Collected Observations Pertaining to Hog Cholera

L. Van Es

J. F. Olney

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L. Van Es and J. F. Olney
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LINCOLN, NEBRASKA
OCTOBER, 1944
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RESEARCH BULLETIN 135
October, 1944. 5M

The University of Nebraska College of Agriculture
Agricultural Experiment Station
W. W. Burr, Director, Lincoln, Nebraska
I. Salmonella suipestifer in Vaccination "Breaks"

The application of the simultaneous serum-virus method of vaccination against hog cholera, introduced in 1908, established the means by which swine could be adequately protected against this malady. It constituted an outstanding achievement in preventive veterinary medicine. On the whole, it brought safety against one of the most damaging animal plagues. But, as in the instances of other methods designed to bring about an artificially induced immunity, undesirable results had to be reckoned with now and then.

Among these results the ones commonly referred to as "vaccination breaks" proved to be the source of the most disappointment, since in cases where such "breaks" were manifest immunity either failed to be established shortly after vaccination or, later on, after exposure to the infection, hog cholera developed.

Veterinarians are wont to speak of early and late "breaks." It is quite probable that most of the former may be attributed either to a faulty vaccination technique or to the missing of some animals in the larger herds. However, in the cases of a number of these "breaks" such factors could be definitely ruled out.

Although it was recognized that a faulty vaccination could be held partially responsible for disappointing results, other factors also could be suspected. One of these, the possible influence of intercurrent Salmonella suipestifer (the cause of pig typhus) infection deserved consideration. It was frequently observed in swine representing apparent vaccination breaks submitted for autopsy that this microbe could be demonstrated in their various organs. In only a limited number of such cases were lesions encountered such as are common in young pigs involved in pig typhus.

The causative microbe of pig typhus is responsible for a relatively high morbidity rate among juvenile swine and apparently it has an ubiquitous distribution in farm hog herds. It would therefore be difficult to exclude it as a potential contributory factor in the etiology of the so-called breaks.
Most of the cases examined by the Department of Animal Pathology and Hygiene were swine weighing 100–150 pounds. They commonly showed a distinct enlargement of the spleen from which \textit{S. suipestifer} could be recovered with a high degree of regularity. Independent of our own observations, the part played by this microorganism was also suspected by veterinarians, many of whom hesitated to proceed with the vaccination against hog cholera of young swine in herds in which pig typhus seemed to be prevalent.

The rather empirical observations related above served as warrant for an experimental attempt to determine whether or not the influence of intercurrent \textit{S. suipestifer} infection may play a part in causing vaccination breaks. These experiments were undertaken in the course of 1939 and 1940. The work done in 1939 was largely for the purpose of orientation whereas the 1940 attempts were designed to secure data, permitting a more definite conclusion with regard to this vexing problem.

**Salmonella suipesiifer Experiments, 1939**

In the 1939 experiments use was made of 30 young pigs which were secured on 5–25–39. On that date a serum sample of each animal was subjected to an agglutination test in which a recently prepared \textit{S. suipesiifer} antigen was used. All of these samples reacted negatively.

For the purpose of this experiment the pigs were divided in five separate groups of six pigs each, in accordance with the type of treatment to which they were to be subjected.

- **Group I.** Pigs susceptible to hog cholera, to be fed with \textit{S. suipesiifer} contaminated feed and to be vaccinated against hog cholera one (1) month after the last infective feeding.

- **Group II.** Pigs susceptible to hog cholera, to be fed with \textit{S. suipesiifer} contaminated feed and to be vaccinated against hog cholera two (2) months after the last infective feeding.

- **Group III.** Pigs susceptible to hog cholera, to be fed with feed contaminated by the addition of 10 cc. of a broth culture of \textit{S. suipesiifer}—this to be immediately followed by vaccination against hog cholera.

- **Group IV.** Pigs susceptible to hog cholera, each to receive an intravenous injection of 0.5 cc. of a broth culture of \textit{S. suipesiifer}—this to be immediately followed by vaccination against hog cholera.

- **Group V.** Pigs susceptible to hog cholera, but vaccinated against this disease on 5–31–39 and to serve as normal exposure controls later.
Each group was maintained separately from the others until 9–6–39 when all the surviving pigs were transferred to Lot No. 2–4 where on 10–31–39 they were subjected to a natural exposure to hog cholera.

The pigs of Groups I, II, and III were exposed to *S. suipestifer* infection by the contamination of their feed by the addition of measured quantities of broth culture in accordance with the following schedule.

<table>
<thead>
<tr>
<th>Dates of contamination</th>
<th>Amount of culture added</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–31–39</td>
<td>10 cc.</td>
</tr>
<tr>
<td>6–14–39</td>
<td>5 cc.</td>
</tr>
<tr>
<td>6–21–39</td>
<td>5 cc.</td>
</tr>
</tbody>
</table>

Two of the six pigs in Group I died on 6–7–39 and 6–12–39, respectively, with lesions of severe gastro-enteritis. From one of these cases *S. suipestifer* was recovered from the organs, whereas in the other case the bacteriologic findings were negative.

The remaining four pigs of Group I were vaccinated against hog cholera on 7–21–39. One of the vaccinated pigs died of a caseous gastro-enteritis on 7–31–39 and two more pigs died from the same cause three days later. All of the three deaths could be attributed to *S. suipestifer* infection. The last pig of this group, while maintaining a negative blood titer against *S. suipestifer* antigen, survived in a much impaired state of health and marked emaciation. It was killed on 1–5–40. Its organs were found to be normal in appearance and bacteriologically negative.

Group II also sustained a loss of two pigs by *S. suipestifer* infection as a result of the artificial exposure. These animals died on 6–21–39 and 7–15–39, respectively.

The four pigs surviving in this group received their vaccination against hog cholera on 8–21–39. Two more pigs died on 8–31–39 and 9–1–39, respectively. *S. suipestifer* was recovered from their organs and the enteric lesions were found to be quite characteristic of pig typhus. An etiologic influence of hog cholera virus could not altogether be ruled out, as at the time it was not possible to secure susceptible pigs for the capture of the virus.

The pigs belonging to Group III were also exposed to *S. suipestifer* infection in a manner similar to that of the preceding two groups. However, the pigs in this group were vaccinated against hog cholera on 5–31–39, the very day on which the first contamination of the feed took place. One of the pigs died on 6–3–39 showing lymphnode lesions suggestive of hog cholera, although *S. suipestifer* was recovered from the mesenteric lymphnodes. On 6–28–39 and 6–30–39, respectively, two more pigs of
this group died. The autopsies revealed pig typhus and not hog cholera as the cause of death.

The pigs in Group IV were vaccinated against hog cholera on 5–31–39 and simultaneously each received an intravenous inoculation of 0.5 cc. of a broth culture of *S. suipestifer*. All these pigs died of an acute *S. suipestifer* infection in the course of one week, indicating the high, if not exalted, virulence of the culture injected.

The pigs of Group V were destined to serve as controls when all the pigs surviving in the experiment had their immunity challenged by a natural exposure to hog cholera. The six pigs were vaccinated against this disease on 5–31–39. They consistently showed a negative blood titer to *S. suipestifer* antigen and maintained a good state of health to the close of the experiment.

On 9–6–39 all pigs which survived in the experiment were transferred to Lot No. 2–4. There, on 10–31–39, they were severely exposed to direct contact infection from hogs actually sick with hog cholera, without a death from this cause occurring among the twelve (12) thus exposed.

**Salmonella suipestifer Experiments, 1940**

Of the thirty-six (36) pigs used in this investigation twelve (12) were inoculated with a suspension of an attenuated culture of *S. suipestifer* on 5–29–40 in order to determine whether or not such treatment may bring about a degree of resistance to this type of specific infection. Apparently this was not the case.

The whole series of 36 pigs was exposed to *S. suipestifer* infection by the contamination of their feed and drinking water. For this purpose on alternating days 0.5 cc. of a freshly prepared broth culture was added to each of three 500 cc. flasks filled with tap water and thoroughly mixed. These flasks were then emptied into the three troughs at feeding time. The contamination of feed and water was commenced on 7–8–40 and continued until 9–13–40.

Beginning on 6–20–40 three (3) of the twelve *S. suipestifer* vaccinated pigs and six of the ones not so treated were vaccinated against hog cholera at intervals of one month. About three weeks after the serum-virus treatment each group of these pigs was transferred to Lot 2–4 where they were maintained until 10–17–40 when all pigs in the experiment were exposed to hog cholera by the introduction of infected pigs among them.

Of the pigs periodically introduced into Lot 2–4 only one died of hog cholera two weeks after it was vaccinated against this disease. *S. suipestifer* could not be demonstrated in its organs, although the agglutination test with an antigen prepared from
this organism on 6-14-40 revealed a positive blood titer of 1:200. Another pig died of pneumonia on 11-16-40, without presenting evidence of either hog cholera or pig typhus.

