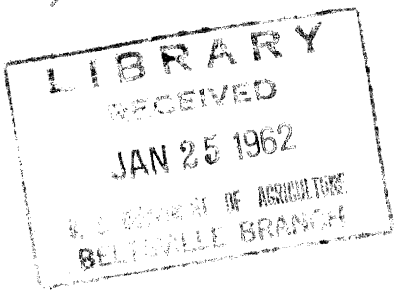

Agriculture Information Bulletin No. 241

HISTORY OF HOG CHOLERA RESEARCH in the U.S. DEPARTMENT OF AGRICULTURE 1884–1960

Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE

Prior to 1954, research work on hog cholera was conducted by the Biochemic and Pathological Divisions of the Bureau of Animal Industry, U.S. Department of Agriculture, Washington, D.C., and at the field experiment station, established first at Sidney, Iowa, and later moved to Ames. Personnel in charge of the work is shown below:

BIOCHEMIC DIVISION

The Biochemic Laboratory was established in 1890 with Dr. E. A. de Schweinitz in charge. On July 1, 1896, the Laboratory became the Biochemic Division.

Dr. E. A. de Schweinitz, Chief, 1896–1904; died Feb. 15, 1904.

Dr. M. Dorset, Chief, 1904–1935; died July 14, 1935.

Dr. J. A. Emery, Assistant Chief, 1904–1935; died July 28, 1935.

Mr. R. M. Chapin, Chief, 1935–1940; died May 6, 1940.

Mr. R. R. Henley, Acting Chief, May–July 1940.

After Mr. Chapin's death, Mr. R. R. Henley was Acting Chief until July 1, 1940, when the Division was abolished and its activities were merged with the Pathological Division.

PATHOLOGICAL DIVISION

Dr. H. W. Schoening, Chief, July 1, 1940, to January 1, 1954.

Mr. R. R. Henley, Biochemist, July 1, 1940, to March 31, 1953.

FIELD EXPERIMENT STATIONS

Hog Cholera Research Station, Ames, Iowa

Dr. W. B. Niles, in charge, 1901–1928; retired 1928.
Dr. C. N. McBryde, in charge, 1928–1942; retired 1942.
Dr. C. G. Cole, in charge, 1942–1952; retired 1952.
Dr. J. P. Torrey, in charge, Jan. 1, 1953 to present.

Hog Cholera Research Station, Live Oak, Fla.

Dr. M. R. Zinober, in charge, Jan. 1, 1956 to present.

In 1942 the Bureau of Animal Industry became a unit of the Agricultural Research Administration. In January 1954 the Agricultural Research Administration was changed to the Agricultural Research Service, and reorganization within the latter Service eliminated the Bureau of Animal Industry.

Since 1954, hog cholera research has been conducted by the Animal Disease and Parasite Research Division of the Agricultural Research Service.

Dr. B. T. Simms, in charge, 1954 to 1956.
Dr. H. W. Johnson, in charge, 1956 to present.

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Washington, D.C. Issued January 1962

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FOREWORD

The subject of hog cholera was closely identified with the United States Department of Agriculture from the time the former Bureau of Animal Industry was established in 1884. Indeed, it was primarily hog cholera, with its ravages to the swine industry, that indicated the need for an organization to study and control animal diseases on a national basis and led to the establishment of the Bureau of Animal Industry.

Dr. D. E. Salmon, the first Chief of the Bureau, became actively engaged in the study of hog cholera, and reported in 1885 that the disease was caused by a bacterium, which was named the "hog cholera bacillus." Immediately following this announcement, experiments were begun to develop a method of treatment or vaccination that would control and prevent hog cholera. This research was based on the belief that the so-called hog cholera bacillus was the causative agent. None of these experiments were successful, although at times the results in the laboratory seemed to encourage the belief that the problem would be solved.

The hog cholera bacillus, which we now know as *Salmonella choleraesuis*, was the principal organism against which these experiments were directed. Because of its close association with the disease, and its pathogenicity under certain circumstances, the significance that was attached to *S. choleraesuis* at that time is readily understandable. Actually the close association of *S. choleraesuis* with hog cholera could still be a fruitful field for study.

Through experiments conducted by Drs. de Schweinitz, Dorset, and their coworkers of the Biochemic Division, it was established that the causative agent of hog cholera is a virus. A method of immunization was developed which has been in use for many years and has been largely responsible for the growth of the swine industry in the United States to the important place it now holds. While the development of the simultaneous virus-serum method of vaccination was a great step forward in the control of hog cholera, certain problems in connection with its use still existed. Research continued, and in later years methods of immunization were developed that avoided the use of live virus.

The research work of the Bureau of Animal Industry was conducted in two Divisions—the Biochemic and the Pathological. In 1940, the Biochemic Division was made a part of the Pathological Division, and the research work on hog cholera from that time was conducted exclusively in the latter Division. The Bureau, from its establishment, had a continuing research program on hog cholera. There was a continuity of thinking from those early research workers—Salmon, de Schweinitz, Dorset, McBryde—through the later workers—Henley, Cole, Dale, Mott, and others of the staff of the Pathological Division. The tireless energy of the early workers and

their careful, conservative evaluation of the work carried down to their latter-day colleagues.

Over the years, the experimental work was conducted at the Hog Cholera Research Station, Ames, Iowa; the Bureau's experimental stations at Bennings, D.C., and Bethesda, Md.; the animal disease station at Beltsville, Md.; and the Bureau's laboratories in Washington, D.C. In 1951 all of the work on this disease was transferred to the Hog Cholera Research Station at Ames.

Hog cholera research activities were expanded in 1955 to include establishment of a pilot plant hog cholera eradication program and testing station at Live Oak, Fla., in cooperation with that State, and the re-establishment of work at Beltsville, Md., to investigate variant viruses, tissue culture-virus adaptation studies, and crystal violet vaccine studies (1955–59).

Hog cholera research work was reported briefly over the years in the Annual Reports of the Chief of the Bureau of Animal Industry and in bulletins and scientific papers. Much of it, however, may not have come to the attention of the scientific world.

This report brings under one cover the hog cholera research work of the Department of Agriculture. Originally, it was intended to be a report of the history of hog cholera research of the former Bureau of Animal Industry from its establishment in 1884 to the end of 1953, when the Bureau lost its identity in the reorganization of the Department. However, hog cholera research has been a continuing function in the Department. An account of the research activities since 1954 is included in an Addendum.

The possibility of eradicating hog cholera in the United States has emphasized the need for further research.

H. W. Schoening, *Assistant Chief*,
Animal Disease and Parasite Research Branch, Agricultural Research Service (Formerly *in charge, Pathological Division, Bureau of Animal Industry*).

H. W. Johnson, *Director*,
Animal Disease and Parasite Research Division, Agricultural Research Service.

CONTENTS

A chronological history of the hog cholera research work in the U.S. Department of Agriculture, as reported in the annual reports of the Chiefs of the former Bureau of Animal Industry, 1903–53, has been printed as a supplement to this report, and is available on request from the Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C.

DATES OF IMPORTANT EVENTS CONCERNING HOG CHOLERA

1833 First hog cholera occurrence as reported by the 1878 Commission.
1860 Division of Agriculture established in the Patent Office.
1862 Department of Agriculture established as an independent agency. First Commissioner of Agriculture appointed.
1878 Commission appointed to study swine diseases.
1884 Bureau of Animal Industry established.
1885 Dr. Salmon discovered an organism which he named *Bacterium suis*, now known as *Salmonella choleraesuis*.
1887 Laboratory to investigate swine diseases established in the Department of Agriculture.
1888 Board of Inquiry appointed to study swine diseases.
1889 Department of Agriculture given executive status. First Secretary of Agriculture appointed.
1890 Establishment of the Biochemic Laboratory.
1896 Biochemic Division established.
1899 Hog cholera eradication experiments continued in Page County, Iowa.
1901 Field experiment station for hog cholera studies established at Sidney, Iowa, with Dr. W. B. Niles in charge.
1903 Discovery of filterable virus.
1905 Hog cholera field station transferred to Ames, Iowa.
1906 Discovery of hog cholera serum announced. Method patented by M. Dorset, and all rights dedicated to the public.
1907 First successful tests of anti-hog-cholera serum on farms.
1908 Demonstration of new hog cholera serum to representatives of 15 States.
1913 Inauguration of inspection of hog-cholera-serum plants, Dr. H. J. Shore, in charge. Hog cholera control work begun in Dallas County, Iowa; Pettis County, Mo.; Johnson and Gage Counties, Nebr.
1914 Field work on hog cholera divided into (1) control work (O. B. Hess, in charge); (2) demonstrational and educational work (U. G. Houck, in charge).
1916 Discovery of methods to clarify serum.
1935 Discovery of crystal violet vaccine. Dr. M. Dorset died July 14, 1935.
1940 Activities of the Biochemic Division transferred to the Pathological Division under the direction of H. W. Schoening.
1942 Bureau of Animal Industry became a unit of the Agricultural Research Administration.
1949 Discovery of variant hog cholera virus.
1951 All of the work on hog cholera was transferred to the Hog Cholera Research Station at Ames, Iowa.
1954 The Agricultural Research Administration was changed to the Agricultural Research Service and reorganization eliminated the Bureau of Animal Industry.

After 1954 hog cholera research in the Department was continued by the Animal Disease and Parasite Research Division of the Agricultural Research Service, at Ames, Iowa. In 1955, the work was expanded to include virus-tissue culture propagation at Beltsville, Md., and hog cholera field trial eradication studies at Live Oak, Fla.

HISTORY OF HOG CHOLERA RESEARCH in the U.S. DEPARTMENT OF AGRICULTURE

By C. G. COLE, R. R. HENLEY, C. N. DALE, L. O. MOTT, J. P. TORREY, and M. R. ZINOBER [1]

Hog Cholera Research in the Former Bureau of Animal Industry, 1884–1953

PART 1. EARLY HISTORY AND RESEARCH WORK

Early History of Swine Diseases

Very little is known about the diseases of swine in the United States prior to 1850. Most of the information now available is contained in the reports of the U.S. Commissioner of Agriculture issued from 1875 to 1878, and in the special reports published by Smith in 1891 (*110*),[2] and by the U.S. Bureau of Animal Industry in 1889 (*119*). The latter report indicates that much of the early information was obtained from replies to circular letters requesting information concerning the first appearance of disease and the health of swine prior to that time. Correspondents, in general, were of the opinion that in the early part of the century the health of swine had been good, and that the swine industry was not then subject to the periodic outbreaks of epizootics that later caused discouraging losses.

Early Outbreaks of Cholera

According to data collected subsequent to 1860, the first outbreak of cholera in this country occurred in Ohio in 1833. It was reported

[1] C. G. Cole, formerly in charge of the Hog Cholera Research Station at Ames, Iowa, retired Dec. 31, 1952; R. R. Henley, formerly Acting Chief of the Biochemic Division, Bureau of Animal Industry, retired March 31, 1953; C. N. Dale, Veterinary Virologist, and L. O. Mott, Head, Viral and Rickettsial Diseases Section, Animal Disease and Parasite Research Division, Agricultural Research Service; J. P. Torrey, in charge, Hog Cholera Research Station, Ames, Iowa; M. R. Zinober, in charge, Hog Cholera Research Station, Live Oak, Fla.

[2] Italic numbers in parentheses refer to Selected References, p. 96.

in South Carolina in 1837, and in Georgia in 1838. By 1845 one outbreak had been reported from each of 10 States, and from 1846 to 1855, 93 outbreaks were reported from 13 States. Between 1856 and 1860, 168 outbreaks were reported from 22 States. The disease continued to spread, and by 1887 cholera had been reported from 35 States.

While the outbreak in Ohio in 1833 was the first reported in this country, early writers suggested the possibility that cholera had occurred prior to that time but was not reported. No official reporting agency existed, and the first statistical data on hog diseases were compiled many years after their first appearance in the United States. Birch (*2*) points out that the first authentic reports of cholera were made when railroads began to operate. He considered this to be significant in that cholera may have existed prior to 1833, but lack of transportation facilities prevented its spread.

Origin of Hog Cholera

It seemed reasonably certain to authorities in this country that hog cholera was brought from Europe, and equally certain to European authorities that it originated in the United States.

Birch's report (*2*) states that an epizootic resembling hog cholera occurred in France in 1822, and similar outbreaks occurred in other parts of Europe prior to 1833.

Hutyra and Marek (*70*) stated: "Hog cholera is supposed to have first appeared in North America in the State of Ohio in 1833, and from there spread over the entire territory of the United States. It was probably carried to Europe in the 60's of the last century."

Law (*75*) stated that the malady had long been known in the Old World, but mistakenly had been placed in the list of malignant anthrax infections.

Report of Early Investigator

In 1858 a disease that was almost unquestionably hog cholera was widespread and was the subject of a report by Sutton (*114*). He reported losses of 11,000 hogs at one distillery plant in Indiana; also heavy losses in New York, Ohio, Illinois, and Kentucky, as well as lesser losses in many other States. Although diseases other than cholera may have caused some of these losses, it seems very likely that hog cholera, as presently recognized, was the chief cause.

Division of Agriculture Established

In 1860 a Division of Agriculture was established in the United States Patent Office and operated as a separate unit in that Office.

By 1861 swine diseases had created national interest and anxiety among swine raisers. Snow (*112*) described the symptoms and lesions of the disease then prevalent, the most important of which he considered to be "inflammation of the lungs, ulceration of the intestines, and pale color of the kidneys." Snow did not consider the disease to be contagious, but felt that it was due to "an epidemic atmospherical poison."

