CONCLUSIONS.

The practical use of horse serum virus in the preparation of hyperimmune serum is open to question. The method, in so far as our results show, has two disadvantages:

1. The animals used for hyperimmunization must be kept under treatment several weeks longer than when treated by the original method.

2. A larger dose of the serum, as compared with that which is recommended by those engaged in preparing hyperimmune serum according to the original formula, must be administered.

This work has resulted in further experimental evidence that horse serum virus represents an activated hog cholera virus. It would scarcely appear possible to produce hyperimmune serum as relatively potent as that which has been used in this work, were similar dilutions of the original cholera virus, in physiologic salt solution *in vitro*, used for the purpose of hyperimmunization in substitution for "horse serum virus."