Analysis of Results

The results of the 1939 experiments do not reveal evidence to indicate that the *S. suipestifer* infection interfered in any way with the resistance to hog cholera or with the acquisition of immunity aimed at by serum-virus vaccination against hog cholera. The pigs which were exposed to *S. suipestifer* infection and which died from this cause before their vaccination against hog cholera, as well as the ones which succumbed after this treatment, all showed to an equal extent the clinical and pathological phenomena by which pig typhus is characterized.

The bacteriologic examination of seventeen of the eighteen pigs which died in the 1939 experiments consistently disclosed the presence of *S. suipestifer* in their organs. The exception was supplied by a pig which died of a severe enteritis twelve days after partaking of the first feeding on contaminated food and water. The pig typhus microbe could not be recovered from the organs of this pig.

In the course of the 1940 experiments only one of the pigs which were vaccinated against hog cholera died of the disease two weeks after the treatment and four days before the inhabitants of Lot No. 2-4 had been exposed to *S. suipestifer* by means of contaminated feed and water. Consideration should here be given to the fact that the attenuation of the culture from which the vaccine used on 5-29-40 was prepared may not have entirely deprived it of pathogenicity. Such a factor may have had a disturbing influence, although the fact that no other deaths from hog cholera occurred seems to warrant the conclusion that *S. suipestifer* had no part in the death of this pig. Be it further noted that the pig typhus bacillus could not be recovered from its organs.

All the other swine which had been gathered together in Lot 2-4 successfully withstood the natural exposure to hog cholera to which they were subjected between 10-23-40 and 11-13-40 when the last one of the exposure controls died.

It does not appear in the 1940 experiments that the more or less constant exposure to *S. suipestifer* infection was at all sufficient to arrest or to disturb the development of a substantial immunity to hog cholera of swine vaccinated against this malady by the serum-virus method.

On the other hand it seems probable that, after all, *S. suipestifer* can not be ruled out with any degree of finality as a source of
hog yard calamities. It is quite possible that such mischief may be attributed to the exaltation of the virulence of \textit{S. suipestifer} by the simultaneous presence of hog cholera virus, rather than to the cancellation of hog cholera immunity on the part of the pig typhus bacillus.

As indicated by some of the results related above, there can seemingly be no doubt that this microbe is fully capable of causing disaster in swine herds. Hence, there is reason to believe that at least a part of the so-called vaccination breaks was due not to the cancellation of immunity to hog cholera but to the innate capacity of \textit{S. suipestifer} to produce disease with or without the help of the filterable virus.

II. Excessive Serum Dosage as a Possible Cause of Vaccination Breaks

In the course of a study pertaining to the immunity of pigeons against swine erysipelas*, it was revealed that in the group of pigeons immunized by the serum-culture method the quality of the immunity declined from the lower serum potency groups to the ones which had been injected with sera of a higher numerical potency.

A similar phenomenon was observed when the immunity of pigeons which received either minimum or maximum serum doses in the vaccination procedure was challenged. Or, stated in another manner, it appeared that any amount of the specific antibodies in excess of the one required to render the use of the culture-vaccine safe tends to shorten or to impair the immunity of the animals treated. This observation supports the empirically established opinion that no more serum should be injected than is needed to remove any hazard arising from the culture-vaccine inoculations.

These observations suggested that a comparable factor in anti-hog cholera vaccination by the use of serum and virus deserved consideration. It was considered to be well within the range of possibility that the so-called vaccination breaks may come about through the use of excessive serum dosages. Therefore, an effort was made to determine whether or not an excessive dosage of anti-hog cholera serum in simultaneous vaccination could be responsible for unsatisfactory vaccination results. These experiments were undertaken in the course of the years 1941, 1942 and 1943.

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The pigs used in the experiments were divided into four serum dosage groups. They were vaccinated against hog cholera by the serum-virus method. They all received a uniform dose of hog cholera virus but variable doses of anti-hog cholera serum, in accordance with the dosage group to which the pigs belonged. The serum doses for each group of pigs were calculated in accordance with the following schedule:

<table>
<thead>
<tr>
<th>Dosage groups</th>
<th>Cc. of serum per pound body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.20</td>
</tr>
<tr>
<td>B</td>
<td>0.43</td>
</tr>
<tr>
<td>C</td>
<td>0.86</td>
</tr>
<tr>
<td>D</td>
<td>1.72</td>
</tr>
</tbody>
</table>

In the 1943 experiments the A and B groups were omitted.

The serum doses administered to the pigs in dosage Group A were approximately half of the ones recommended for vaccination practice. The amounts of serum injected into the Group B pigs corresponded to the ones used in routine vaccination. The doses administered to the Group C pigs were about double the ones prescribed in practice, and the Group D pigs received a quadruple amount of serum.

The four dosage groups were maintained in separate yards throughout the experiment. Food and water supplies were the same for all groups. At the termination of the season’s experiments, the immunity to hog cholera of the inhabitants of each yard was challenged by the introduction of pigs actually affected with hog cholera.

Serum Dosage Experiments, 1941

The fifty-five (55) pigs destined to serve in the 1941 experiments were procured on 5-23-41. A week later they were vaccinated against swine erysipelas, because one of the pigs had developed an arthritis of doubtful origin and in order to protect the experiments against interference by an intercurrent infection. These pigs were divided into four groups. The ones marked as A, B, and C, were each composed of 14 pigs, and D of 13 pigs.

On 6-25-41 all the pigs belonging to the four dosage groups were vaccinated against hog cholera; and on 10-2-41 all four dosage groups were exposed to hog cholera by introducing into each yard four pigs, two of them infected by hog cholera virus inoculation to serve as infection sources and two pigs not artificially infected to serve as exposure controls. All of these pigs succumbed to hog cholera in from 7 to 17 days after their introductions into the yards, whereas none of the inhabitants of the
yards contracted the disease. Neither did they do so when their immunity was tested again on 11-1-41 by the injection of 5 cc. of hog cholera virus into each of the pigs used in the experiment. The experiment was concluded on 11-24-41.

In the course of 1941 two cases of cholera were recorded—one pertaining to a Group A pig which died 19 days after vaccination and the other to a pig of Group B, which died 35 days after its treatment. Both these pigs were the smallest ones in their respective groups. The one from Group A at the time of vaccination weighed 35 lbs. and received only 7 cc. of serum. The pig which developed hog cholera in Group B weighed only 23 lbs. and was injected with 9.98 cc. of serum. These small amounts of serum were apparently insufficient to cope with the 2 cc. of hog cholera virus used in the vaccination procedure.

The results of the 1941 experiment failed to reveal evidence to indicate that excessive serum dosage in anti-hog cholera vaccination impaired either the development of an adequate degree of immunity or that it materially shortened its duration.

Serum Dosage Experiments, 1942

In the experiments conducted in the course of the year 1942, the procedure was similar to the one applied in the preceding year. Each dosage group was composed of 15 pigs. They were vaccinated against hog cholera on 4-29-42. The experiment was concluded on 10-26-42 when all the surviving pigs were exposed to hog cholera by having introduced among them a number of pigs actually infected with hog cholera virus.

None of the pigs thus exposed contracted the disease. Nor did they do so when their immunity was further challenged by virus inoculations.

Commenting on observations made in the course of the 1942 experiments, it seems quite clear that the serum dosage (0.2 cc. per lb. live weight) administered to the Group A pigs was not a sufficient safeguard against the simultaneously inoculated 2 cc. of hog cholera virus. This indicates that, whereas excessive serum doses may upset vaccination results, underdosing constitutes an even greater hazard.

The observation in Group D of the 1942 experiments tend to show that the excessive amount of serum apparently interfered with the acquisition of immunity by the two pigs which died of hog cholera 42 and 57 days after vaccination.

The 1942 results are summarized in Table 1.
Table No. 1

<table>
<thead>
<tr>
<th>Dosage groups</th>
<th>Died of cholera</th>
<th>Died of other causes</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Serum dose 0.2 cc. per pound live weight</td>
<td>Pig No. 323 324 329 330</td>
<td>Pig No. 319</td>
<td>Aside from the pigs which died of hog cholera, a number also developed the disease but either recovered or remained unthrifty.</td>
</tr>
<tr>
<td>B Serum dose 0.43 cc. per pound live weight</td>
<td>0</td>
<td>334 343</td>
<td>This group excelled all others in gains and thrift.</td>
</tr>
<tr>
<td>C Serum dose 0.86 cc. per pound live weight</td>
<td>0</td>
<td>0</td>
<td>This group, although in constant good health, failed to show the gains observed in Group B.</td>
</tr>
<tr>
<td>D Serum dose 1.72 cc. per pound live weight</td>
<td>364 368</td>
<td>373</td>
<td>The pigs in this group showed a considerable degree of variance in body weight at the conclusion of the experiment. One of the pigs died of hog cholera 42 days and the other one 57 days after vaccination.</td>
</tr>
</tbody>
</table>

Serum Dosage Experiments, 1943

The results obtained in the 1942 experiments warranted a continuation of these studies during 1943. In these efforts dosage Groups A and B were omitted and the investigation continued as Groups C and D.