First Commissioner of Agriculture

In 1862 Isaac Newton[3] of Pennsylvania, Chief of the Division of Agriculture in the Patent Office, was appointed the first Commissioner of Agriculture by President Lincoln.

Losses Caused by Swine Diseases

Heavy losses continued to occur throughout the country, and practically nothing was known regarding prevention or treatment of swine diseases.

A preliminary report of the Commissioner of Agriculture in 1877 stated that the value of animals lost during the preceding year, principally from contagious diseases, was $16,653,428, and that approximately two-thirds of that sum was due to losses in swine.

Investigation Commission Appointed

In 1878 Congress appropriated $10,000 to defray the cost of a Commission to investigate the cause and develop remedies for some of the more contagious diseases of domestic animals. Because losses were much greater in swine than in all other animals, the Commissioner of Agriculture deemed it best to use the greater portion of the sum for an investigation of the diseases of swine.

The men appointed by the Commissioner of Agriculture were Drs. N. J. Detmers of Illinois, James Law of New York, D. W. Voyles of Indiana, D. E. Salmon of North Carolina, Albert Dunlap of Iowa, R. I. Dyer of Illinois, A. S. Payne of Virginia, H. N. McNutt of Missouri, and C. M. Hines of Kansas. The researches were to be made in the field in different sections of the country, and various remedies suggested for the treatment of epizootic diseases were to be tried.

The results of the investigations may be summed up briefly: (1) No evidence was discovered to show that more than one disease prevailed as an epizootic; (2) swine disease was found destructive in the most widely separated districts of the country; (3) symptoms and lesions were similar to those already reported by Drs. Sutton, Law, and Snow; (4) there was a preponderance of opinion that the disease was communicable, and that outbreaks were due to contagion; (5) the remedies tested either produced no effect, or were of doubtful value for the treatment of affected animals, or for guarding against contagion.

Extensive reports were made by members of the Commission (*121*) of many experiments on the cause, remedies, transmission to other animals, freezing and drying of blood and tissues, sanitation, disinfection, and many other phases of swine disease.

Detmers (*30*) reported that he found an organism (*Bacillus suis*), which he considered to be pathogenically connected with the disease. Law (*76*) reported that he had transmitted the disease to rabbits, rats, and sheep. Salmon later questioned both of these observations, considering them premature and based on insufficient evidence.

[3] Mr. Newton served until his death, June 19, 1867.

In the light of present knowledge it is of interest to note the following statement made by the Commissioner of Agriculture regarding the transmission of the disease of swine to other animals: "The results of these experiments have convinced Dr. Law, as they must convince others, that the rabbit is itself a victim of this disease, and that the poison can be reproduced and multiplied in the body of this animal and conveyed back with undiminished virulence to the pig. . . . Once infected, the rabbit may carry the disease long distances. . . ."

Another statement by Law was that "freezing does not destroy virulent matter for 1 or 2 months when it is closely packed in dry bran." In his report he frequently referred to the "virus of hog cholera." His statements concerning the preservation of hog cholera virus by freezing, and the transmission of the disease to other animals, are particularly interesting, in view of the knowledge on these subjects that has been obtained in recent years. It appears that Law has not been given credit for his original observations on these two phases of hog cholera research.

Law's report and supplemental report (*76*) were published in the report of the Commissioner of Agriculture for the year 1878. The following statements are quoted from his supplemental report:

> As an addendum to my former report, I would respectfully submit the following further observations on the fever of swine, commonly known as hog cholera. . . .
>
> In two cases I have successfully inoculated virulent products which had been frozen hard for 1 and 2 days, respectively. In both instances the resulting disease was a very violent type, and would assuredly have proved fatal if left to run its course. The freezing had certainly failed to impair the virulence; it had rather sealed it up to be opened and given free course on the occurrence of a thaw; for, once it is frozen, it is manifest that no further change could take place until it was again thawed out, and if it was preserved for 1 night unchanged in its potency, it would be equally unaffected after a lapse of many months, provided its liquids had remained in the same crystalline conditions throughout. In this way undoubtedly the virus is often preserved through the winter in pens and yards, as well as in cars and other conveyances, to break forth anew with the returning spring. This is precisely what we find to be the case with the other fatal animal plagues, the virus of rinderpest, lung fever, anthrax, and aphthous fever, being often bound up through the winter with frozen manure to reappear with undiminished power on the access of warmer weather. This is a matter of no small moment, inasmuch as the long-continued frosts of our northern States prevent any such destruction of the poison as takes place so readily in summer in connection with the alternate wetting and drying and the resulting putrefaction.
>
> I have had instances brought under my notice in which, after the prevalence of the fever in a herd in early summer, new swine were introduced into the open yard a month or two after all trace of the disease had disappeared and had continued to preserve the most perfect health. This is quite in keeping, too, with my failure in the attempts to convey the disease by feeding and inoculating with a semi-putrid intestine. It serves, moreover, to explain my failure, as the exposure and wet at a moderately high temperature would lead in both cases alike to decomposition and destruction.

Of infection of animals other than hogs, Law (*76*) stated his experience as follows:

> I consider the most important part of my researches to be that which demonstrates the susceptibility of other animals than swine to the fever we are investigating. Dr. Kline of London, England, claimed nearly a year ago, that he had conveyed the disease "with difficulty" to rabbits, guinea pigs, and mice, but he gives no hint as to whether he had subjected the question to the crucial test of reinoculation from these animals back upon the pig. This test seemed very important to apply, so that the identity or otherwise of the two diseases

might be determined. I have accordingly instituted experiments on the rabbit, two sheep, a rat, and a puppy, the three former of which have turned out successfully.

In some of Law's experiments it is obvious that his apparently successful results were due to inoculation with contaminated material. In other experiments the indications are that he actually transmitted the virus of hog cholera to rabbits and sheep. His comments on these experiments are as follows:

> It is not surprising that the disease should have been hitherto unrecognized in the sheep and rabbit. The most obvious symptoms in pigs—the pink, purple, violet, or black spots and patches of the skin—were never observed in these animals, unless we can consider the eruption on the ears of the lamb as of this nature. In the sheep, to which alone much attention would be paid, the constitutional disturbance was so slight as to be easily overlooked, the appetite even, and rumination scarcely suffering for a day. . . .
>
> It is no wonder, therefore, that the mildness of the hog-fever in the sheep should have masked its true nature, and that the universal disregard of the disease of the small rodents should have led us to ignore it in these as well.

The descriptions of the disease by the different investigators were very similar, and very much the same as hog cholera is described today. Law was probably the first to call attention to the petechiae, which he stated were common on all internal organs.

Investigations were carried out by one or more of the above-named men until 1883.

Name for the Disease

There was some difference of opinion among the early investigators as to the proper name for the disease. Some suggested swine fever; some, swine plague; and some, hog cholera. Sutton considered the name "hog cholera" to be a misnomer. He believed the disease was a contagious inflammation, not confined to a particular tissue. Law objected to the name "hog cholera" since that term, he said, had only diarrhea to support it, and "that feature is mainly remarkable for its absence."

Establishment of the Bureau of Animal Industry

In his report to Congress in 1869, the Commissioner of Agriculture [4] had strongly recommended the establishment of a Division of Veterinary Surgery in the Department of Agriculture. On May 1, 1883, Dr. D. E. Salmon was called to Washington to head such a Division. An Act of Congress May 29, 1884, established the Bureau of Animal Industry, and Dr. Salmon became its first Chief.

In 1885 he reported the discovery of a micro-organism of hog cholera, which he named *Bacterium suis.*

In 1887 he obtained permission to equip a small room in the attic of the Department of Agriculture building as a laboratory, and to rent a small tract of land in the suburbs for work with animals. This was the beginning of systematic research work by the Bureau on animal diseases.

Early Research

In the 7 years beginning with 1885, a prodigious amount of work was carried out by the Bureau of Animal Industry. Most of this

[4] General Horace Capron, appointed by President Johnson, Nov. 29, 1867.

work was directed by Dr. Salmon, but practically all the laboratory work was done by Dr. Theobald Smith and his assistant, Dr. Veranus A. Moore, with Dr. F. L. Kilbourne in charge of the field station, at that time located in northeast Washington. Although cholera had been prevalent in the United States for many years, very little was actually known about it and other swine diseases when these men began their work. They conducted many experiments and made many important observations regarding the nature of swine diseases. While it is true that they were mistaken in their conclusion as to the causative agent of hog cholera, this does not obscure their many important contributions to the knowledge of swine diseases. The results of their work were published in the reports of the Chief of the Bureau (*117*) beginning in 1885 and continuing through 1892, and in separate publications issued in 1889 and 1891 (*119*, *110*).

The first definite accomplishment of these investigators was the differentiation of the disease so widely prevalent in America from a disease prevailing in Europe known as Rouget. To accomplish this differentiation, Rouget vaccine was obtained from Pasteur and used to vaccinate hogs in this country. All animals so treated died when exposed to hog cholera. At that time the American disease was described as "swine plague."

One of the first organisms studied was *Bacterium suis*, called variously "swine plague bacterium," "hog cholera bacillus," "*Bacterium suipestifer*," and now known as "*Salmonella choleraesuis*" in honor of Dr. Salmon. Work with this organism led to the conclusion that there were two distinct diseases affecting swine in this country. This discovery was announced in the report of the Chief of the Bureau (Salmon, *105*) for 1886, in the following words:

> In view of the results of investigations, which have shown the existence of two distinct infectious diseases in swine, perhaps of equal virulence and distribution, a change in nomenclature became necessary in order to avoid any confusion in the future. Since these two diseases have been considered as one in the past, and the names "swine-plague" and "hog cholera" have been applied indiscriminately, we prefer to retain both names, with a more restricted meaning, using the name "hog cholera" for the disease described in the last report as "swine-plague," which is produced by a motile bacterium, and applying the name "swine-plague" to the other disease, the chief seat of which is in the lungs. This change is the more desirable since recent investigations have shown that the latter disease exists in Germany, where it is called swine-plague ("Schweiniseuche").

Further, two forms of hog cholera were recognized—one an acute hemorrhagic septicemia with ecchymosis in various organs and tissues, particularly in the kidneys; the other, a chronic form, the most characteristic lesions being button ulcers in the large intestines, particularly around the ileo caecal valve.

The organism, now known as *Pasteurella suisepticus*, that produced the pneumonic lesions, to which disease the name swine-plague was assigned, was also isolated and thoroughly investigated.

Early Vaccines

Another early accomplishment was the use of killed cultures of the bacteria now known as *Salmonella choleraesuis* to immunize pigeons against injections of the live organism. This experiment was concluded February 13, 1886, and was the first successful use

of killed cultures to immunize animals. It pointed the way to the development of methods to immunize against typhoid, whooping cough, and other important diseases of man, as well as diseases of animals, such as anthrax and hemorrhagic septicemia. This discovery is considered of fundamental importance, and one of the outstanding contributions of the Bureau of Animal Industry.

Several pathogenic organisms were isolated from the tissues of sick pigs, and studied with respect to their growth characteristics, susceptibility to various disinfectants, and infectivity for various animals. Attempts were made to immunize animals with the various organisms, their products, or both. The work was of great value in later studies of other diseases of animals and man.

Board of Inquiry Appointed

In December 1888, a Board of Inquiry was appointed by Norman J. Colman—the last Commissioner and the first Secretary of Agriculture—to investigate certain contested questions related to the epizootic diseases of swine. The members of this Board were Dr. C. E. Shakespeare of Philadelphia, Chairman, Prof. T. J. Burrill of the University of Illinois, and Prof. B. Mead Bolton of the University of South Carolina.

Conclusions by Board of Inquiry

The conclusions reached by this Board (*109*) were, in part, as follows: (1) There are at least two widespread diseases of swine in this country that are caused by different micro-organisms and that have clinical histories and pathological lesions more or less similar and very difficult to distinguish without the aid of a microscope and resort to bacteriological methods. These two diseases have been fairly well described in the Bureau of Animal Industry reports, except that it does not appear that the "hog cholera" of these reports has its special and exclusive seat in the digestive tract, as distinct from the lungs. The disease called "swine plague" is far less prevalent than the one called "hog cholera." (2) The disease called "hog cholera" by the Bureau is caused by the "hog cholera germ" discovered by Salmon. The epizootic disease called "swine plague" has as its specific cause a certain microbe possessing characteristics which distinguish it both biologically and pathologically from the hog cholera germ. (3) Disinfection as a general practical means of preventing the enormous losses of swine could not be made effective under conditions existing where hog raising is extensive. (4) Treatment of existing cases is utterly futile. Some incomplete experiments strongly indicate that the chemical products of hog cholera germs possess the same power to create immunity as the living germs themselves. (5) The Board earnestly advises that a thorough and exhaustive investigation be made as rapidly as possible to determine if vaccines would be practical.

Serum Prepared From Cultures

After thorough investigation of the two organisms commonly found in diseased swine, attempts were made to produce serums and vaccines for combating them. Cattle, horses, mules, and donkeys

were hyperimmunized by injecting into them cultures of the hog cholera and swine plague organisms, separately and combined. It was found that the swine plague serum would produce some immunity against later infections with the organism; that the serum made from the hog cholera germ would sometimes protect against that organism; and that animals hyperimmunized by cultures of both organisms produced serum that would sometimes protect against infection by both organisms. The potency of these serums was determined by the amount necessary to protect or cure guinea pigs inoculated with hog cholera or swine plague germs sufficiently virulent to kill the control animals within a week or 10 days.