Twenty pigs were placed in each of their appropriate yards and there kept separate throughout the experiment. On 5–8–43 the pigs belonging to dosage Group C were vaccinated against hog cholera by the injection of 2 cc. of hog cholera virus each, simultaneously with a dose of anti-hog cholera serum at the rate of 0.86 cc. per pound body weight. At the same time, the pigs which composed Group D were likewise vaccinated with this difference, that their serum doses were determined at the rate of 1.72 cc. per pound body weight.

Both groups were maintained on identical rations. On 10–25–43 all survivors in the two yards were exposed to hog cholera by the introduction of pigs recently inoculated with 3 cc. each of hog cholera virus. As an additional challenge to their resistance the pigs, on 12–17–43, were each injected with 2 cc. of hog cholera virus. The summary of results of the 1943 experiments are exhibited in Table No. 2.
Table No. 2

Results of Serum Dosage Experiments, 1943

<table>
<thead>
<tr>
<th>Dosage groups</th>
<th>Died of cholera</th>
<th>Died of other causes</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig No.</td>
<td>Pig No.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Serum dose</td>
<td>0.86 cc. per</td>
<td>493</td>
<td>Pig typhus (1), Peritonitis (1) and Pneumonia and liver cirrhosis (1)</td>
</tr>
<tr>
<td>pound live</td>
<td>497</td>
<td>498</td>
<td></td>
</tr>
<tr>
<td>weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D Serum dose</td>
<td>1.72 cc. per</td>
<td>510</td>
<td>Hypoparathyroidism (1) Pig Typhus</td>
</tr>
<tr>
<td>pound live</td>
<td>513</td>
<td>518</td>
<td></td>
</tr>
<tr>
<td>weight</td>
<td>521</td>
<td>525</td>
<td></td>
</tr>
</tbody>
</table>

From the evidence presented it appears that in the 1943 Group D experiments, hog cholera developed in three of the 20 pigs within 22 to 31 days after the vaccination.

Referring to the results of the 1941, 1942 and 1943 experiments pertaining to serum dosage it was found that the 1941 experiments failed to show any unfavorable influence arising from excessive serum dosage in anti-hog cholera vaccination.

That such an influence may have to be reckoned with was revealed by the 1942 experiments in dosage Group D in which two of the 15 pigs (13.33%) developed hog cholera.

In dosage Group D of the 1943 experiments three of the pigs (15.00%) succumbed to hog cholera. Thus in the dosage Group D populations of the three years during which the experiments were conducted, 5 pigs among a total of 48 animals (10.41%) developed hog cholera in the course of the investigation. It is deemed quite possible that these deaths may be attributed to the use of excessive amounts of anti-hog cholera serum. The death losses in this group of pigs somewhat approximate the rate of breaks reported from the field.

Aside from the cases of hog cholera which, owing to inadequate serum dosage, developed in the dosage Group A, only one other case developed in Groups B and C.

From the results of the experiments, it seems that in anti-hog cholera vaccination practice:

1. Serum dosage at the rate of 0.2 cc. per pound live weight is likely to bring disappointing results.

2. Serum dosage at the rate of 0.43 cc. per pound live weight can be recommended as safe.

3. Serum dosage at the rate of 0.86 cc. per pound live weight may not constitute a hazard, but seems to be a waste of serum.
4. Serum dosage at the rate of 1.72 cc. per pound live weight is not only wasteful but may also be an important factor in disappointing vaccination results.

III. Experiments with Crystal-Violet Hog Cholera Vaccine

A press release by the U. S. Department of Agriculture in December, 1935, entitled, “Tentative Outline of Production of Crystal-Violet Vaccine Against Hog Cholera,” aroused considerable interest in the subject among veterinarians and swine producers. This interest induced this Station to undertake a limited number of experiments in order to determine, if possible, the practical value of the product.

Crystal-violet vaccine was originally prepared in the following manner:

A. The vaccine mixture. With 90 parts of virulent defibrinated virus blood drawn from pigs sick from acute, uncomplicated hog cholera, preferably between the seventh and ninth days, inclusive, after inoculation with virus, is thoroughly mixed with first 10 parts of an aqueous one (1) per cent phenol solution and second five (5) parts of an aqueous one (1) per cent solution of pure crystal-violet.

B. Attenuation. The vaccine mixture is held at 37.5°C. until tests by subcutaneous injection into susceptible pigs fail to produce symptoms of hog cholera. A period of two weeks at 37.5°C. is regularly employed for attenuation. After attenuation, the product is stored in a refrigerator.

After April 1, 1937, the method described above was revised to read as follows:

A. Materials. (a) Defibrinated virulent virus blood, freshly drawn with aseptic precautions from pigs sick with acute, uncomplicated hog cholera induced by the injection of virulent virus. This blood should be drawn from the pigs from the sixth to the eighth days inclusive after injection of virus. (b) Crystal-violet of commercially high purity, equal to “DuPont Crystal Violet Extra Pure.” A 0.5 per cent solution is prepared by thorough digestion with hot water, the substance being somewhat difficultly soluble. The solution should be stored at a temperature not below 20°C. to avoid deposition of crystals. It may be filtered if desired. (c) Anhydrous dibasic sodium phosphate, equal in quality to exsiccated Sodium Phosphate U.S.P. It should be free from moisture, which it readily absorbs from the air, or be freshly dried to constant weight at 110°C. A three (3) per cent aqueous solution is prepared, which should be sterilized by heat and protected against bacterial contamination.
B. *The vaccine mixture.* With aseptic precautions, to 800 cc. defibrinated virus blood (while being stirred) is added 100 cc. of the crystal-violet solution and 100 cc. of the sodium-phosphate solution. The mixture is preserved under aseptic conditions.

C. *Attenuation.* The vaccine mixture is held at a temperature of 37.5°C. for fourteen (14) days, being agitated sufficiently to distribute sediment uniformly, at least once each day. At the expiration of this period the containers are placed in cold storage.

In the pursuit of the experiments two methods of procedure were followed which may be designated as pen experiments and yard experiments.

The pen experiments were carried on with several groups of pigs maintained in small enclosures constructed throughout of concrete. Each group consisted of three pigs, two of which were vaccinated, the third one serving as virus control in order to determine whether or not any actively virulent virus could be transmitted from the vaccinated animals.

After the injections of the vaccine to be tested, the pigs were left undisturbed, with the exception of the recording of body temperatures, for a period of one month. At the conclusion of this period all three pigs in a given pen were inoculated with an appropriate amount of virulent swine blood. The body temperature of each pig was recorded morning and evening, as well as such other observations as may be of clinical import. Each experiment was terminated one (1) month after the virulent inoculations, unless the subjects were eliminated earlier by death.

In connection with the yard experiments, larger groups of pigs were used. The yards concerned were populated early in the season and the occupants appropriately vaccinated with the crystal-violet vaccine, with the intention of subjecting them to a natural exposure to hog cholera toward the close of the season.

A small number of pigs were periodically removed from the yard and their immunity challenged in order to determine its status.

In the earlier experiments the crystal-violet vaccine was prepared in accordance with the original method. In the later tests the revised method of vaccine preparation was followed.

**Pen Experiments**

*Vaccines Prepared in Accordance with Original Formula*

Crystal Violet Vaccine No. 85.231

**Pig tests.** On 8–11–36 (thirty-eight days after the preparation of the c.v. vaccine) Pigs No. 301 and 302 were each injected with 10 cc. of vaccine No. 85.231, leaving Pig No. 303 as virus control in
the same pen. A bacteriologic examination of this vaccine eleven days later revealed the latter to be contaminated by *S. suipestifer*, a factor capable of upsetting the experiment.

On 9–11–36, the three pigs were each injected with 3 cc. of hog cholera virus blood. The following results were obtained. Pig 301. Immediately after the initial c.v. vaccine inoculation, this pig showed conspicuous rise of temperature (Max. 104.4°F.) accompanied by a moderate degree of anorexia lasting about eight days. The hog cholera virus injection was followed by a rise of temperature enduring for 12 days. The subject recovered completely and was discharged on 10–5–36 while in excellent health.

Pig 302 also experienced similar reactions to both inoculations, somewhat milder in character, from which it promptly recovered. Control Pig No. 303 succumbed to hog cholera 17 days after the virus injection.

On 7–12–37 (three hundred and thirty-seven days after the preparation of the c.v. vaccine) Pigs No. 63 and 64 were each injected with 5 cc. of vaccine No. 85.231, leaving Pig No. 65 unmo­lested so that it might serve as virus control later. On 8–13–37 the three pigs each received an injection of 3 cc. hog cholera virus. The following observations were made.

Pig No. 63 was not at all disturbed by the c.v. vaccine inoculation, whereas the virus injection brought about an attack of hog cholera from which it fully recovered after a period of illness lasting about sixteen days. Pig No. 64 showed only a transitory, mild illness after virus injection followed by recovery. Control Pig No. 65 developed hog cholera after the virus injection and died as a result.