Since, after hyperimmunizing these animals, from 6 to 8 months was required to produce serum sufficiently potent to provide immunity, and because of the irregular results obtained, the serum was not considered practical for general use in the field.

In experiments by the Bureau, in which cultures of *Salmonella choleraesuis* were injected into hogs in an attempt to produce immunity, it was observed that in certain cases the animals receiving injections would be made sick and would sometimes die. Associating with these animals were others that had not received injections and were susceptible to cholera but did not, as would be expected in true hog cholera, contract the disease. It was noted also that some hogs that recovered after being made sick by the cultures, showed no evidence of having acquired immunity, whereas hogs that recovered from true cholera were almost invariably immune.

Smith (*110*), in his special report on swine plague, stated that he had observed many cases among swine in which the animals exhibited all appearances of hog cholera without the bacilli being present in the internal organs. In another outbreak, only hog cholera bacilli were found in some of the affected animals, only swine plague organisms in others, and in still others both kinds of organisms were identified.

These findings failed to suggest to the investigators that some factor other than *S. choleraesuis* might be responsible for hog cholera. Indeed, their conviction that the hog cholera bacillus was the responsible agent was not shaken by their repeated observation that large amounts of cultures of the bacillus could be injected subcutaneously without producing illness.

Biochemic Laboratory

Attempts to protect hogs against cholera by means of vaccines and serums prepared from the hog cholera organism or its products were continued through the 1890's. To aid in this work a chemist, Alexander de Schweinitz, was employed in 1890, at which time the Biochemic Laboratory was established. De Schweinitz studied the hog cholera bacillus and published a number of articles (*22–26*) soon after entering the Bureau.

In 1894 Dr. Marion Dorset, also a chemist, became one of Dr. de Schweinitz's assistants. They found that serum obtained from horses inoculated with either *S. choleraesuis* or *P. suisepticus* protected guinea pigs against the homologous but not against the heterologous culture. They then prepared mixed anti-serum which

protected guinea pigs against both hog cholera and swine plague bacilli. Their findings were similar to those of Salmon and Smith.

Only mixed or polyvalent serum was practical for field use because bacteriological examinations were required to differentiate between hog cholera and swine plague. Such examinations could not, of course, be made in field work with hogs. The success of the work with guinea pigs led to field work with swine.

The Biochemic Laboratory at first served chiefly as an agent for obtaining chemical data relating to problems of investigation by other sections of the Bureau of Animal Industry, but it was found that really effective results could best be obtained by placing in close contact, in the same Division, trained bacteriologists and chemists. On July 1, 1896, the Biochemic Laboratory was made a separate Division of the Bureau and the name was changed to the Biochemic Division. Dr. de Schweinitz became the first chief. After this change the greater part of the research work on hog cholera was directed by that Division of the Bureau.

First Field Experiments

The first field experiments were begun in Page County, Iowa, in 1897. Dr. Dorset was placed in charge. In that year 196 animals were treated with mixed serum and 161, or 82 percent, were saved, whereas in the check herds containing 429 untreated animals, only 15 percent survived. What was considered cholera at that time was present in both the treated and the untreated control herds.

The field work in 1898 was under the direction of Dr. John McBirney. Of 1,727 animals treated that year, 402, or 23.16 percent, died and of 3,197 in the untreated control herds 2,597, or 81.24 percent, died.

The use of mixed serums was continued in 1899. All were prepared at the Bureau experiment station in Washington. About 21,000 hogs were treated, of which about 70 percent survived, whereas about 70 percent of the untreated control hogs died.

Doubts as to Cause of Cholera

The failure of these later, tested-on-a-large-scale, mixed serums to protect, raised doubts in the minds of de Schweinitz and Dorset that so-called hog cholera was caused by the bacillus of Salmon and Smith. These doubts were further emphasized, according to Dorset, by observation that, although the cholera bacillus could infect and produce lesions resembling those of cholera, other characteristics of the natural disease were different. For example, the disease produced by the cultures was only slightly contagious and recovered pigs were not immune, but pigs that recovered from the natural disease, as observed in the field, were permanently immune. Further, he noted that blood from hogs inoculated subcutaneously with the bacillus was not infectious for other hogs, whereas it was known that the real etiological factor of cholera was in the blood of sick hogs.

Discovery of Filterable Virus

Realizing that the disease produced by cultures was different from the natural disease, de Schweinitz and Dorset sought an ex-

planation. They had observed a very small micrococcus in primary cultures from sick pigs, and thinking that this might be the causative agent, they filtered serum to separate the micrococcus from the hog cholera bacillus. The filtrate was found to be free from bacteria, yet produced cholera when injected into susceptible pigs, and had no effect on rabbits and guinea pigs. It was then realized that the disease was caused by a filtrable virus, and that hog cholera and swine plague organisms were only secondary invaders.

De Schweinitz and Dorset (*27*) reported their epoch-making findings with considerable caution, as follows:

> There is an infectious disease among hogs in this country which cannot be distinguished clinically from hog cholera, and which may be reproduced by infecting with material which contains no hog cholera bacilli.

The authors, both chemists, exercised restraint in making their announcement because the leading bacteriologists of that time had, for many years, accepted the hog cholera bacillus as the cause of hog cholera. Although Circular 41, announcing the fundamental discovery, was published in September 1903, the Annual Report of the Bureau of Animal Industry, 1903—the formal report of progress by the Chief of the Bureau for that year—merely states, "Work on hog cholera is being continued." No mention was made of de Schweinitz's and Dorset's discovery. Later, Salmon (*106*) made the following statement:

> On account of the often discordant results which were secured . . . when the Bureau was treating diseased hogs with serum from animals which, in their turn, had received large and repeated doses of hog cholera and swine plague cultures, it appeared that some other factor must be considered in the efforts to produce immunity.

Salmon was one of the first to accept the evidence produced by de Schweinitz and Dorset (*28*) although he had previously criticized de Schweinitz for questioning the possibility that hog cholera bacillus was not the cause of hog cholera.

Advance in Research

The discovery that hog cholera was not due to the hog cholera bacillus ended the period of doubt and misunderstanding and opened the period of advancement in knowledge, not only of the cause but also of the transmission, treatment, and prevention of hog cholera, that has continued to the present time. After the discovery that hog cholera could be produced by a filtrable virus, the research work was largely devoted to studies of the blood of sick hogs.

Death of Dr. de Schweinitz

On February 15, 1904, Dr. de Schweinitz died, and Dr. Dorset was appointed Chief of the Biochemic Division. During the next few years numerous experiments were conducted to study the filtrable virus, and the relation of the hog cholera bacillus to diseases of swine.

Filtration Experiments

The results of many filtration experiments conducted by the Bureau were published in 1905 (*47*):

(1) The results of the subcutaneous injections of hog cholera blood filtered through Chamberland and Berkefeld cylinders show that these injections produced disease quite as uniformly as those with the unfiltered blood. On the whole, it is true that hogs inoculated with the unfiltered blood became sick more quickly after inoculation than the hogs inoculated with the same serum after filtration, but this was not always the case. . . .

It is not surprising, however, that the bloods after filtration should show less potency than blood before filtration; for, whatever the nature of the infectious agent, it is but natural to suppose that some of it is removed by the filter.

(2) Our experiments have shown that pure cultures of *B. choleraesuis* injected subcutaneously into hogs usually produce but slight disturbance, although after intravenous injections or feeding a severe illness frequently results. The disease produced in this manner may present the symptoms and lesions seen in acute hog cholera, but does not possess the characteristics of contagiousness nor of infectiousness of the blood; nor are those hogs which have recovered from such illness immune when exposed subsequently to the natural disease.

(3) While our experiments establish beyond question that the filtrable virus was present in all the outbreaks of hog cholera studied experimentally by us, it is also true that *B. choleraesuis* was present almost as uniformly.

(4) The exact role played by *B. choleraesuis* in outbreaks of acute hog cholera is difficult to define. That the fatal result in many instances is materially influenced by the presence of that organism cannot be doubted, and, in addition, we would emphasize the fact that although the filtrable virus appears to have been the primary invader in those cases of acute hog cholera which we have studied, we do not deny the possibility of independent disease being caused by *B. choleraesuis*. . . .

(5) The fact that *B. choleraesuis* was found in a large proportion of the cultures taken from hogs inoculated with filtered serum seems to indicate strongly that that organism has its normal habitat on or in the bodies of healthy hogs, and that in the case of lowered resistances on the part of the hogs, this organism enters the circulation. . . .

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

The bloods used in these experiments came from eight outbreaks of natural disease. The outbreaks occurred at different times in different parts of Iowa and in Maryland.

In all, 24 filtration experiments were carried out, and the filtrates were found to be free from *Salmonella choleraesuis* by culture tests in all experiments and by inoculation of rabbits and guinea pigs in most cases. Ninety-seven pigs were used to test the filtered and unfiltered blood. Only three pigs remained normal. *S. choleraesuis* was usually found in the blood or tissues of all hogs that died, whether filtered or unfiltered blood had been injected.

Confirmation of Work of de Schweinitz and Dorset

The announcement by de Schweinitz and Dorset that hog cholera was caused by a filtrable virus did not meet with ready acceptance by some workers. However, their findings were confirmed later by research workers in this country and abroad.

Among foreign investigators who confirmed their work were Ostertag and Stadie of the Hygienic Institute of the Veterinary High School of Berlin, Uhlenhuth and coworkers of the Imperial Board of Health of Berlin, Hutyra of Austria, Stockman and McFadyean in England, Theiler in South Africa, and Carré, LeClainche, and Vallée in France. It was confirmed in the United States by McClintock, Boxmeyer, and Siffer, among others.

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Further Filtration Experiments

Although the filtration experiments by the Bureau were generally accepted by scientific investigators throughout the world, certain criticisms had been made. In 1907, Lourens (*78*), Subdirector of the State Serum Institute at Rotterdam, published an article on the filtrability of *Bacillus suipestifer* in which he said that the so-called hog cholera bacillus, *B. choleraesuis* or *B. suipestifer*, was capable of passing through filters of the Chamberland and Berkefeld types composed, respectively, of unglazed porcelain and infusorial earth. He claimed that the ability of *B. choleraesuis* to pass through filters of the types mentioned was due to a property which this bacillus possesses of breaking up into granules sufficiently small to pass through the pores of the filter. He also asserted that *B. choleraesuis* was the cause of hog cholera, and that none of the investigators who had conducted filtration experiments to show that hog cholera was due to a filtrable virus had afforded sufficient and convincing proof that the filtrates they employed did not contain *B. choleraesuis*.

While there was no doubt as to the accuracy of the early filtration experiments, or that hog cholera was due to a filtrable virus, the Bureau decided to conduct additional experiments to combat these criticisms.

These experiments were reported by McBryde (*79*). They confirmed the earlier experiments and brought out the following conclusions:

(1) That the Pasteur-Chamberland filters F and B effectually prevent the passage of *Salmonella choleraesuis*.
(2) That the smaller Berkefeld laboratory cylinders vary in permeability.
(3) That certain of the Berkefeld laboratory cylinders will prevent the passage of *S. choleraesuis* when a limited amount of material is filtered.
(4) That the granules noted in cultures of *S. choleraesuis* have no significance in filtration experiments with this organism.
(5) That in the filtration experiments described in Bulletin 72 the filtrates employed did not contain *S. choleraesuis*.
(6) That hog cholera is due to an ultra-visible virus sufficiently small to pass through the pores of the Chamberland filters.

Granules were found in the cultures and in filtrates but did not develop in the filtrates nor in cultures made from the filtrates. The granules were incapable of development in the bodies of rabbits or hogs. It was found that organisms occasionally pass through unglazed filters (Berkefeld) but not through glazed bougies (Chamberland).

Part 2. Anti-Hog-Cholera Serum: Discovery, Production, and Research

Discovery of Anti-Hog-Cholera Serum

After it was determined that the filtrable virus was the cause of hog cholera, the work of the Bureau was directed toward a means of protecting hogs from invasion by the virus. To facilitate this work a field station was established at Sidney, Iowa, in 1901, with Dr. W. B. Niles in charge. In 1905, the station was moved to Ames,

Iowa, where it is still maintained. By far the greater part of the Bureau's research work on hog cholera was carried on there.

Attempts were made to develop a vaccine (1) by attenuating the disease-producing blood in liquid or dried form, and (2) by using a mixture of virulent blood and the blood from immune hogs. The vaccines so prepared were in some cases successful, but in some cases pigs given injections developed cholera. In other cases the pigs remained well after treatment, but succumbed when later exposed to cholera. In this connection, Dorset, McBryde, and Niles (*54, p. 7*) made the following statement:

> The efforts to attenuate the virus in blood from diseased hogs by drying, by heat, and by chemical agents, have not led to satisfactory results. It may be that later investigations will show that it is possible to obtain a satisfactory vaccine for hog cholera by attenuating the virus, but our own results were so discouraging that efforts in this direction were abandoned, and our entire efforts turned to the production of a serum. . . .

The First Hyperimmune Hog

The first attempt to produce a potent, anti-hog-cholera serum was begun by injecting into a hog that was known to be immune, disease-producing blood in increasing doses, the object being to impart additional protective power to the blood of the immune hog—or hyperimmunize him (*54, p. 7*). It had been shown previously that blood from an ordinary immune hog—one which had not been hyperimmunized—possessed very little, if any, protective power.