In spite of the *S. suipestifer* contamination of the vaccine, the latter proved to be capable of establishing a degree of immunity even after storage of almost one year.

**Crystal Violet Vaccine No. 85.652**

**Pig tests.** On 8–11–36 (twenty-five days after the preparation of the c.v. vaccine) Pigs No. 304 and 305 were each injected with 10 cc. of c.v. vaccine No. 85.652, and Pig No. 306 was left without treatment in the same pen so as to eventually serve as virus control. The vaccine used also proved to be contaminated by *S. suipestifer*.

On 9–11–36 each of the three pigs was inoculated with 3 cc. of hog cholera virus blood.

Pig No. 304. The c.v. vaccine injection was followed by a severe reaction lasting 13 days, during which the body temperature ranged between 104.6°F. and 108°F. with a corresponding degree
of anorexia. At the close of this period of illness, the feces were found to contain shreds of necrotic tissue.

Another febrile period followed the injection of hog cholera virus. On 10–5–36 the subject was destroyed in a state of emaciation. The autopsy disclosed no evidence of hog cholera and S. suipestifer was isolated from its organs. Apparently this animal contracted pig typhus by S. suipestifer injected with the vaccine and this alone was responsible for its condition. Its survival after the virus inoculation may indicate that after all some immunity had developed in this subject.

Pig No. 305. A pyrexia enduring about six days accompanied by inappetence followed the c.v. vaccine inoculation. The hog cholera virus injection provoked a transitory rise of temperature, not accompanied by anorexia. The subject, in a good state of health, was discharged on 10–5–36. Control Pig No. 306 promptly sickened with hog cholera after injection with virus to which it succumbed 17 days later.

On 7–12–37 (three hundred and eighty days after the preparation of the c.v. vaccine) Pigs No. 66 and 67 were each injected with 5 cc. of c.v. vaccine No. 85.652. Pig No. 68 was not injected in order to serve as a virus control in the same pen.

On 8–13–37, Pigs No. 66, 67, and 68 were each injected with 3 cc. of hog cholera virus, as a result the three pigs developed hog cholera and died from this cause.

Whatever protective qualities c.v. vaccine No. 85.652 may have originally possessed, it evidently was lost in the course of a storage period of more than one year.

Crystal Violet Vaccine No. 87.144

Pig tests. On 11–7–36 (twenty-three days after the preparation of the c.v. vaccine) Pigs No. 311 and 312 were each injected with 5 cc. of c.v. vaccine No. 87.144, and Pig No. 313 was left uninjected in order to serve as virus control in the same pen.

On 12–7–36 these three pigs were each inoculated with 3 cc. of hog cholera virus blood. The following results were observed. Pig No. 311 showed a transitory pyrexia and a somewhat reduced appetite after the c.v. vaccine injection. After the virus injections, the subject developed a nine-day febrile period with a slight and transitory anorexia. The pig made a good recovery.

Pig No. 312. The c.v. vaccine injection apparently caused a temperature response and impairment of appetite lasting about three days. The hog cholera virus inoculation precipitated an attack of the disease which terminated fatally eleven days later. In control Pig No. 313 the virus inoculation was followed by a
five day period of fever without loss of appetite. The subject fully recovered.

On 11–7–36 (twenty-three days after the preparation of the c.v. vaccine) Pigs No. 314 and 315 were each injected with 10 cc. of c.v. vaccine No. 87.144, and Pig No. 316 was left without this treatment to serve as virus control in the same pen.

On 12–7–36, Pigs No. 314, 315, and 316 were each inoculated with 3 cc. of hog cholera virus. The following results could be recorded. In Pig No. 314 the c.v. vaccine injection was followed by a rather marked febrile reaction (Max. 106° F.) and a temporary reduction of appetite. The injection of blood virus caused the subject to sicken with hog cholera from which it made a good recovery.

Pig No. 315 showed a rather mild and transitory fever and loss of appetite after the c.v. vaccine injection, whereas the inoculation of hog cholera virus failed to cause even the slightest disturbance. The subject had acquired a solid immunity. As a result of the virus blood injection, control Pig No. 316 developed hog cholera with a lethal termination after two weeks of sickness.

On 2–13–37 (ninety-eight days after the preparation of the c.v. vaccine) Pigs No. 317 and 318 were each injected with 3 cc. of c.v. vaccine No. 87.144, and Pig No. 319 was left without treatment so as to be used as virus control in the same pen.

On 3–13–37 these pigs were each injected with 3 cc. of hog cholera virus. The following results were observed:

Pig No. 317 reacted only to the virus inoculation which brought about a period of pyrexia and a somewhat reduced appetite lasting nine days. Recovery was complete and the subject was discharged on 4–13–37 while in excellent health.

Pig No. 318. The c.v. vaccine injection was followed by a more or less intermittent rise of temperature for about eleven days during which time the appetite was but slightly reduced. After the virus blood inoculation, this animal again developed fever (Max. 106.4° F.) for a period of four days, followed by complete recovery.

Control Pig No. 319 contracted hog cholera after the virus injection which terminated in death seven days after the inoculation.

On 2–13–37 (ninety-eight days after the preparation of the c.v. vaccine) Pigs No. 320 and 321 were each injected with 5 cc. of c.v. vaccine No. 87.144, and Pig No. 322 destined to serve as virus control in the same pen was left without treatment.

On 3–13–37 Pigs No. 320 and 321 and 322 each received an injection of 3 cc. of hog cholera virus. The following observations were recorded.

Pig No. 320 showed a sudden but transitory rise of temperature
(Max. 106.2°F.) on the sixth day after the treatment. The blood virus inoculation precipitated hog cholera with fatal termination on the sixth day after the injection.

Pig No. 321 reacted only to the virus inoculation which caused hog cholera and death on the twelfth day after the injection. Control Pig No. 322 developed hog cholera subsequent to the virus injection and died as a result.

On 2-13-37 (ninety-eight days after the preparation of the c.v. vaccine) Pigs No. 323 and 324 were each injected with 10 cc. of c.v. vaccine No. 87.144, and Pig No. 325 was left uninjected so as to serve as virus control in the same pen.

On 3-13-37, these three pigs were each inoculated with 3 cc. of hog cholera virus, which caused hog cholera and death in all of the three pigs.

On 4-23-37 (one hundred and fifty-two days after the preparation of the c.v. vaccine) Pigs No. 7 and 8 were each injected with 5 cc. of c.v. vaccine No. 87.144, and Pig No. 9 was left without vaccination to serve as virus control in the same pen.

On 5-22-37, the three pigs were each inoculated with 3 cc. of hog cholera virus, resulting in the development of hog cholera and death from this cause in these three subjects.

Note. Apparently c.v. vaccine No. 87.144 was endowed with a moderate degree of immunizing qualities. It is, however, difficult to account for the unexpected results in a series of pigs respectively injected with 3 cc., 5 cc., and 10 cc., of vaccine in which the two pigs which had received the smaller vaccine doses were the only ones which survived the hog cholera virus inoculations.

Crystal Violet Vaccine No. 88.703

Pig tests. On 4-23-37 (fifty-one days after the preparation of the c.v. vaccine) Pigs No. 1 and 2 were each injected with 5 cc. of c.v. vaccine No. 88.703, leaving Pig No. 3 uninjected so as to serve as virus control in the same pen.

On 5-22-37, Pigs No. 1, 2, and 3 were each injected with 3 cc. of hog cholera blood virus. The following observations were made:

Pig No. 1 showed a transitory rise of temperature without impairment of appetite after the c.v. vaccine inoculation. It successfully withstood the hog cholera virus injection and on 6-22-37 was discharged while in a good state of health.

Pig No. 2. The results of both inoculations were identical to those observed in the preceding subject. The latter also was discharged while in excellent health.

Control Pig No. 3 sustained a transitory thermic reaction (Max. 106°F.) without loss of appetite and fully recovered.

On 4-23-37 (fifty-one days after the preparation of the c.v. vac-
COLLECTED OBSERVATIONS PERTAINING TO HOG CHOLERA

Cine) Pigs No. 4 and 5 were each injected with 10 cc. of c.v. vaccine No. 88.703, whereas Pig No. 6 was not treated in order to serve as virus control in the same pen.

On 5-22-37 Pigs No. 4, 5, and 6 were each injected with 3 cc. of hog cholera virus. The following results were observed.

Pig No. 4 revealed brief thermic reactions after both inoculations and remained in good health throughout the experiment.

Pig No. 5. This animal sustained a brief and moderate rise of temperature, not accompanied by anorexia after the c.v. vaccine injection. The virus inoculation failed to cause any disturbance and the subject was discharged on 6-22-37 in a good state of health. Control Pig No. 6 promptly developed hog cholera after the virus inoculation and died of this cause thirteen days later.

On 5-10-38 (four hundred and eight days after the preparation of the c.v. vaccine) Pigs No. 100 and 101 were each injected with 5 cc. of c.v. vaccine No. 88.703, and Pig No. 102 was left uninjected in order to serve as virus control in the same pen.

On 6-10-38, the three pigs were each injected with 3 cc. hog cholera virus. The following developments were observed:

Pig No. 100 reacted to the c.v. vaccine injection with a transitory and moderate rise of temperature without anorexia. The reaction to the hog cholera virus inoculation was more severe and a febrile period of 9 days followed the inoculation, while the desire for food was much reduced. The pig recovered and was discharged while in good health on 7-10-38. Similar results were observed in connection with Pig No. 101.

Control Pig 102 developed hog cholera after the virus inoculation and died from this cause fifteen days later.

Note. C.v. vaccine No. 88.703 was found to possess rather remarkable immunizing qualities, which after a storage of 408 days still protected the pigs inoculated with it.

Crystal Violet Vaccine No. 92.040B

Pig tests. On 9-20-37 (fifty days after the preparation of the c.v. vaccine) Pigs No. 78 and 79 were each inoculated with 5 cc. of c.v. vaccine No. 92.040B, and Pig No. 80 was left without treatment to become the virus control in the same pen.

On 10-20-37, Pigs No. 78, 79, and 80 were each injected with 3 cc. of hog cholera virus with the following results. Pigs No. 78 and 79 failed to show any significant reactions to the two inoculations and were discharged on 11-20-37 while in good health. Control Pig No. 80 developed a very acute hog cholera after the virus inoculation and succumbed to it nine days later.

On 5-10-38 (two hundred and eighty-two days after the preparation) Pigs No. 109 and 110 were each injected with 5 cc. of c.v.
vaccine No. 92.040B, and Pig No. 111 was not treated, being destined to serve as virus control in the same pen.

On 6–10–38, Pigs No. 109, 110, and 111 were each inoculated with 3 cc. of hog cholera virus. The following observations were made on the results.

Pig No. 109 did not react to the c.v. vaccine injection. The virus inoculation provoked a brief thermic reaction (Max. 106.2 °F.) with a slight reduction of the appetite. The subject was discharged on 7–10–38 while in good health.

Pig No. 110 developed an 18-day period of fever after the c.v. vaccine injection (Max. 105 °F.) not accompanied by anorexia. The virus injection was followed by a brief thermic reaction (Max. 105 °F.) The subject was discharged in a good state of health.

Control Pig No. 111 developed hog cholera after the virus inoculation and died 13 days later.

Table No. 3

Summary of results of vaccination against hog cholera by means of crystal-violet vaccine prepared in accordance with original formula including the reactions of non-vaccinated control pigs against hog cholera virus.

<table>
<thead>
<tr>
<th>Number of pigs and their reactions to C.V. Vaccine</th>
<th>Number of pigs and their reactions to hog cholera virus</th>
<th>Cases of hog cholera followed by</th>
<th>Total of pigs surviving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrexia</td>
<td>Anorexia</td>
<td>Pyrexia</td>
<td>Anorexia</td>
</tr>
<tr>
<td>---------</td>
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<td>+</td>
</tr>
<tr>
<td>+</td>
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<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>

Reactions to Virus Injections of Controls

<table>
<thead>
<tr>
<th>Pyrexia</th>
<th>Anorexia</th>
<th>Results of virus inoculations</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>Death Recovery Negative</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>0 2 0 2 13 2 0</td>
</tr>
</tbody>
</table>
Pen Experiments

Vaccines prepared in accordance with revised formula

Crystal Violet Vaccine No. 90.122

**Pig tests.** On 6–4–37 (nineteen days after the preparation of the c.v. vaccine) Pigs No. 10 and 11 were each injected with 5 cc. of c.v. vaccine No. 90.122, and Pig No. 12 was left without treatment so as to serve as the virus control in the same pen.

On 7–6–37, the three pigs were each inoculated with 3 cc. of hog cholera virus. The following results could be recorded:

Pigs No. 10 and 11 showed intermittent rises of temperature, not accompanied by anorexia, between the 5th and 8th day after the c.v. vaccine injections. The virus inoculations were also followed by a transitory period of fever without loss of appetite. These animals were discharged on 8–6–37 while in a normal state of health.

Control Pig No. 12 acquired hog cholera as the result of the virus injection and died ten days later.

On 5–10–38 (three hundred and fifty-nine days after the preparation of the c.v. vaccine) Pigs No. 103 and 104 were each injected with 5 cc. of c.v. vaccine No. 90.122, and Pig No. 105 was not treated being reserved as virus control in the same pen. On 5–18–38 Pig No. 103 died after a febrile period which began two days after the c.v. vaccine injection. The autopsy as well as the bacteriologic examination of the organs failed to reveal the cause of death.

On 6–10–38 Pigs No. 104 and 105 were each inoculated with 3 cc. of hog cholera virus with the following results:

Pig No. 104 showed no reaction to either one of the two inoculations. Apparently it had acquired a substantial immunity.

Control Pig 105 developed hog cholera after the virus blood injection and died from this cause 17 days later.

Crystal Violet Vaccine No. 92.040a

**Pig tests.** On 9–20–37 (fifty-one days after the preparation of the c.v. vaccine) Pigs No. 75 and 76 were each injected with 5 cc. of c.v. vaccine No. 92.040a, and Pig No. 77 was left without vaccination to become the virus control in the same pen.

On 10–20–37 these three pigs were each inoculated with 3 cc. of hog cholera virus. The following results were observed:

Pig No. 75. There was a transitory febrile reaction to the c.v. vaccine injection without impairment of appetite. The virus inoculation caused a thermic reaction persisting during a period of seven days and accompanied by a marked anorexia. The subject recovered and was discharged on 11–20–37 while in a normal state of health.
Pig No. 76 showed some fever without loss of appetite soon after the vaccination. A similar observation could be recorded after the inoculation with hog cholera virus. The subject was discharged in a normal state of health at the conclusion of the experiment.

Control Pig No. 77 developed hog cholera from which it recovered.

On 5–10–38 (two hundred and eighty-three days after the preparation of the c.v. vaccine) Pigs No. 106 and 107 were each injected with 5 cc. of c.v. vaccine No. 92.040a, and Pig No. 108 was left untreated being destined to serve as virus control in the same pen.

On 6–10–38, Pigs No. 106, 107, and 108 were each inoculated with 3 cc. of hog cholera virus. The following developments could be recorded:

Pig No. 106 failed to react to either one of the two inoculations and apparently had developed a solid immunity.

Pig No. 107 sustained a very brief thermic reaction after the vaccination and no disturbance followed the hog cholera virus inoculation.

Control Pig No. 108, as a result of the virus injection, developed a severe attack of hog cholera from which it eventually recovered.

Crystal Violet Vaccine No. 93.434

Pig tests. On 12–7–37 (thirty-two days after the preparation of the c.v. vaccine) Pigs No. 88 and 89 were each injected with 5 cc. of c.v. vaccine No. 93.434, and Pig No. 90 was left untreated in order to become the virus control in the same pen.

On 1–7–38 these three pigs were each injected with 3 cc. of hog cholera virus. The following results were recorded.

Pigs No. 88 and 89 exhibited no significant reactions after each of the two inoculations and were discharged on 2–6–38 while in good health.

Control Pig No. 90 succumbed to hog cholera nine days after the inoculation with virus.

On 5–10–38 (one hundred and eighty-six days after the preparation of the c.v. virus) Pigs No. 112 and 113 were each injected with 5 cc. of c.v. vaccine No. 93.434, and Pig No. 114 was left without vaccination being destined to serve as virus control in the same pen.

On 6–10–38, Pigs No. 112, 113, and 114 were each inoculated with 3 cc. of hog cholera virus. The following results were observed:

Pig No. 112 manifested a brief rise of temperature 48 hours after the c.v. vaccine injection. The virus inoculation caused an
8-day fever, accompanied by a marked, although not complete loss of appetite, from which it completely recovered.

Pig No. 113 failed to react to either one of the two treatments. Apparently the vaccine had conferred a solid immunity.

Control Pig No. 114 sickened with hog cholera after the virus inoculation and died of this disease eight days later.

**Crystal Violet Vaccine No. 94.866a**

**Pig tests.** On 2–28–38 (twenty-six days after the preparation of the c.v. vaccine) Pigs No. 91 and 92 were each injected with 5 cc. of c.v. vaccine No. 94.866a, and Pig No. 93 was left without treatment serving as virus control in the same pen.

On 3–28–38, the three pigs were each inoculated with 2 cc. of hog cholera virus. The following developments were observed:

Pigs No. 91 and 92 showed no reaction to the c.v. vaccine injections, whereas the virus inoculations provoked a mild thermic reaction accompanied by some irregularity of appetite. Both pigs were discharged on 4–28–38 in a good state of health. Control Pig No. 93 developed a mild case of hog cholera after the virus inoculation from which it made a complete recovery.