The first attempt to produce a potent anti-serum in the blood of a hog hyperimmunized with large doses of blood from sick hogs indicated that such a method was possible. The first hog was hyperimmunized in 1903. This hog, No. 844, which had recovered from an attack of hog cholera, was first given an injection of 1 cc. of hog cholera blood; then the dosage was gradually increased to 400 cc. During this time the hog remained normal. Eleven days after the last injection of disease-producing blood, it was bled from the tail, and the defibrinated blood was tested for potency. One pig was given an injection of 20 cc. of No. 844 blood alone; another was given 20 cc. of No. 844 blood and 1 cc. of disease-producing blood. Both pigs remained normal and were exposed to cholera 5 weeks later. The pig that received 20 cc. of No. 844 blood alone, died; the one that received 20 cc. of No. 844 blood and 1 cc. of disease-producing blood remained normal. Hog No. 844 was therefore considered a hyperimmune hog, and the defibrinated blood from such hogs was thereafter referred to as anti-hog-cholera serum.

Hog No. 844 was again given several injections of disease-producing blood ranging from 400 to 490 cc. Blood was again drawn from the tail and tested from time to time for potency, or its ability to protect against hog cholera. It was found that the serum from hyperimmune hogs was capable of protecting susceptible swine against virus administered simultaneously, but failed to protect against virus administered several weeks after treatment with serum. In other words, serum alone produced immunity of short duration, but when administered simultaneously with hog cholera virus, it produced lasting immunity.

In the spring of 1905, after the experiment station was moved to Ames, this work was continued, in an effort to find the best means

of producing potent and dependable antiserums such as that afforded by hog No. 844.

Serums were prepared from strains of virus obtained from outbreaks of cholera at Schribner, Nebr., and at Syphax, Va. (*54, p. 19*). Thirteen hogs were hyperimmunized with these strains of virus, and serum was obtained from them; also from two immune hogs that had not been hyperimmunized. These hogs were bled and the serums were subjected to a series of tests for potency. The bloods of the hyperimmunes afforded adequate protection, but the bloods from the immune hogs gave only slight protection. The results of the tests of serum obtained from hyperimmune hogs caused a great deal of interest among the men who had, after many years of experimentation, devised a method by which hogs could be immunized against the dreaded hog cholera.

Although it appeared certain that the serum would immunize hogs against cholera, many similar experiments were conducted to confirm this observation.

Methods of Hyperimmunizing

Two methods were used to hyperimmunize hogs for serum production; one was called the slow, and the other the quick, method. In the slow method, which was used first, immune hogs were given injections subcutaneously with virus blood three times at intervals of 10 to 14 days. The amount injected was first 1 cc. per pound of body weight of the immune hog, then 2½ cc., then 5 cc. The quick method consisted of injecting 10 cc. of diseased blood per pound of body weight all at one time. In about 3 weeks after hyperimmunizing, blood was drawn from the tail and tested for potency or ability to protect susceptible pigs.

Only the first bleeding was used in the first experiments. The blood was defibrinated and mixed with sufficient carbolic acid to give 0.5-percent concentration to the finished serum.

Repeated experiments were conducted at Ames, and the following time-proven conclusions drawn (*54*):

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

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

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

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

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

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

(7) Immunity in hogs treated by the serum-virus vaccination lasts 3½ months and probably longer.

(8) In serum-virus vaccination it is not necessary to produce illness in the vaccinated hog in order to secure immunity lasting for at least 3 months.

(9) If a sufficient dose of serum is given, hogs are not injured by the serum-simultaneous vaccination.
(10) Hogs vaccinated by the serum-virus method do not communicate disease to susceptible pigs unless they themselves become sick.
(11) Serum administered alone may not furnish complete protection for a longer period than 3 weeks.
(12) The serum probably can be used successfully as a curative agent if administered in the early stages of the disease.

This experimental work was done in the early stages of the development of anti-hog-cholera serum, but the conclusions have proved to be substantially correct after nearly half a century.

The process of producing anti-hog-cholera serum was patented by Dorset (*33*), and the patent was dedicated to the public.

First Farm Experiments

Prior to 1907 all experimental work with the new hyperimmune serum was conducted at the Bureau experiment station at Ames. The results of the first experiments were so uniformly satisfactory that arrangements were made to conduct experiments on farms under varying conditions of feeding and management.

In the early fall of 1907, 47 herds containing approximately 2,000 head were selected for treatment, some with serum alone and some with serum and virus. The sera used were prepared in the manner previously described except that the hogs were bled four times at weekly intervals after hyperimmunization, and the different bleedings were mixed and tested for potency.

Field tests were planned to obtain information on three important points:

(1) What could be accomplished toward diminishing losses in a herd in which hog cholera had appeared?
(2) What could be gained by treating a healthy herd after it had been exposed to the disease, but before any of the pigs became sick?
(3) Could the spread of hog cholera be arrested by treating all healthy herds on farms bordering on the center of infection that had not been exposed to the disease at the time of treatment?

A number of untreated pigs were left in each herd to serve as controls. Details of these experiments, and the results and conclusions were reported by Niles (*98*) as follows:

RESULTS

In a majority of the healthy herds which had not been exposed at the time of treatment with serum and virus, the disease did not appear in either the treated or the untreated controls. In a few of these herds, hog cholera appeared among the controls some weeks later. The average loss in such herds was 68 percent of the controls, and none of the treated pigs died. In the exposed herds, 89 percent of the controls died and only 4 percent of the treated pigs died. In the infected herds, 75 percent of the controls and 13 percent of the treated pigs died.

CONCLUSIONS

(1) The serum of properly hyperimmunized hogs, when administered in sufficient doses, will protect nonimmune hogs of all ages against hog cholera in the following doses: For suckling pigs, 10 to 15 cc.; for shoats weighing from 30 to 200 pounds, 20 to 30 cc.; for old hogs, 40 to 60 cc.
(2) Healthy herds treated by the simultaneous method are rendered immune against hog cholera.

(3) In herds recently infected, where only a few have become sick, nearly all losses may be prevented by the use of serum alone or the simultaneous method, but the duration of immunity may be longer when the simultaneous method is used.

(4) In badly infected herds, a number of animals may be saved by the serum, the number saved depending upon the extent of the infection.

(5) By prompt use of serum in infected herds and the prompt vaccination of surrounding herds, hog cholera may be quickly stamped out when it first appears in new territory.

(6) The treatment of healthy shoats by the simultaneous method rarely causes the appearance of disease, and should it do so, it may be quickly controlled by use of serum alone.

After experiencing losses up to 100 percent from cholera in past years, herd owners considered it almost miraculous when 50 percent or more of their hogs could be saved by this new treatment.

Conference on Practical Application of Serum

A conference of veterinarians was held at Ames, Iowa, on May 30, 1908, to discuss the practical application of the new serum and virus treatment for immunizing swine.

State and Federal representatives agreed unanimously that the serum should be prepared by each of the States for distribution to hog raisers. There appeared to be sufficient evidence to justify a request for State aid, and State representatives expressed their intention to undertake the work as soon as funds could be obtained.

In view of the publicity given in the press, it was recognized that "quack" vaccines might be placed on the market, and would need to be guarded against.

Question was raised about the production of serum by private individuals, to which Dr. Dorset replied that the production of serum was free to all individuals.

As a result of this and other conferences, serum production was begun by States and individuals, and eventually developed into a very important industry.

Demonstrations for the Public

Following these practical experiments, the Chief of the Bureau of Animal Industry, Dr. A. D. Melvin, invited representatives of the important hog-raising States to visit the experiment station at Ames to observe methods of producing and using serum and virus. The station immediately became a Mecca for representatives of the swine industry and for State control officials.

In the Annual Report of the Chief of the Bureau for 1909, it was stated that scientific workers in Germany and Hungary had confirmed the effectiveness of the hyperimmune serum developed by the Bureau for combating hog cholera, and that the serum was on sale in Germany. Eleven States in this country had appropriated money for starting serum-producing plants, and others would do so in the near future. It was also noted that 25,000 hogs had been vaccinated.

The demand for information and for public demonstrations increased. Publications of the Bureau of Animal Industry contain records of demonstrations held to acquaint the public with the efficiency of the serum treatment. One conducted at South Omaha,

Nebr., is described here to acquaint present-day readers with the kind of demonstration conducted. The Nebraska Swine Breeders' Association and the leading swine breeders of the State obtained the services and cooperation of the Bureau of Animal Industry and the Union Stock Yards Company of South Omaha to conduct this demonstration. The latter furnished all necessary facilities, including the animals.

The South Omaha Stock Yards was selected as the best location because it offered the most convenient facilities for swine breeders, farmers, stockyard officials, and veterinarians to observe the demonstration.

The demonstration was conducted by Dr. W. B. Niles, assisted by Dr. H. J. Shore, and was started on July 23, 1910. For this demonstration, 30 pigs were purchased from Mr. Jacob Armbrust, a farmer living west of Omaha, who reported that there had been no cholera on his farm during the previous 3 years. Four of the pigs were brought to the stockyards and given injections of hog cholera virus. They all developed cholera about the fifth day and eventually all died. At this time (the fifth day) the other 26 pigs were delivered. Eighteen were given injections of serum alone and 8 were left as controls and placed in the pen with the sick pigs. The 8 untreated controls developed cholera; 2 were stolen at night while sick, and the other 6 died. The 18 serum-treated pigs remained normal after exposure to 12 sick pigs. During the course of the experiment many persons visited the pen and observed the progress of the test.

The number of Middle Western States undertaking the production of serum increased, and by 1913 serum was produced on a large scale in Ohio, Indiana, Missouri, Kansas, Minnesota, Michigan, North Dakota, and California. Iowa, Nebraska, Illinois, and Kentucky were soon added to the list. Later, when commercial serum plants had increased in number and were being operated on a sound basis under Government supervision, the States abandoned serum production. Commercial serum production continued to increase until, in 1951, it had reached 1,762,053,000 cc.—sufficient to vaccinate approximately 50 million hogs in a single year.

The importance of the discovery of a method of producing long-time immunity cannot be overestimated. Prior to this time, if hog cholera broke out in a herd, practically nothing could be done except bury or burn the hog carcasses. Many so-called cures or preventives were sold and used, but tests of many of these products by the Bureau of Animal Industry and other agencies proved that they were of no value.

By the use of serum alone, or serum and virus simultaneously, many hogs in herds where cholera was present could be saved. The earlier the treatment, the more hogs could be saved by either the serum-alone or the simultaneous treatment. Experiments indicated that if hog cholera was the only disease affecting the herd, results of treatment were no different regardless of whether the serum-alone or the simultaneous method was used. A distinct advantage of the simultaneous method over the serum-alone method, even in sick herds, was that the disease was less likely to recur. While the results of early experiments were considered satisfactory, they probably would have been still more so if larger doses of serum had been used. The question of dosage will be discussed later in more detail.

Area Immunization of Swine

During 1912, State officials showed increased interest in the possibility of combating hog cholera by the methods of immunization developed by the Bureau. Thirty States began to distribute serum—either manufactured by the State or purchased from commercial sources—to State officials, veterinary practitioners, and, in some cases, to farmers.

Accurate statistics on the use of serum and virus were not obtainable, but replies to inquiries indicated that more than 1 million doses of serum had been used by the end of 1912, and that results were usually satisfactory.

The Bureau's appropriation for the fiscal year 1913–14 contained a provision that "the sum of $75,000 be set aside for demonstrating the best methods of preventing and eradicating hog cholera." Therefore, it was necessary to organize a force of trained inspectors, to increase facilities for serum production, and to plan a campaign for demonstration.

The plan was developed, the organization was formed, and several areas in important hog-raising States were selected to undertake the control of hog cholera. Veterinarians were selected and sent to Ames for training in the production and use of serum and virus. The work was carried out with the cooperation of State colleges, State Live Stock Sanitary Boards, and State veterinarians. State officials were largely responsible for educational work and the enforcement of necessary livestock sanitary regulations. The serum and virus were administered by Bureau veterinarians.

The counties selected for the work were Dallas County, Iowa; Pettis County, Mo.; Montgomery County, Ind.; and Gage and Johnson Counties, Nebr. Work was started in three of these counties in the summer and fall of 1913. The results in 1913 in the three counties were as follows:

In herds in which 70 percent of the pigs were sick, 16,152 pigs were vaccinated; of these, 3,930, or 24.3 percent, died. This was a remarkable demonstration of what could be accomplished when control work was organized and treatment applied in the early stages of the disease. In herds where sick hogs were present but only the well ones were treated, of 11,776 head only 343, or 2.9 percent, died. In healthy herds on farms adjoining infected premises, 13,578 pigs were treated and 81, or 0.5 percent, died. After the work was well organized in these three counties, losses from hog cholera were materially reduced. A total of 41,506 hogs had been treated when these statistics were compiled.

The county-area work was extended until, in 1914, it included 17 counties in different sections of the country. An outbreak of foot-and-mouth disease in the fall of 1914 interfered with the work to some extent and made it necessary to drop two counties, but work was conducted in the following: Decatur County, Ga.; Twin Falls County, Idaho; Hendricks and Montgomery Counties, Ind.; Clay and Dallas Counties, Iowa; Marshall, Kans.; Henderson, Ky.; Branch, Mich.; Renville, Minn.; Pettis, Mo.; Gage and Johnson, Nebr.; Muskogee, Okla.; Davison, S. Dak.; and Maury, Tenn.