**Crystal Violet Vaccine No. 96.210a**

**Pig tests.** On 8–13–38 (one hundred and nineteen days after the preparation of the c.v. vaccine) Pigs No. 170 and 171 were each injected with 5 cc. of c.v. vaccine No. 96.210a, and Pig No. 172 was left without treatment so as to become the virus control in the same pen.

On 9–17–38, Pigs No. 170, 171, and 172 were each inoculated with 2 cc. of hog cholera virus. The following were the results:

Pigs No. 170 and 171. The c.v. vaccine injections did not cause any reaction in either one of the two subjects. On the other hand the virus inoculations produced hog cholera in both pigs and caused their deaths from this cause after a period of illness of approximately two weeks.

Control pig No. 172 developed hog cholera as a result of the virus inoculation and died from this cause fourteen days later.

**Crystal Violet Vaccine No. 96.573a**

**Pig tests.** On 8–13–38 (one hundred and one days after the preparation of the c.v. vaccine) Pigs No. 176 and 177 were each injected with 5 cc. of c.v. vaccine No. 96.573a, and Pig No. 178 was left without treatment in order to become the virus control in the same pen.

On 9–17–38 the three pigs were inoculated with 2 cc. of hog cholera virus. The following results were recorded:
Pigs No. 176 and 177 developed a pyrexia (Max. 105.8° F.) which with some intermissions persisted for about 13 days. There were no other physical disturbances. The hog cholera virus inoculations caused hog cholera to which both subjects succumbed after a period of illness of about two weeks.

Control Pig No. 178 also developed hog cholera as a result of the virus inoculation and died from this cause 10 days later.

**Crystal Violet Hog Cholera Vaccine No. C–15.480**
*Expiration date, 12–14–40. Sold under U.S. Veterinary License 52 (limited)*

**Pig tests.** On 6–10–40, Pigs No. 235 and 236 were each injected with 10 cc. of c.v. vaccine No. C–15.480, and Pig No. 237 was left unvaccinated in order to serve as virus control in the same pen.

On 7–10–40 Pigs No. 235, 236, and 237 were each inoculated with 2.5 cc. of hog cholera virus. The following results were obtained:

Pigs No. 235 and 236 failed to manifest any reaction to the c.v. vaccine injections. On the other hand both these subjects responded to the virus inoculations by pyrexia and anorexia, from which after a period of ten and eight days they fully recovered.

Control Pig No. 237 developed hog cholera induced by the virus inoculation and died of this cause seventeen days later.

On 6–10–40, Pigs No. 238 and 239 were each injected with 10 cc. of c.v. vaccine No. C–15.480, and Pig No. 240 was not treated being reserved to become the virus control in the same pen.

On 7–10–40, the three pigs were each injected with 2.5 cc. of hog cholera virus. The following observations were recorded:

Pig No. 238 showed a brief temperature reaction (Max. 107° F.) on the day following the vaccination. The virus injection provoked a 13-day illness marked by pyrexia (Max. 107° F.) and loss of appetite. The subject had apparently sickened with hog cholera but recovered completely.

Pig No. 239 developed a moderate rise of temperature without loss of appetite. The virus inoculation resulted in clinical manifestations of hog cholera from which this animal recovered.

Control Pig No. 240 died of hog cholera seventeen days after the virus inoculation.

**Crystal Violet Hog Cholera Vaccine No. C–19.077**
*Expiration date, 5–13–41. Sold under U.S. Veterinary License 52 (limited)*

**Pig tests.** On 11–18–40, Pigs No. 291 and 292 were each injected with 10 cc. of c.v. vaccine No. C–19.077, and Pig No. 293 was left uninjected in order to serve as virus control in the same pen.

On 12–18–40, the three pigs were each inoculated with 2.5 cc. of hog cholera virus. The following observations were recorded:

Pigs No. 291 and 292 revealed no significant reactions to either
one of the two treatments, having apparently acquired immunity.

Control Pig No. 293, as a result of the virus inoculation, developed a typical case of hog cholera from which it eventually recovered.

On 11–18–40, Pigs No. 294 and 295 were each injected with 10 cc. of c.v. vaccine No. C–19.077, and Pig No. 296 destined to become the virus control in the same pen was not injected.

On 12–18–40, Pigs No. 294, 295, and 296 were each inoculated with 2.5 cc. of hog cholera virus. The following results were recorded:

Pig No. 294 showed no reaction to the c.v. vaccine injection and only a moderate one to the virus inoculation from which it promptly recovered.

Pig No. 295 reacted by a transitory pyrexia (Max. 105°F.) to the vaccine injection and a similar one was induced by the virus inoculation. Recovery was uneventful.

Control Pig No. 296 developed a rather severe and typical case of hog cholera after the virus inoculation but recovered after a fifteen-day period of illness.

Table No. 4

Summary of results of vaccination against hog cholera by means of crystal-violet vaccine prepared in accordance with revised formula including the reactions of non-vaccinated control pigs against hog cholera virus.

<table>
<thead>
<tr>
<th>Number of pigs and their reactions to c.v. vaccine</th>
<th>Number of pigs and their reactions to hog cholera virus</th>
<th>Cases of hog cholera followed by</th>
<th>Total of pigs surviving</th>
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<tbody>
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<td>Pyrexia</td>
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</tr>
</tbody>
</table>

Reactions to Virus Injections of Controls

<table>
<thead>
<tr>
<th>Pyrexia</th>
<th>Anorexia</th>
<th>Results of virus inoculations</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ +</td>
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</tr>
<tr>
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<td>2</td>
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</tr>
</tbody>
</table>
Yard Experiments, 1937

On 6–1–37 fifty-nine (59) pigs were placed in the experimental yard, tagged and each injected with 5 cc. of c.v. vaccine No. 90.122. This vaccine had manifested protective qualities in a pen experiment.

As it was deemed advisable to determine periodically the immunity status by the inoculation of hog cholera virus, four of the pigs taken at random were removed to the stable and there each was inoculated with 2 cc. of hog cholera virus on 7–6–37. The four pigs died of hog cholera 9, 14, 18, and 22 days, respectively, after the virus inoculation.

Another lot of four pigs was removed from the yard on 8–3–37 and placed in an indoor pen where each was inoculated with 2 cc. of hog cholera virus on 8–6–37. These pigs succumbed to hog cholera 10, 12, 15 and 18 days, respectively, after the inoculations with hog cholera virus.

As apparently c.v. vaccine No. 90.122 had failed to confer protection, it was deemed proper to change the original experimental program in order to prevent a needless waste of experimental animals.

In pursuit of the changed plan, the remaining pigs were divided in two lots of 20 pigs each. On 8–17–37 each of the pigs in one lot was injected with 5 cc. of c.v. vaccine No. 92.040a, and the ones of the other group with a similar quantity of c.v. vaccine No. 92.040b.

The immunity of two pigs from each lot was challenged on 9–17–37 by the injection of each with 2 cc. of hog cholera virus. The four pigs were found to be fully resistant to the infection. Similar results were obtained with an additional four pigs, each inoculated with 2 cc. of hog cholera virus.

Exposure to hog cholera under farm yard condition was brought about by the introduction into the yard on 11–3–37 of three pigs immediately after each of them had been inoculated with 3 cc. of hog cholera virus. At the same time four susceptible pigs were admitted to the yard, there to serve as exposure controls. The artificially infected pigs died on the 7th, 22nd and 34th day, respectively, after being inoculated. The four exposure control pigs died of cholera on the 28th, 34th, 36th and 38th day after being exposed to the artificially infected pigs.

At the close of the experiment on 12–29–37, thirty-seven of the forty pigs surviving in the yard had acquired adequate immunity to hog cholera. Three pigs of the forty succumbed to swine erysipelas. This accident could be traced to a pen mate of the seven exposure pigs which, prior to the latter's admission to the
yard, had died of chronic endocarditis. After anti-swine erysipelas treatment, no more pigs were lost.

**Yard Experiments, 1938**

Owing to the fact that the pigs to be used in this experiment proved after the experiment got under way to be involved in pig typhus and tuberculosis, this vitiation of the experiment caused the results to be rejected as unreliable even though 21 of the 55 original inhabitants were saved in an apparently good state of health.

**Notes and Comments**

Although the results of the experiments described above showed that on the whole the products used did not engender an immunity comparable to the one induced by the simultaneous serum-virus method still widely used, there can be no doubt that crystal violet has a decided attenuating influence on the virus of hog cholera. There seems to be good reason to believe that this means of attenuation may eventually lead to the development of a true vaccine, the use of which would result in a stable and constant immunity of pigs treated with it.

Nor can there be any doubt of the elimination of the virulence of the hog cholera virus in the course of the preparation of the vaccine. In the twenty-eight separate pen experiments, the untreated control pigs, which for a period of 30 days cohabited with the vaccinated ones, in not a single instance showed evidence of active virus transmission.