The methods employed in these counties were purposely varied to meet different problems in different localities. In some counties

serum alone was used; in others, serum and virus; in still others, a combination of the two methods was used. In some counties the apparently sick hogs were given serum alone, whereas those that appeared to be well were given serum and virus.

The combined results for 1913 and 1914 were as follows: Of 9,686 hogs in herds exposed but in which no visible sickness was observed when treated with serum alone, 34, or 0.35 percent, died. In the same kind of herds treated with serum and virus, of 8,010 head, 14, or 0.17 percent, died. Of 53,485 pigs sick when treated, 15,570, or 29.1 percent, died. Of 44,277 pigs in herds infected but apparently well when treated with serum and virus, 1,298, or 2.9 percent, died. Of 40,462 pigs in herds infected but apparently well when treated with serum alone, 2,077, or 5.1 percent, died. Although approximately 38 percent of the hogs were sick when treated, the sickness being indicated either by visible symptoms or high temperatures, only 13.7 percent died. Some of the high temperatures might have been due to other causes; nevertheless, the results clearly showed that serum had a distinct curative action when administered in the early stages of the disease. One disadvantage of the serum-alone treatment was the recurrence of cholera in 5.55 percent of the herds treated, whereas cholera reoccurred in only 1.3 percent of the herds treated with serum and virus or given combined treatment previously described.

In addition to the important data obtained from these extensive field experiments, confidence in the treatments resulted in higher pork production and stabilization of the hog industry. In the counties under supervision, swine production increased from 975,400 head in 1912 to 1,256,729 in 1914, and the losses decreased from 178 hogs per thousand raised in 1912 to 49 head per thousand raised in 1914.

Conclusions on Field Experiments

Of the conclusions reached after the completion of these field experiments, the following are considered the most important:

(1) The results of the experiments indicate clearly that by pursuing one or the other of these systems the losses from hog cholera could be reduced to a minimum and hog raising could be greatly increased with comparative safety.

(2) Cooperation is needed between farmers, State officials, the Federal authorities, and the practicing veterinarians.

(3) More education is needed on the nature, prevention, and treatment of hog cholera.

(4) Until a real attempt is made to eradicate hog cholera, the Federal Government should be an advisory agency.

(5) Eradication of hog cholera would require many years of effort. No plan promising a reasonable degree of success can be suggested.

Up to the time the results and conclusions were reached and published, the field work was regarded primarily as a research project, and was directed by Dr. Dorset, Chief of the Biochemic Division. After the field work had conclusively demonstrated the value of anti-hog-cholera serum, it was no longer regarded as a research project and was abandoned.

Research Work on Serum Production

Although field work with serum and virus had demonstrated the practical value of the antiserum then prepared, many problems remained to be solved and improvements in methods worked out. The work of the Bureau was therefore concentrated on these phases of research.

Hyperimmunizing Experiments

One important project, carried out in 1906 and reported in 1908, was to determine whether or not immune hogs could be hyperimmunized by injecting hog cholera blood intravenously. Only half the amount of virulent blood was required, by this new method, and the technique of hyperimmunization was materially improved (*54*).

Potency of Serum

Early experiments showed that in serum production hyperimmune hogs could be bled four times at weekly intervals, the different bleedings mixed, and the potency of the serum would be satisfactory. However, they did not show the relative potency of the different bleedings. Later experiments indicated that first and second bleedings were distinctly more potent than fourth and fifth bleedings.

Effects of Heavy Bleeding

Eighteen hyperimmune hogs were bled about 8 cc. per pound of body weight, and 18 were bled about 4½ cc. per pound. The individual bleedings from heavily bled and lightly bled hogs, and the mixtures, were tested for potency. The individual bleedings from the heavily bled hyperimmunes, with few exceptions, appeared to be of lower potency than the bleedings from regularly or lightly bled hogs. No difference was found in the mixed sera from the heavily and lightly bled hogs. The physical condition of the hyperimmune hogs bled lightly was better during the bleeding process than that of hogs from which a large amount of blood was drawn.

Effect of Hydrogen-ion Concentration on Potency of Serum

Several lots of anti-hog-cholera serum known to be potent were adjusted to hydrogen-ion concentration between pH 2 and pH 8.83. The serums were incubated at 37.5 ° C. for from 30 to 271 days and then tested for potency. The portions adjusted to pH values between 5 and 8 appeared to retain their potency, whereas the portions adjusted to 4 or below, and from 8.3 to 8.83, had partly or completely lost their potency.

Reduced Hypering Dose

Extensive experiments were carried out to determine whether or not the hypering dose could be reduced below 5 cc. per pound of body weight without lowering the potency of the serum. Variations in doses between 1 cc. and 4.6 cc. per pound were tried, and the resulting sera compared with regularly prepared sera. In one experiment all hogs furnished serum that protected in 15- and 20-cc.

doses except the hyperimmune that received 1 cc. per pound of body weight. This indicated that the cost of serum could be reduced. However, before drawing any conclusions, the sera were again tested in 8-, 12-, and 15-cc. doses. The latter test produced evidence that the serum made by hypering with lower doses was of lower potency than that made from hogs hypered with 5 cc. of virus blood per pound of body weight.

Hyperimmunizing With Tissues From Virus Pigs

Livers and spleens from cholera-infected pigs were finely ground, suspended in a salt-glycerol solution, and injected subcutaneously into immune hogs. The serum obtained from hogs hyperimmunized in this manner possessed considerable potency but less than serum prepared by the standard method. Also, it was found that tissue injections sometimes caused abscess formation.

Hypering With Muscle Extract

Muscle tissue from cholera-infected pigs was finely ground and mixed with physiological salt solution, frozen, then thawed and filtered through infusorial earth filters. The clear extract obtained by this process was used for hyperimmunizing hogs. The serum obtained from some hogs hypered in this manner was of excellent potency, but the potency of serum from other hogs seemed definitely lower than that of serum produced by the standard method.

Hypering With Citrated Blood

As in other methods tried, the serum made from hogs hyperimmunized with citrated blood was unsatisfactory as to potency (*58*). The standard method of producing serum, as outlined by the Bureau, has not been materially changed.

Time To Hyperimmunize

Experiments were conducted to determine the proper time after immunizing to hyperimmunize hogs. Tests demonstrated that hog cholera serum could not be improved by following the methods for improving rinderpest serum developed by Holmes (*69*), an investigator in the service of the Government of India. Holmes was able to produce more potent serum for rinderpest when the immune, serum-producing animal was hyperimmunized within a short time following simultaneous treatment. He found also that a more potent serum could be obtained when the virus need for hyperimmunization was previously diluted with a weak solution of potassium citrate.

Because of the similarity of the methods of producing rinderpest and hog cholera serums, it was thought desirable to study the process used for rinderpest in respect to hog cholera. Nonimmune hogs were hyperimmunized at various times following simultaneous inoculation. It was found that large amounts of virus could be administered to immune hogs almost immediately after vaccinating, but the serum so hypered was of low potency. Little or no protection could be expected from serum derived from hogs hyperimmunized within 10

days after simultaneous treatment. The serum from hogs hyperimmunized between the 10th and 42d days possessed more potency than that derived within 10 days, yet was not so satisfactory as that from animals held for a longer time before hyperimmunizing (49 to 56 days). As a result of the Bureau's experiments it was concluded that the interval between immunizing and hyperimmunizing should be not less than 7 weeks. It was also found that citrated blood, when used for hyperimmunizing did not produce as potent serum as was obtained by the use of ordinary defibrinated blood.

Keeping Qualities of Serum

A sample of clear serum produced in 1916 and a sample of defibrinated blood serum produced in 1919 were tested for potency in 1931. Twenty-cc. doses of serum and 2-cc. doses of virus were administered. All treated pigs remained normal, and the controls died of cholera. Thus, two samples of serum still retained their potency 12 and 15 years, respectively, after they were produced.

Six serial numbers of commercial serum were tested for potency from 3 months to 1 year after the expiration date for their use in the field. Three were defibrinated blood and three were clear sera. All six were found to be of satisfactory potency.

Development of Clear Pasteurized Serum

The occurrence in 1914–15 of foot-and-mouth disease in hogs following serum-virus treatment led to the discovery of the virus of that disease in a lot of hog cholera serum, and also in a small lot of hog cholera virus. A method of preventing a recurrence of this mishap therefore became desirable.

It was known that the virus of foot-and-mouth disease could be destroyed by yeast or pasteurization. However, the hog cholera serum produced prior to 1915 was made from defibrinated blood, and it could not be pasteurized without materially thickening the product and thereby rendering it unsuitable for use. Research was begun to develop a serum that could be heated sufficiently to kill the virus of foot-and-mouth disease and also any bacteria that might be present without adversely affecting its original potency against hog cholera.

This research resulted in the development of several methods of producing serums that could be heated to 60° C. for half an hour, which is believed to be sufficient to destroy the virus of foot-and-mouth disease but does not destroy the protective properties of anti-hog-cholera serum (*49*). Four of the processes were patented, and the patents dedicated to the public or assigned to the Secretary of Agriculture by the patentees (*51*, *52*, *53*, *66*).

These processes made it possible to separate the clear serum from cells and cellular debris in defibrinated blood. The clear portion of the serum could then be pasteurized without any material physical change or loss of potency.

Two of the processes were used to clarify old phenolized, defibrinated-blood serum that had failed to pass a satisfactory test for purity and potency. One of these processes was never used commercially. In the other process (*63*, *66*, *68*), chloroform was added to old phenolized, defibrinated-blood serum kept at 40° F. The mix-

ture was held for 4 or more hours and then centrifuged. The clear serum so obtained was then heated at 58° to 60° C. for one-half hour and 5-percent phenol was added in the proportion of one-fifteenth of the volume of serum. This process was used commercially to some extent but fell into disuse because of the general adoption of another process (*51*) for use with freshly drawn, defibrinated blood from hyperimmunized hogs. This process consisted of the addition of an extract of bean and a salt solution to the blood, followed by centrifuging the mixture, and decanting the clear serum. The bean extract agglutinated the red blood cells and the salt contracted them. The clear serum could easily be poured off from the corpuscles and cell debris. The yield of serum approximated 70 percent of the whole blood. The serum was heated at 58° to 60° C. for 30 minutes, cooled, and phenolized. A modification of this process, using salt alone, was also patented (*53*).

The bean used in the bean extract-salt method was a pea or navy bean, particularly the Wisconsin pea bean. However, a number of other beans of the same family (*Phaseolus vulgaris*) also agglutinated hog blood. In connection with the work on bean extracts, 54 varieties of beans or seeds were tested for their agglutinating properties on 11 different kinds of blood. Many of the extracts agglutinated many bloods, but a number of differences were noted. For example, no extract agglutinated cow's blood. It was possible by means of the extracts to differentiate one fresh blood from another, but not one dried blood from another. The agglutinins from beans resembled albumin and were destroyed by heat at about 80° C. The only records of these bean tests were published in the Annual Reports of the Chief of the Bureau of Animal Industry for 1917 and 1918 (*117*).

Nonsporeforming, pathogenic bacteria, including tubercle bacilli, were added to clear serum, obtained by the bean extract-salt method, and heated at 58° to 60° C. for 30 minutes. At the end of that time it was found that the added bacteria were destroyed. It was found also that tubercle bacilli added to clear serum containing 0.5-percent phenol were apparently attenuated after 2 weeks of storage at refrigerator temperature, and were destroyed in 4 weeks.

Shortly after the bean-salt method of preparing serum was published in 1916 (*49*), commercial production of clear serum was begun, and it gradually replaced the defibrinated-blood serum. In 1935 the Bureau required that all anti-hog-cholera serum be heated, which, in effect, required that it be prepared by the bean-salt method.

Bean extract as originally prepared did not keep well, and a new method of preparation was devised (*50*). This method involved extracting bean meal at 60° C. with 0.5-percent phenol solution containing 0.85 percent sodium chloride, and filtration through bacteria-proof filters.

Although pasteurization of clear serum rendered it safe against the virus of foot-and-mouth and many other diseases, no effective method of safeguarding hog cholera virus against contamination by other viruses has been found.

The method of producing pasteurized serum was adopted by all commercial serum producers and is the standard method in use at the present time.

Effect of Heating Serum

To determine the effects of heating on the potency of hog cholera serum, two lots of clear serum were each divided into three portions, and the portions of one lot were heated at 59°, 60°, and 62° C., respectively, for 30 minutes. Each portion was then compared for potency with the same serum that was not heated. The serum was administered in doses of 5 cc., 10 cc., and 15 cc. The pigs that received the unheated serum and the serum heated at 59° or 60° remained normal. The pigs that received serum heated at 62° were visibly sick and their temperatures were elevated, but all recovered.

In early experiments it was found that the antibodies in old defibrinated-blood serum would withstand heating at 50° C. for 24 hours. It was also found that the antibodies were not destroyed by heating to 60° for 1½ hours, but heating this kind of serum to 60° caused coagulation and was not practical.

Antibodies in Serum not Adsorbed by Virus

To find out whether the antibodies in serum could be removed by virus, clear hyperimmune serum and washed red blood cells of virus pigs were mixed in equal parts. The mixture was incubated for 2 hours at 37.5° C., then centrifuged, and the clear serum removed. Pigs were then treated with 1 cc. of virus and the hyperimmune serum that had been in contact with the virus cells. Others were treated with 1 cc. of virus and a portion of the regular serum. All pigs remained well, which indicated that the hyperimmune serum did not lose antibodies when mixed with blood cells from virus pigs.