The unfortunate experience with the first two preparations of the series certainly showed the need of preliminary bacteriologic examinations by which the hazard of intercurrent infections, such as pig typhus, swine erysipelas and others may be eliminated. Apparently *S. suípestifer* is not readily destroyed in the process of c.v. vaccine preparation. The fact that the presence of the pig typhus bacillus apparently did not materially interfere with antigenic functions should not prevent such blood from being promptly discarded.

The retention of immunizing qualities of the vaccines used in the experiments seems to be subject to variation. It appears that the c.v. vaccines may be no longer dependable after a storage period of approximately one hundred days, even if some of the vaccines prepared were still valid after storage periods of 282, 283, 337, 359 or even 408 days.

As exhibited by Tables 3 and 4 various degrees of pyrexia and anorexia were often observed to follow the inoculations of c.v. vaccine and hog cholera virus. They were somewhat less marked
for the products prepared in accordance with the revised formula than in those made as directed in the original formula.

Observations made in connection with the yard experiment suggest the possibility that the immunity engendered by the c.v. vaccine may possibly be of a rather transitory character and be completely lost after a given period. On the other hand, if the pigs concerned happen to be exposed to the infection during the period when their resistance is still intact a more enduring resistance could develop as a result of such an exposure.

IV. Experiments with Hog Cholera Vaccine (Tissue Origin)

While the experiments pertaining to crystal-violet hog cholera vaccine were still in progress, another type of anti-hog cholera vaccine appeared on the market. The latter product is prepared from the tissues of swine affected with hog cholera and encouraging results of its application were being reported.

Because of the fact that hog cholera remains as one of the chief causes of swine mortality, pork producers and veterinarians began to make inquiry with reference to the value of this new type of vaccine. In order to be prepared to answer such inquiries, a series of experiments with this product were undertaken. The procedure investigation did not differ from the one followed in the case of crystal violet vaccines.

In the administration of the tissue vaccine the producers' recommendations were adhered to and all samples procured for the purpose were tested before their expiration dates were reached.

Pen Experiments

**Hog Cholera Vaccine (Tissue Origin) No. C-15.479**

Expiration date, 11-29-40. Sold under U.S. Veterinary License 52 (limited)

**Pig tests.** On 5-27-40, Pigs No. 229 and 230 as well as Pigs No. 232 and 233 each received an intramuscular injection of 5 cc. of tissue vaccine No. C-15.479, and Pigs No. 231 and 234 were left uninjected in order to serve as virus controls in each of the two pens occupied by the pigs during the experiment.

On 7-10-40, the six pigs occupying the two pens were each inoculated with 2.5 cc. of hog cholera virus. As a result of the virus inoculation, Pig No. 230 developed hog cholera and succumbed to it thirteen days later. Control Pigs No. 231 and 234 also developed hog cholera and died from this cause after a period of illness of nine days. Pigs No. 229, 232, and 233 were discharged on 8-10-40 while in good health, having apparently acquired a substantial immunity.
**Hog Cholera Vaccine (Tissue Origin) No. C-19.076**

Expiration date, 2–24–41. Sold under U.S. Veterinary License 52 (limited)

**Pig tests.** On 11–2–40 Pigs No. 285 and 286 were each injected with 5 cc. of tissue vaccine No. C-19.076, and Pig No. 287 was left without vaccination to serve as virus control in the same pen. On 11–18–40 these pigs were subjected to an identical treatment.

On 11–2–40 Pigs No. 288 and 289 were each injected with 5 cc. of tissue vaccine No. C-19.076, and Pig No. 290 was not vaccinated, being destined to become the virus control in the same pen. On 11–18–40 the pigs were again treated in a similar manner.

On 12–18–40 each of the six pigs occupying the two pens were inoculated with 2.5 cc. of hog cholera virus with the following results:

Pigs No. 285, 286, 288 and 289 failed to sicken after the virus injections whereas control Pigs No. 287 and 290 developed hog cholera to which they succumbed 18 and 11 days, respectively, after the virus injections.

**Hog Cholera Vaccine (Tissue Origin) No. C-32.476**

Expiration date, 4–10–42. Sold under U.S. Veterinary License 52 (limited)

**Pig tests.** On 1–24–42, Pigs No. 301 and 302 were injected with 10 cc. and 5 cc., respectively, of tissue vaccine No. C-32.476, and Pig No. 303 was left untreated to serve as virus control in the same pen. These inoculations did not provoke any reaction in the two pigs treated.

On 2–24–42, Pigs No. 301, 302 and 303 were each inoculated with 2.5 cc. of hog cholera virus. As a result Pig No. 301 showed a moderately high temperature reaction and some anorexia for a period of about one week from which the subject promptly recovered.

The virus injection into Pig No. 302 induced a rather marked rise of temperature (Max 107.2° F.) which was accompanied by alarming depression and loss of appetite. After a period of illness of ten days' duration, the subject fully recovered.

Control Pig No. 303 developed hog cholera after the virus injection and was killed in a moribund state on 3–24–42.

**Hog Cholera Vaccine (Tissue Origin) No. C-32.477**

Expiration date, 4–27–42. Sold under U.S. Veterinary License 52 (limited)

**Pig tests.** On 1–24–42, Pigs No. 304 and 305 were each injected with 5 cc. of tissue vaccine No. C-32.477, whereas their pen mate Pig No. 306 was not treated so as to serve later as virus control in the same pen. The vaccine injections were not followed by any disturbance of health in the animals treated.
On 2-24-42, the three pigs were each inoculated with 2.5 cc. of hog cholera virus with the following results:

Pig No. 304 developed a thermic reaction of moderate severity, a considerable degree of depression and a marked anorexia, from all of which the subject completely recovered after an illness persisting for about two weeks.

Pig No. 305 reacted more mildly to the virus inoculation, although the subject showed some loss of appetite and a moderate degree of lassitude which endured for a period of five days. The pig recovered completely. Control Pig No. 306 developed hog cholera which terminated in death on 3-9-42.

**Hog Cholera Vaccine (Tissue Origin) No. C–32.478**
*Expiration date, 5–14–42. Sold under U.S. Veterinary License 52 (limited)*

**Pig tests.** On 1–24–42, Pigs No. 307 and 308 were each injected with 5 cc. tissue vaccine No. C–32.478 and their pen mate No. 309 was not treated, being reserved for control purposes in the same pen. The injections did not precipitate any reaction in these two subjects.

On 2-24-42, these three pigs were each inoculated with 2.5 cc. of hog cholera virus which brought about the following results:

Pigs No. 307 and 308 developed sickness of alarming aspects, marked by fever, loss of appetite and depression, from all of which they completely recovered. Control Pig No. 309 succumbed to hog cholera sixteen days after the inoculation with virus.

**Hog Cholera Vaccine (Tissue Origin) No. C–32.479**
*Expiration date, 7–31–42. Sold under U.S. Veterinary License 52 (limited)*

**Pig tests.** On 1–24–42, Pigs No. 310 and 311 were each injected with 5 cc. of tissue vaccine No. C–32.479, and Pig No. 312 was not vaccinated being destined to serve as virus control on a later date. The vaccine injections did not bring about any reaction in the two pigs injected.

On 2-24-42 the three pigs of this group were each inoculated with 2.5 cc. of hog cholera virus after which the following results were recorded:

Pigs No. 310 and 311 developed a rather marked pyrexia, anorexia and depression from which they fully recovered after a period of illness lasting 11 and 12 days, respectively. Control Pig No. 312 developed hog cholera and was killed when near death on 3-24-42.

**Hog Cholera Vaccine (Tissue Origin) No. C–32.480**
*Expiration date, 8–28–42. Sold under U.S. Veterinary License 52 (limited)*

**Pig tests.** On 1–24–42 Pigs No. 313 and 314 were each injected
with 5 cc. of tissue vaccine No. C-32.480, and Pig No. 315 was left without vaccination to serve later as virus control in the same pen. Pigs No. 313 and 314 did not manifest any reaction after the vaccination.

On 2-24-42, Pigs No. 313, 314, and 315 were each inoculated with 2.5 cc. of hog cholera virus, as a result Pigs No. 313 and 314 developed a transitory reaction consisting of some fever and inappetance from which they promptly recovered.

Control Pig No. 315 responded to the virus injection by developing hog cholera to which the subject succumbed after an illness enduring for 18 days.

Yard Experiments

Hog Cholera Vaccine (Tissue Origin) No. C-38.215

Expiration date, 9-6-42. Sold under U.S. Veterinary License 52 (limited)

On 4-29-42, twenty-seven (27) pigs were each subcutaneously injected with 5 cc. of tissue vaccine No. C-38.215 and were then turned into the enclosure reserved for them until the conclusion of the experiment. During their sojourn in this environment the pigs remained in good health and a normal state of vigor until 10-26-42 when the inhabitants of the yard were exposed to hog cholera in the following manner:

On 10-26-42 two pigs susceptible to hog cholera were each inoculated with 2.5 cc. of hog cholera virus and placed in the yard. An additional two susceptible pigs, which received no virus inoculations, were also introduced into the yard to serve as exposure controls.