Variations in Potency of Serums From Hyperimmunes

Early experiments showed that the serum from individual hogs varied in potency. Low doses were used to demonstrate this point. One out of 13 hyperimmunes produced serum that was not satisfactory. The general practice was, and still is, to mix the serum from a number of hogs and test the mixture for potency. The mixture is usually found sufficiently potent to meet test requirements; however, if too many lots of low-potency serum are included, the entire batch is sometimes found to be of low potency.

Curative Value of Anti-Hog-Cholera Serum

In the early stages of serum development it was recognized that serum was not a cure for hog cholera. Most of the hogs treated were visibly sick, and some were in advanced stages of the disease. Such hogs apparently were not benefited by serum treatment, even in doses up to 300 cc.

In later experiments pigs were given injections of virus, followed on the first, second, third, fourth, and fifth days, respectively, by serum treatment. Some of the pigs infected 1 or 2 days prior to the administration of serum did not get sick. The number of pigs protected gradually decreased as the period of infection increased. About 50 percent of the pigs treated 4 days after infection were not protected, although some were not showing visible symptoms when treated. Some of the injections were given subcutaneously

and some intravenously, but no difference was noted in results (*89*).

It is difficult to make a prognosis when treating sick herds in the field because it is impossible to know how many pigs are infected or exactly when infection occurred. In all early experiments, defibrinated-blood serum was used. A few experiments were carried out in 1929 to test the curative value of clear and defibrinated-blood serums. The results indicated that clear serum was somewhat superior, but the tests were too few to warrant definite conclusions. In field experiments, where only a few pigs in a herd were sick, many were saved by the early administration of serum.

Dosage of Serum

In the first stages of the development and use of hog cholera serum, a dosage of 20 cc. was adopted as more or less standard for pigs weighing up to 90 pounds; the maximum dose was 40 cc. It was soon learned that a wider range of dosage was necessary, and there developed a tendency to increase the dosage for each weight group to afford adequate protection. It was also learned that different lots of pigs, and individual pigs in the same lot, varied in susceptibility and in their response to immunizing treatment. In order to administer sufficient serum to compensate for these differences, many pigs received larger doses than actually would have been required to protect.

At the present time the minimum doses recommended are:

Suckling pigs	20 cc.
20 to 40 lb.	30 cc.
50 to 90 lb.	35 cc.
90 to 120 lb.	45 cc.
120 to 150 lb.	55 cc.
150 to 180 lb.	65 cc.
Over 180 lb.	75 cc.

Since serum produced at present is clear, and the first serum used was defibrinated-blood serum, the recommended dose for some weights is considerably more than double the amount in early tests and experiments.

In 1934, Dorset (*45*) stated, "Excessive doses of serum do not prevent the development of an active immunity," and he strongly advocated the use of "a suitable dose of highly potent virus and . . . sufficient hyperimmune serum to prevent the development of any visible reaction in the inoculated animal." Dorset's statement and recommendations were based on the Bureau's experiments and on the results obtained by treatment of more than 300 million farm pigs with commercial anti-hog-cholera serum.

Proportions of Serum to Virus

In experiments conducted at Ames (*10*), it was shown that pigs were immunized by serum and virus in the proportion of 200,000 parts of serum to 1 part of virus, and that pigs could also be immunized with 20 parts of serum to 50 parts of virus. The minimum amount of serum (10 cc.) required to protect against 2 cc. of virus would also protect against 100 cc. of the same virus. In the same experiment doses of 20 cc. of serum failed to protect against 2 cc. of certain virus. The discovery of variant virus, which will be

discussed later, resulted in the official recommendation to increase the hitherto recommended doses of serum by 25 percent.

Although permanent immunity was produced experimentally with doses of virus as low as 1/10,000 cc., such procedure is not advisable in practice when the exact virulence of the virus used is unknown. The virus used in the experiment herein described was known to be sufficiently virulent to produce cholera in 1/1,000,000 cc. doses. In certain other experiments, viruses that would not produce cholera even in 1/10,000 cc. doses did produce lasting immunity when used in 2 cc. doses with regular doses of serum.

Practical Results of Heavy Dosage

Investigations of unfavorable results following use of the serum-virus treatment have shown that veterinarians who regularly use large doses of serum have had better results than those who use the minimum dosage.

It is important to determine, if possible, whether any debilitating condition is present in a herd to be treated. If such conditions are present, larger doses of serum are indicated in order to keep the reaction following treatment to a minimum. At times conditions are found which contraindicate treatment by the simultaneous method. In such herds, if treatment cannot be deferred until the pigs are in normal condition, serum alone should be administered. If immunity for more than 3 weeks is needed, the herd should be treated again by whatever method is preferred.

Vaccination Shock

In 1929, reports were received from veterinarians that alarming symptoms had been observed following the use of clear hog cholera serum. The symptoms usually followed the intraperitoneal injection, particularly in young pigs. The reactions usually occurred within a few minutes after the serum was administered, and varied from shivering, rapid breathing, and muscular trembling, to convulsions and sometimes death.

It was first considered that the reaction might be anaphylactic shock due to sensitization by the proteins in soybean meal. To check this possibility, pigs were fed equal parts of cracked corn and soybeans for 2 weeks. A control group was fed corn only. Both groups were given injections of clear serum intraperitoneally; virus was injected intramuscularly. No reaction occurred in either group.

Munce and Hoffman (*96*) reported a series of experiments in which they produced shock experimentally by heating serum to a degree slightly higher than that required by Government regulations. To obtain additional data on this subject, experiments were carried out by the Bureau.

In the Bureau's experiments, serum was heated for 30 minutes at 57.6°, 58.2°, 60.6°, 61.6°, and 62.4° C. One sample was heated at 58.1° for 60 minutes. When injected into pigs, the unheated serum and those heated at 57.6° or 58.2° for 30 minutes did not produce shock symptoms. That heated at 60.6° produced shock in 90 percent of the pigs treated; that heated at 61.6° or 62.4° produced shock in 100 percent of the pigs treated. The regulations govern-

ing the production of clear serum require a heating period of 30 minutes at 58° to 59°. The sample heated at 58.1° for 60 minutes produced shock in 60 percent of the pigs treated. These experiments confirmed the work of Munce and Hoffman.

Other experiments were conducted on farms and the same results were obtained. In one farm experiment, a number of brands of commercial serum were used. Shock was more likely to occur following the use of serum obtained from certain laboratories. It was considered that this might be due to slightly different methods of heating in the different laboratories, or to variations in the different thermometers. There was individual susceptibility of pigs in the same herd, and some herds reacted differently from other herds treated with the same serum. Chemical studies indicated that the shock-producing substance was precipitated by ammonium sulfate in the concentration used to precipitate euglobulins.

Anemia and Shock

Hemoglobin tests were made of a large number of pigs at vaccination, and their reactions were observed. There was a close correlation between anemia and vaccination shock (*83*). Pigs with a low hemoglobin showed severe shock and several died. Shock following administration of clear serum is more common in spring pigs than in fall pigs, as is anemia. In practice, it is well to watch for symptoms of anemia when vaccinating pigs, and if symptoms are well marked, treat the herd for anemia before vaccinating.

Absorption of Serum

Seven experiments were conducted to determine whether the effectiveness of serum when administered with virus would be influenced by the rate of absorption, or the method of administration. The doses of serum were small enough to permit a reaction in the pigs but large enough to prevent death. The results indicated that the intraperitoneal administration of serum was slightly less effective than the subcutaneous or intramuscular administration. In practice, it has been observed that when large doses of serum are administered subcutaneously or intramuscularly, too much serum should not be injected in one place, in order to aid absorption and to prevent pressure necrosis.

Duration of Serum-Alone Immunity

The duration of serum-alone immunity varies greatly, even among pigs in a single herd. In 1915, Dorset (*38*) made the following statement: "Now we know from experimental work that hogs treated by serum alone may remain immune from 3 weeks to 3 months. . . ." This statement is perhaps as nearly accurate as any statement that can be made at present regarding the duration of immunity following the use of serum alone. In conducting experiments wherein it is necessary to hold pigs until serum-alone immunity has expired, they are still held for 3 months. It has been observed that when pigs from the same lot, part of which have been given serum alone and part left untreated, are held 3 months and then exposed, the serum-

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treated pigs sometimes show less susceptibility than the untreated pigs.

An experiment was conducted to determine if serum-alone immunity could be prolonged by increasing the dose of serum. Twelve pigs were given doses of 20 cc., 12 were given 100 cc., and 6 were left untreated to serve as controls. It was intended to hold these pigs for later exposure, but one of the untreated controls developed cholera 3 weeks after the experiment was started and exposed the entire group. Of the 12 pigs that received 20 cc., 1 remained normal and 11 developed cholera, 10 of which died. Of the 12 pigs that received 100 cc., 6 remained normal, and 6 reacted but recovered. This experiment was repeated, the same dosage (20 cc. and 100 cc.) being given, followed by exposure 4 weeks after treatment. All pigs developed cholera and very little, if any, difference was noted between the two groups.

Considering the first experiment alone, it might be concluded that the larger dose did produce a longer lasting immunity. However, judging the results of both experiments, it appears that the larger dose might have produced a stronger early immunity without actually prolonging it. In the second experiment, wherein the exposure was made only 1 week later, the larger dose definitely did not prolong the serum-alone immunity.

PART 3. HOG CHOLERA VIRUS, HOG CHOLERA SERUM, AND SIMULTANEOUS TREATMENT

Hog Cholera Virus

Defibrinated blood from cholera-infected pigs plus 0.5-percent phenol is usually referred to as hog cholera virus, and it is used with anti-hog-cholera serum to immunize swine. It is referred to in commercial serum plants as "simultaneous virus."

Preservatives

A number of chemicals were tested for their effectiveness in destroying contaminants in virus blood without destroying the virulence of the virus. Among these were phenol, ether, chloroform, formaldehyde, ammonia, and glycerine. Phenol was used from the beginning of the work, with both virus and serum, and was a satisfactory preservative when used in 0.5-percent solution. None of the other chemicals tested were more satisfactory, and phenol is still the standard preservative.

Etherized virus retained virulence in some experiments longer than phenolized virus, but bacterial counts were slightly higher in the etherized-virus blood. In one case an etherized virus retained virulence when 2 years old. In early work, virulence was determined by injecting into susceptible pigs comparatively large doses of virus to see if it would produce disease. Later, virulence was determined by titration of the blood.

Glycerine in 40-percent strength was compared with 0.5-percent phenol in two experiments. The glycerinated portions of the virus had lost virulence when they had been held for 183 and 188 days, respectively, whereas the corresponding portions preserved with

phenol were still virulent after 240 days. It was clear that the use of glycerine as a preservative significantly curtailed virulence in comparison with the virulence of phenolized virus. Later, when virus was treated with glycerine in 40-percent strength and held at incubator temperature at 37.5° C., the virulence was destroyed in some experiments in only 5 days.

Formaldehyde in a weak solution destroyed virulence in 30 to 55 days at room temperature. Some of the pigs treated with formaldehyde virus did not get sick, but were later found to be unusually resistant to cholera. As a result of this experiment, attempts were made to produce vaccine from formaldehyde virus, but the vaccines did not prove to be satisfactory, chiefly because of separation or thickening of the final product after storage.

Ammonium hydroxide (NH_4OH) in low concentration (0.125 percent) destroyed the virus of hog cholera within a comparatively short time. Pigs treated with attenuated ammonia virus that remained normal showed considerable but irregular immunity when later given injections of virulent hog cholera virus.

These experiments with preservatives led to experiments in vaccine production, which will be described later.

PRESERVING VIRUS BY FREEZING

As work on vaccines progressed, it became desirable to develop a method whereby a virus could be held for prolonged periods without impairment of virulence and thus permit its use as a standard challenging virus for successive lots of vaccine. Challenging viruses were frequently found to be different and constituted an undesirable variable in testing and retesting vaccines for keeping qualities. For this reason, a mechanical refrigerator that would maintain a temperature of –40° F. was obtained. Phenolized and unphenolized viruses were titrated in doses ranging from 1 cc. to 1/5,000,000 cc. and held at –40° for periods up to 425 days and again titrated for virulence in the same doses. With few exceptions, no loss of virulence was detected in either phenolized or unphenolized viruses. A detailed report of these experiments was made by Cole and Henley *(13)* in 1951. This method was adopted for the storage of test viruses and has since been adopted for preserving commercial inoculating and simultaneous viruses. By this method, large quantities of virus could be produced and thoroughly tested for virulence and for variant characteristics, thereby assuring a uniform and dependable virus that would last for many months and obviate many tests of smaller lots of virus.

Amount of Virus Required To Produce Hog Cholera

From the time of its discovery, investigators were interested in determining the smallest amount of hog cholera virus that would produce disease. In the first studies, relatively large doses (from 5 cc. to 20 cc.) were used, but it was later decided that 2 cc. of virus blood invariably produced cholera when injected alone, and produced immunity when injected with serum. A standard dose of 2 cc. was therefore used in the first field tests. In 1922 an attempt was made to determine the minimum lethal dose of hog cholera virus. At that time, cholera was produced by doses of 1/25,000 cc.

In 1930, McBryde (*81*) reported that experiments indicated that the minimum lethal dose of the strain of virus used was between 1/300,000 cc. and 1/400,000 cc. There was no direct relation between the size of the dose and the length of the period of incubation. Powick (*100*), in work carried out at Ames on the distribution of hog cholera virus among the fractions of blood, produced cholera in 1/50,000 cc. doses but failed to produce disease in 1/300,000 cc. doses.

Later, it became desirable, for several reasons, to obtain information as to the amount of virus present in the blood of artificially infected pigs used in vaccine production. To obtain that information, Cole, Henley, and Hubbard (*14*) carried out a series of experiments in which virus blood was tested in doses varying from 1/25,000 cc. to 1/10,000,000 cc. Blood for these experiments was drawn on the sixth to the ninth day after inoculation. Hog cholera was produced in 8 pigs in doses of 1/500,000 cc., in 8 pigs in doses of 1/750,000 cc., in 8 pigs in doses of 1/1,000,000 cc., in 2 pigs given injections of 1/2,500,000 cc., and in 1 out of 6 pigs given injections of 1/5,000,000 cc. All pigs not made sick by these small doses of virus were later challenged by injection of 2 cc. of virus, and with very few exceptions they all died. Later, Dale carried out a series of titrations of spleen tissue from cholera-sick pigs. Cholera was produced in doses as small as 1/1,000,000 of a gram.

Clear Virus

Although clear anti-hog-cholera serum has been produced since 1916, and has been the only kind produced since 1935, clear hog cholera virus has not been produced commercially. It has, however, been produced and used to a limited extent experimentally. Although experiments have indicated that the greater part of the virus of hog cholera is found in the cellular elements, some is present in the clear virus serum.

Experiments with clear virus were carried out in six farm herds containing 322 pigs. Lots of virus were prepared in the usual way, and then divided into two portions. One portion was clarified and the other was used as regular or phenolized defibrinated-blood virus. Half of each herd was treated with serum and clear virus, the other half with serum and defibrinated-blood virus. From 3 to 9 months later, 40 of the pigs treated with serum and regular virus, and 40 treated with serum and clear virus, were given injections of 5 cc. of virulent virus. All pigs exposed remained normal, which would indicate that clear virus is as efficacious as defibrinated-blood virus in the immunization of pigs by the serum-virus simultaneous method.

Virus Dosage in Simultaneous Treatment

In 1917, the doses of virus officially recommended for use in the simultaneous treatment varied from ¼ cc. for small pigs to 2 cc. for pigs weighing 100 pounds or over. In 1921, 34 pigs were treated with standard doses of serum and doses of virus varying from 1/100 cc. to 2 cc. The pigs were isolated, free from other exposure, for 3 months and then exposed to cholera. All pigs remained well, which led to the conclusion that virus doses may be exceedingly small and at the same time produce permanent immunity.

Distribution of Virus in Blood Fractions

It was found that virus blood cells retained virulence after seven washings with physiological salt solution.

Following attempts to determine which fraction of blood contained the most virus, Powick (*100*) reported as follows:

(1) Most of the virus of hog cholera blood was associated with cellular elements.

(2) Probably no important part of the whole quantity of virus in hog cholera blood was associated with the leucocytes.

(3) The erythrocyte stroma of hog cholera blood carried an important part of the virus in relatively high concentration.

(4) Blood cells of normal pigs, when mixed with virus serum, readily and firmly adsorbed a considerable part of the hog cholera virus that the serum contained.

Effects of Repeated Washings of Red Blood Cells

Red blood cells obtained from the blood of cholera-infected pigs were divided into three portions; one was washed 6 times, one 7 times, and one 8 times with physiological salt solution. Washing was accomplished by suspending the cells in salt solution and separating them by centrifugation. After the different portions of cells were separated, they were injected into susceptible pigs and all pigs developed hog cholera, which indicated that it was not possible to remove the virus from the red blood cells by the method followed.

Cultivation of Virus

Attempts were made to cultivate the virus of hog cholera by methods developed in the laboratory, and by methods recommended by outside investigators.

Powick attempted, without success, to cultivate the virus on tissue from pigs, and also in chick embryos. A number of successive transfers were made on chick embryos, sufficient to eliminate a carry-over of virus, and in the final transfer, no virus was found.

An attempt was made at Ames to cultivate the virus by the method proposed by Frosch and Dahmen (*61*) for cultivation of foot-and-mouth disease virus. This method involved the use of a pork infusion medium, to which was added peptone, freshly prepared from pigs' stomachs. Cultures were made from eight pigs on the second to the sixth day following inoculation, but no evidence of growth was obtained. Attempts were made to cultivate virus by the methods described by Proescher and Seil (*101*) and by TenBroeck (*115*) but, like other methods tried, they resulted in failure.

Effects of Various Agents on Virus

LYOPHILIZATION

Phenolized and unphenolized viruses were lyophilized and tested shortly after their preparation in 1 cc. doses only. They were held in a refrigerator and retested over a period of 7 years, and continued to produce disease. The test made of one lyophilized virus after 7 years of storage showed that it produced cholera in 1 cc., 1/10,000 cc., and 1/100,000 cc. doses. In the latter dosage, only one of two

pigs developed cholera. Some of these viruses were distributed from time to time to officials in foreign countries, and their reports indicated they were satisfactory for virulence.

EFFECTS OF AGE ON VIRUS

From the beginning, it has been recognized that phenolized virus deteriorates with age. How fast deterioration proceeds may depend, in part, on the particular virus and the manner in which it is stored. As stated under preservatives, a phenolized virus stored in a refrigerator for 2 years produced cholera. Two viruses, 2 years 11 months old, were found to have completely lost virulence.

In work reported by Cole and Henley (*13*), a number of phenolized and unphenolized viruses were titrated, held for varying periods at different temperatures, and then retitrated to determine if loss of virulence had occurred or if immunogenic properties were retained. The results of these experiments indicated that viruses held at 32° to 50° F. deteriorated; that is, they partly or completely lost virulence. For example, one virus, when first prepared, produced disease in doses of 1 cc. down to 1/5,000,000 cc., but after storage for 226 days at refrigerator temperature, it produced disease in 1-cc. doses, but not in doses of 1/10,000 cc. or less.

Results of tests of frozen phenolized viruses held at 20° to 25° F., closely paralleled those obtained with viruses held at refrigerator temperatures. On the other hand, portions of the same viruses stored at −40° for periods up to 425 days retained practically all of their original virulence. Portions of these viruses that had been frozen and held at −40° for long periods were thawed, held in a refrigerator for 60 to 105 days, and then tested for immunogenic properties. After storage in the refrigerator, the viruses were again titrated, and showed considerable loss in virulence, but all produced disease in 1 cc. doses.

IMMUNOGENIC TEST

Four pigs were treated with an ordinary dose of hog cholera serum and 2 cc. of the virus that had been stored for long periods at −40° F., then thawed, and held in a refrigerator. Two hogs from the same lot were given serum alone and two were left as untreated controls. All treated hogs and the controls were held free from exposure for 90 days; then exposed to cholera by injections of 2 cc. of virus.

The experiment was repeated with 9 different viruses, and in all experiments, all serum-and-virus-treated hogs remained normal. Those treated with serum alone, and the untreated controls all developed cholera. These experiments indicated that, although virus had decreased in virulence, it would still produce permanent immunity when used in 2-cc. doses with serum.

EFFECT OF HEAT ON HOG CHOLERA VIRUS

In 1915, eight unphenolized viruses were heated at 50° C. for periods of 6 to 12 hours, and then tested for virulence. The conclusion reached was that hog cholera virus is slightly attenuated when heated to 50° for 12 hours.

In 1924, 28 pigs were used in an experiment to determine the effects of heating phenolized virus to temperatures from 82° to 100.7° F. for different periods. Holding virus at 82° to 86° for 18 hours apparently did not destroy the virulence, but of 2 pigs treated with the same virus held for 48 hours, 1 developed cholera and 1 did not. Virus held at 92.3° to 100.7° for 7 days produced sickness, but the pigs recovered. Virus held at 92.3° to 100.7° for 15 days failed to produce cholera.

It was concluded that virus for field work should not be exposed to summer heat for more than a few minutes.

Survival of Virus in the Blood and Lymph Glands

At intervals during the Bureau's research on hog cholera, pigs were treated with serum and virus and bled later to determine how long the virus could survive in the blood. The pigs were given regular doses of serum along with 2 cc. of virus, and blood was drawn at intervals and tested for the presence of virus. Blood drawn up to the 14th day was invariably infectious, and in some cases it was infectious on the 21st day. Blood drawn on the 28th day was, in all cases, free of virus. During some of these experiments untreated pigs were placed in the pens with the pigs that were being tested for virus in the blood. Although virus was known to be in the blood of the serum-virus-treated pigs, the disease was not transmitted to the susceptible pigs in the same pen unless the treated pigs became sick.

After a European investigator claimed that virus could be found in the lymphatic glands for 10 months after treatment, an experiment (*85*) was conducted on the persistence of virus in the bodies of serum-virus-treated pigs. In this experiment the blood and lymph glands were tested at intervals for the presence of virus. Virus was not found in the blood but was found in lymph glands 3 weeks after treatment. Virus was not found in either the blood or the lymph glands 6 weeks after treatment.

Adsorption of Virus by Chemicals

Experiments were conducted to determine whether or not certain agents could adsorb the virus of hog cholera.

The first experiment was carried out with calcium carbonate and kieselguhr. The virus used was a clear serum obtained by centrifuging defibrinated-blood virus. It was diluted with sterile physiological salt solution in the proportion of 1 part of virus serum to 9 parts of salt solution. To one portion, calcium carbonate was added in the proportion of 1 gram to 10 cc. To a second portion, kieselguhr was added in the same proportion. Each portion was stirred for about an hour, then allowed to settle; the supernatants were pipeted off, filtered through paper, and 20 cc. of each portion was injected into susceptible pigs. The precipitates of both portions were resuspended in normal saline solutions, and injected into susceptible pigs. All pigs that received injections of the supernatents and precipitates developed cholera.

A second experiment was carried out along the same line except that 1 part of clear virus serum was diluted with 99 parts of salt solution. The mixtures were stirred for 30 minutes and then allowed

to settle overnight. Doses used in testing were 40 cc., which was equivalent to 0.4 cc. of original virus serum. Symptoms of cholera appeared in the control pigs on the third day, in the pigs that received calcium carbonate-treated virus on the 7th day, and in those that received kieselguhr-treated virus on the 11th and 14th days. Judging by the incubation period, some adsorption occurred with calcium carbonate, but the most adsorption occurred with kieselguhr.

In another experiment, "Nuchar," a commercial vegetable charcoal, was used in the proportion of 1 part to 20 parts of virus. Some pigs were given an injection of clear virus serum separated from the "Nuchar"; others were given an injection of a suspension of the twice-washed carbon. All pigs developed cholera, which indicated that the carbon may have adsorbed some of the virus.

Adsorption of Virus by Normal Blood Cells "In Vitro"

Normal blood cells obtained from a healthy, susceptible pig were added to clear virus serum in the proportion of 1 to 10, and allowed to stand at room temperature (72° F.) for 2½ hours. The mixture was then centrifuged and the virus serum was withdrawn. The residue of cells was then washed four times with normal salt solution to remove all traces of the virus serum from the cells. After the final washing, the blood cells were suspended in a small amount of normal salt solution. Two pigs were then given injections of 2 cc. of the cells. Both pigs developed cholera, which was an indication that the normal cells adsorbed virus from the virus serum.

Virus Contaminants

Following an announcement by a State experiment station investigator that vaccination breaks had been caused by the use of serum or virus contaminated with spores of *Clostridium botulinum*, the following experiment was conducted: Pigs were given injections of serum and virus to which such spores had been added. Other pigs were treated with a commercial serum reported to contain spores of *Cl. botulinum*. All pigs remained well following treatment, and when exposed 90 days later, were found to be immune. It was concluded that the presence of spores of *Cl. botulinum* in serum and virus was not an important cause of so-called "breaks" in immunity.

Disinfectants Against Hog Cholera Virus

In early work it was found that a compound solution of cresol was more effective than phenol in destroying hog cholera virus. For reasons of economy, other disinfectants were tested; also a number of soaps, but these failed to destroy the virus. Sodium hydroxide had been found to destroy the virus of foot-and-mouth disease, so a series of tests were made to determine its effectiveness against the virus of hog cholera. These tests showed that 3 percent of sodium hydroxide in combination with 2 percent of milk of lime destroyed hog cholera virus within 15 minutes (*90*). The milk of lime was used chiefly to indicate the thoroughness of coverage when used on pens and buildings; it also tended to preserve the sodium hydroxide, and retard its conversion into carbonate.

Relation Between pH Values of Virus and Retention of Virulence

A comprehensive survey of simultaneous, commercially produced, viruses indicated that the hydrogen-ion concentration seldom falls outside the range of pH 7.0 to pH 7.4. Samples of defibrinated virulent blood preserved with either 0.5 percent of phenol or 40 percent of glycerine were adjusted by the addition of acid or alkali to cover the range of pH 3 to pH 9, and were stored at about 25° C. Periodic tests were made on susceptible pigs to determine when virulence had been lost. The results indicated that pH 5.0 to pH 5.5, representing a slightly acid medium, was optimum for the preservation of virulences. Under these conditions virulence appeared to persist three times as long as at the normal pH of 7.0. Unfortunately, the improvement in retention of virulence could not be applied directly to ordinary virus because the mixtures became too thick. Preliminary experiments indicated that the difficulty could be overcome by the addition of glycerine (*5*). This work seemed to have favorable possibilities, but was not further pursued.