As a result of this exposure, hog cholera declared itself among the original occupants. The first death from this cause occurred on 11-14-42. When the experiment was concluded on 12-29-42, a total of 23 pigs (85.14%) of the original inhabitants had died of hog cholera.

Hog Cholera Vaccine (Tissue Origin) No. C-45.622

Expiration date, 12-16-43. Sold under U.S. Veterinary License 52 (limited)

In this experiment two groups of pigs (A and B) were utilized, one group being injected with 5 cc. of the tissue vaccine, whereas the pigs of the other group were injected with 10 cc. of the same product—this in order to determine, if possible, whether or not any advantage could be gained by the administration of a larger dose of vaccine. The details of this experiment were as follows:

Group A, on 4-26-43, twenty pigs were each injected with 5 cc. of tissue vaccine No. C-45.622.
Group B, on the same date, twenty-one pigs were each injected with 10 cc. of tissue vaccine No. C-45.622.

The two groups of pigs were maintained in separate yards. Up to the time when all the pigs were exposed to hog cholera infection they remained in good health and made normal gains.

On 10-25-43 the pigs in each enclosure were simultaneously exposed to hog cholera. Two susceptible pigs were injected with 3 cc. of hog cholera virus and with two other susceptible pigs, as exposure controls, were added to each yard. The following results were observed:

Group A (5 cc. vaccine doses), the infective exposure brought about the death from hog cholera of fourteen (70%) of the twenty pigs, which were vaccinated on 4-26-43.

Group B (10 cc. vaccine doses), seventeen (about 81%) of the original vaccinated pigs succumbed to hog cholera as a result of the infective contacts.

In order to challenge the immunity of the ten original inhabitants which survived in both yards each of these pigs were inoculated with 2 cc. of hog cholera virus. They were found to have acquired a solid immunity against the disease.

**Notes and Comments**

In the pen experiments seven (7) different samples of hog cholera vaccine (tissue origin) were subjected to the tests, in which a total of eighteen (18) pigs were utilized for vaccination. The experiments included nine (9) virus controls.

In none of the vaccinated pigs did the injection of the vaccine provoke a systematic reaction. The subsequent virus inoculations caused hog cholera to develop in one pig only, and seventeen (17) or 95% of the treated animals acquired a solid immunity. It is of interest to note that several of the subjects reacted to the virus inoculations, some even to a rather alarming extent (see Table 5). It appears as probable that thirty (30) days after the vaccination, a complete immunity had not yet been fully established or, stated in another manner, that resistance induced by the vaccine developed rather slowly. In only one of the pigs could freedom from reaction to the virus be observed.

The reactions to the virus inoculations consisted principally of rises of the body temperature and although the loss of appetite was also conspicuous, as a whole it was not so complete as the anorexia which is such a marked feature in spontaneous or artificially induced hog cholera. On this point, the difference between that which followed the virus inoculations in the control pigs and in the vaccinated ones is quite illuminating. All of the nine (9) control pigs developed hog cholera accompanied by complete
loss of appetite, even though two of them eventually recovered.

In the yard experiments, samples of two different serial numbers of hog cholera vaccine (tissue origin) were used. They were purchased from a dependable firm and used several weeks before their expiration dates.

As in the yard experiment with crystal violet vaccine, the results in the pigs vaccinated with the tissue vaccine were highly unsatisfactory. The possibility suggests itself that the immunity induced by the two newer vaccines may be of a transitory character disappearing gradually. Successful results may depend on actual exposure to hog cholera during the period when a measure of immunity is still available.

**Table No. 5**

**Summary of observations and results of vaccination against hog cholera by means of a vaccine of tissue origin including the reactions of the virus control pigs.**

<table>
<thead>
<tr>
<th>Number of pigs and their reactions to tissue vaccine</th>
<th>Number of pigs and their reactions to hog cholera virus</th>
<th>Cases of hog cholera followed by</th>
<th>Total of pigs surviving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrexia</td>
<td>Anorexia</td>
<td>Pyrexia</td>
<td>Anorexia</td>
</tr>
<tr>
<td>+ + + 0 + + 0</td>
<td>+ + + 0 + + 0</td>
<td>0 + + 0 + + 0</td>
<td>7 5 5 1 1 8 9 0</td>
</tr>
</tbody>
</table>

**Reactions to Virus Injections of Controls**

<table>
<thead>
<tr>
<th>Pyrexia</th>
<th>Anorexia</th>
<th>Results of virus inoculations</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ + + 0 + + 0</td>
<td>+ + + 0 + + 0</td>
<td>Death</td>
</tr>
<tr>
<td>+ + + 0 + + 0</td>
<td>+ + + 0 + + 0</td>
<td>Recovery</td>
</tr>
</tbody>
</table>

**V. Phenolized Hog Cholera Virus as a Possible Vehicle for Pathogenic Contaminants**

Soon after swine erysipelas became a problem in Nebraska, the fact that the disease mentioned manifested itself in swine recently vaccinated against hog cholera aroused, here and there, the suspicion that the hog cholera virus may have been the source of erysipelas in the pigs concerned. The occasional finding of E.
rhusiopathiae in the blood of swine used by producers for the preparation of the virus to be used in anti-hog cholera vaccination was likewise considered as warrant for investigation. The investigational efforts pertained especially to the determination of the longevity of E. rhusiopathiae and S. suipestifer in phenolized hog cholera virus.

For this purpose the following mode of procedure was followed: 1. The procurement of 90 cc. of non-phenolized fresh virus blood and the establishment of its sterility. 2. The addition of 0.5 cc. of the washings from an agar slant culture of the organism to be studied. 3. The immediate addition of 10 cc. of a 5.0% solution of phenol in physiologic salt solution. 4. The determination of the virulence and growth vigor by the bacteriologic examination and animal inoculation within one hour after its completion.

The mixtures were kept under refrigeration during the course of each experiment. Beginning the day after preparation and continuing every other day, small quantities of the mixture were removed with due precautions in regard to asepsis and subjected to bacteriologic examination as well as challenged for its virulence for pigeons.

This procedure was continued until a number of the tests had consecutively shown negative results. The maximum survival period was expressed by the number of days which elapsed between the date of the first test and that of positive findings by culture and animal inoculation. The results are summarized in Tables 6 and 7.

They showed conclusively that pathogenic contaminants must be reckoned with in the preparation of phenolized hog cholera virus destined to be used in the simultaneous vaccination against hog cholera.

It may be of interest to call attention to the behavior of E. rhusiopathiae and S. suipestifer. Whereas the swine erysipelas bacillus disappeared from the contaminated phenolized virus quite suddenly when the maximum survival period was reached, S. suipestifer frequently showed intermissions of negative findings and the recurrence of positive results in subsequent tests.

This phenomenon may probably be interpreted as evidence that the organisms were gradually dying and that the numbers were reduced to the extent that some of the inocula contained only dead bacilli.

Because of the evidence obtained by the experimental efforts described above, it seemed advisable to challenge the hog cholera virus in general use and to determine to what extent contamination by the two organisms concerned, does or does not prevail in the hog cholera virus of commerce. For this purpose 252 sam-
Table No. 6

Survival periods of E. rhusiopathiae in phenolized hog cholera virus

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>Maximum survival periods (days)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>88.705</td>
<td>32</td>
<td>Culture 87184</td>
</tr>
<tr>
<td>96.573</td>
<td>54</td>
<td>Culture 87193</td>
</tr>
<tr>
<td>90.122</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>90.892</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>92.040</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>93.436</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>94.866</td>
<td>59</td>
<td></td>
</tr>
</tbody>
</table>

Average—43.28 days.

Table No. 7

Survival periods of S. suipestifer in phenolized hog cholera virus

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>Maximum survival periods (days)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>88.704</td>
<td>23</td>
<td>Culture 87.985</td>
</tr>
<tr>
<td>90.122</td>
<td>16</td>
<td>Culture 88.721</td>
</tr>
<tr>
<td>90.892</td>
<td>15</td>
<td>Culture 88.715</td>
</tr>
<tr>
<td>92.040</td>
<td>...</td>
<td>Accidentally miscarried</td>
</tr>
<tr>
<td>92.133</td>
<td>17</td>
<td>Culture 88.715</td>
</tr>
<tr>
<td>93.435</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>94.866</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>96.573</td>
<td>56</td>
<td></td>
</tr>
</tbody>
</table>

Average—20.28 days.

Samples of hog cholera virus, produced by 42 different concerns operating in the United States, were obtained by purchase in the field. The 252 samples included eleven (11) duplicate serial numbers and one sample of which the expiration date had been reached sometime before.

All these samples were subjected to bacteriologic examination and inoculation into pigeons. The results of these tests were quite reassuring, since in not one of the virus samples was E. rhusiopathiae found and from only one of the 252 samples could S. suipestifer be isolated.

Apparently regulatory authorities as well as producers operating in swine erysipelas territory were on the alert in order to keep anti-hog cholera vaccination free from intercurrent infection hazards.