Virus Bottles

A survey made it clear that bottles used to store and distribute virus were often of such poor quality that the alkalinity and insoluble matter could seriously affect the contents. This finding was brought to the attention of producers, and steps were taken to use glass of better quality, which resulted in better quality virus.

Virulence of Virus at Different Stages of Cholera

Pigs were inoculated with hog cholera virus and bled on different days between the 3d and the 12th day after inoculation (*14*). The bloods were tested in doses between 1/25,000 and 1/10,000,000 cc. These tests indicated that the maximum amount of virus was found on the 6th, 7th, and 8th days after inoculation.

Distribution of Virus in Tissues of Cholera Pigs

Livers, spleens, kidneys, and lymphatic glands of cholera-infected pigs were finely ground and suspended in physiological salt solution. The suspensions were then diluted and administered to susceptible pigs in doses of 1/150,000 gram, 1/350,000 gram, and 1/450,000 gram, respectively. The liver and spleen tissue produced disease in 1/450,000 gram, and the kidney in 1/350,000 gram; the gland tissue did not produce disease even in the largest dose. These experiments indicated that the livers and spleens of cholera-infected pigs contained large amounts of virus.

Period of Incubation of Hog Cholera Virus

Experiments carried out in 1922 (*40*) showed that following the subcutaneous injection of fresh hog cholera virus, 50 percent of the pigs showed visible symptoms on the fourth day, and 90 percent showed visible symptoms by the end of the sixth day. From these experiments it was concluded that the blood from pigs showing symptoms and fever between the fourth and sixth day, and con-

tinuing to show progressive symptoms, would furnish virus suitable for hyperimmunizing and for simultaneous immunization.

Simultaneous Treatment

For many years the standard and accepted method of immunizing swine had been the administration of anti-hog-cholera serum and virus at the same time but injection separately. Some modifications of this method were tried and in some cases recommended. One of these methods was the so-called follow-up method, in which serum alone was administered, followed in from 1 to 2 weeks by the simultaneous treatment. Another was referred to as the delayed-virus method, in which serum was administered first, followed in a few days by virus. These two methods were used in problem herds in which some abnormal condition existed, and virus might be contraindicated.

Delayed Virus Experiments

In 1928, 15 pigs were given serum alone, followed in 7 days by doses of 3 cc. of hog cholera virus. Three months later they were exposed to cholera and all remained well. In the second test, a group of farm pigs were given serum and virus; another group received serum alone, followed in 7 days by the injection of 2 cc. of virus. Five months later 5 pigs from each group were exposed to cholera by injection of virus in 5-cc. doses, and all remained normal.

Serum Alone Followed by Simultaneous Treatment

In this experiment a herd of farm pigs, weighing an average of 65 pounds, were given doses of 45 cc. of serum alone, followed 11 days later by serum in doses of 45 cc. and virus in doses of 2 cc. Three months later 5 of the pigs were exposed by virus injection. All remained well, which indicated that prior doses of serum did not interfere with the production of an active and persistent immunity by a later treatment with serum and virus.

Effects of Simultaneous Treatment

ON PREGNANT SOWS

The following statistics were compiled from field experiments in the treatment of pregnant sows with serum alone and by the simultaneous method in cholera-infected herds, and in herds exposed to cholera but not showing symptoms of the disease. In the infected herds, 3,235 sows were treated with serum alone and 261, or 8 percent, aborted. In the same herds 1,357 sows were given the simultaneous treatment and 98, or 7.2 percent, aborted. In the healthy exposed herds, 126 pregnant sows were treated with serum alone and 2, or 1.5 percent, aborted. In the same herds 38 were treated by the simultaneous method and none aborted. There was practically no difference in results of vaccination with serum alone and by the simultaneous method.

ON FERTILITY

In these field investigations it was found that of 2,362 healthy brood sows in 181 herds treated with serum and virus, 95 percent produced litters the following year. Of 1,840 healthy untreated sows in 148 comparable herds, 94 percent produced litters the following year. It was concluded that the simultaneous treatment does not cause infertility the following year.

ON YOUNG PIGS

As the use of serum and virus increased, the possibility of reducing the dose of vaccination by vaccinating baby pigs was investigated. A number of investigators and observers stated that the simultaneous treatment of young pigs did not confer lasting immunity against cholera.

An experiment to obtain data on this subject was reported by the Chief of the Bureau of Animal Industry in 1918. In this experiment, 29 pigs a few days old were treated by the simultaneous method. They were exposed to cholera 5 months later and found to be immune. In another experiment reported in 1919, 139 pigs from 1 to 3 weeks old were given serum and virus, and exposed 6 months later (*99*). Three pigs died, but it could not be determined that the deaths were due to cholera. Since these results did not agree with results of other researchers, it was decided to extend the investigations.

Work was carried out on six widely scattered farms under control of the Animal Husbandry Division, and covered a period of 5 years beginning in 1921 (*48*). Pigs from immune sows were vaccinated with serum and virus when 1 day to 9 weeks old, and were exposed 3 to 7 months later. At least 30 percent of all pigs immunized were less than 3 weeks old when treated. Prior to 1924, the doses of serum ranged from 20 to 30 cc. and the doses of virus from ¾ to 2 cc. After 1924, serum doses were as low as 10 cc. and virus doses ranged from 1 to 4 cc. Altogether, 3,187 pigs were treated. Of these, 1,645 were exposed, and 211, or 12.8 percent, died.

Of the 211 deaths after exposure, 195 occurred in 1924. Eliminating that year, of 2,181 pigs vaccinated and 980 exposed, only 16, or 1.6 percent, died. The cause of heavy losses in 1924 could not be definitely determined, but suspicion was directed toward the virus. The virus had passed satisfactory tests at Ames, was shipped to Beltsville, and stored there for some time before use. It may have deteriorated in the interim between test and use.

On the whole, the results of these experiments indicated that lasting immunity can be produced by vaccinating young pigs. However, it is known that pigs farrowed by immune sows have considerable resistance to hog cholera while very young, and many practicing veterinarians still consider that the safest time to vaccinate is shortly before or after weaning.

Immunity of Pigs Farrowed by Immune and Susceptible Sows

Thirty-five litters of pigs from immune sows and four litters farrowed by susceptible sows were given injections of virus when the pigs were from 3 days to 3 weeks of age. All the pigs farrowed

by susceptible sows died of cholera, and all farrowed by immune sows remained normal. After the pigs from immune sows were weaned they were re-exposed to cholera. Approximately 75 percent developed cholera, and approximately 50 percent died.

It is probable that the injection of virus into very young pigs increased their immunity. More information would have been obtained on this subject if only half of each litter had been given injections of virus and the other half left without virus to serve as controls. However, a very great number of other pigs farrowed by immune sows have been treated with virus after weaning time, and a large percentage of them have been susceptible to cholera. Pigs farrowed by susceptible sows are somewhat more susceptible than pigs farrowed by immune sows, and for this reason, such pigs are preferred for experimental work.

Effects of Feeding Protein

Some investigators thought that feeding high protein feeds might prevent the establishment of immunity, or cause serum-virus-treated pigs to lose immunity.

In experiments to obtain data on this subject, pigs were placed on high protein diets before, after, and at the time of simultaneous treatment. There were no indications that high protein feeding prevented the production of, or caused the loss of, immunity. The results of these experiments should not be construed to mean that a proper diet at vaccination time is not considered good practice.

Neutralization of Virus by Serum

Clear serum and clear virus were mixed in the proportion of 1 part of virus to 10 parts of serum. The mixtures were incubated for 2 hours, or held at room temperature for 2 hours. Pigs were given injections of 1 cc. of the mixture. At the same time control pigs were given injections of 0.09 cc. of virus alone. The pigs treated with 1 cc. of the mixture remained normal and the controls developed cholera. This indicated that 0.9 cc. of serum neutralized 0.09 cc. of virus.

In a similar experiment, pigs were given injections of 0.9 cc. of serum and 0.09 cc. of virus in different parts of the body. All pigs developed cholera. These experiments indicated that 0.9 cc. of serum will neutralize 0.09 cc. of virus *in vitro*, but not *in vivo*.

In another series of tests, washed red blood cells from virus blood were mixed with hyperimmune serum in the proportion of 1 to 10 and injected into susceptible pigs. In some experiments some neutralization appeared to have occurred, but the results were erratic.

Finely ground spleen tissue from a cholera-infected pig was mixed with serum in the proportion of 1 to 10 and injected into susceptible pigs. All developed cholera, which showed that complete neutralization had not occurred.

In a later experiment red blood cells from virus blood were washed seven times with normal salt solution and mixed with hyperimmune serum in the proportion of 1 to 10. The mixtures were held 2½ hours at 72° F., the serum was removed by centrifugation, and the cell residue washed twice with salt solution. The cells were injected

into susceptible pigs and all developed cholera. The experiment was repeated in the same manner, except that the mixtures were held at 37.5° C. for 2 hours. The results were the same; there was no neutralization of cells by serum, either heated or unheated.

The foregoing experiments showed that virus in cell-free virus serum could be neutralized by hyperimmune serum in the proportion of 1 to 10, but virus in red blood cells or minced spleen tissue was not neutralized.

In an experiment conducted in 1916, serum and virus were mixed before administration. The mixture contained 30 cc. of serum to 2 cc. of virus and was kept for 17½ hours before it was administered to susceptible pigs in doses of 32 cc. Ten days later the pigs were bled, and their blood was injected into susceptible pigs. The pigs sickened promptly, which showed that the virus had not been neutralized by the serum either *in vitro* or *in vivo*. The pigs that received the mixture were held free from exposure for 3 months, then tested for immunity by virus injection, and found to be immune. Since this method had no particular advantage over the regular simultaneous treatment except in ease of administration, and because a large amount of experimentation would have been necessary before the method could be recommended, it was not further pursued.

Vaccination Reaction

When virulent hog cholera virus is injected into susceptible pigs, even in conjunction with large doses of potent serum, a reaction always follows. The reaction, in the majority of cases, is invisible, but the natural resistance of the pigs is lowered, due perhaps to a leucopenia which occurs from 4 to 8 days after virus injection. This lowering of resistance may render the animals susceptible to bacterial infection and other debilitating conditions, and sickness sometimes occurs. This sickness is referred to as a "break" and heavy losses are at times sustained, even when ample doses of potent serum are administered. Cholera is often the cause of death in these cases, perhaps because the combination of the reaction from vaccination and secondary infection, or a debilitating condition, is more than the animal can withstand. This condition has been observed occasionally since the beginning of the serum-virus treatment, and is one good reason for careful examination of a herd before treatment is administered.

Heavy losses have occurred in hogs previously treated for immunization, because of delays in diagnosis. Veterinarians are reluctant to make a diagnosis of cholera in a previously treated herd, but cholera in such herds is not uncommon. If indications of cholera are found, the herd should be re-treated without delay.

Leucocyte Counts in Normal, Cholera-Infected, and Serum-Virus-Treated Pigs

In 1914, Dinwiddie (*31*) reported on the white cell counts of blood of normal and cholera-infected pigs. From 7 normal pigs he obtained counts as low as 7,000 per cubic millimeter, and placed the average at approximately 11,000 per cubic millimeter. He made blood counts of 14 cholera-infected pigs at different stages of the

disease, and found a distinct leucopenia, which was usually so marked as to be discernible in stained smears.

Lewis and Shope (*77*), in 1929, stated they found that the white cell count of normal pigs varied from 14,000 to 24,000 per cubic millimeter.

The foregoing citations, which by no means exhaust the literature on the subject, show that leucopenia is a well-recognized phenomenon in hog cholera. They also show that the white cell count of normal hog blood has been found to vary within a considerable range.

In Bureau experiments, 131 counts were made on 48 apparently normal pigs. The counts varied between 12,000 and 39,000, with an average of 20,000 per cubic millimeter (*6*).

Sixty-five counts were made on 15 pigs known to be infected with cholera. One hundred and forty counts were made on other pigs in various stages of cholera. In a large number of cholera-infected pigs the white cell count was lowered as the disease progressed. Counts as low as 3,300 per cubic millimeter were noted in cholera pigs, although high counts were observed in some pigs known to be infected. It was concluded that leucopenia was a usual, although not constant, feature in hog cholera, and that counts below 8,000 per cubic millimeter were an indication of the disease, although counts above 8,000 could not be considered a certain indication of the absence of that disease (*6*, *7*). Powick (*100*) calculated that the probability of diagnosing hog cholera correctly by means of white cell counts was 81 percent.

WHITE CELL COUNTS FOLLOWING SERUM-VIRUS TREATMENT

Twenty-eight pigs were used in three experiments to determine if leucopenia following serum-virus treatment is a usual phenomenon. The dosage of serum was varied from 20 cc. to 60 cc. Three daily counts were made before serum and virus were administered to obtain the average white cell count of each pig. Counts were made from the 1st to the 14th day after treatment. There was a distinct but transitory leucopenia in all 28 pigs treated with serum and virus. The severity of the leucopenia appeared to be related to the dose of serum, it being more marked and of longer duration in the lower dosage. In several cases the count was below 8,000, although the pigs showed no visible symptoms of sickness. After this discovery, it was considered by many that the leucopenia, which is equivalent to lowered resistance, explained why some breaks occur following serum-virus treatment.

WHITE CELL COUNTS FOLLOWING SERUM ALONE

No leucopenia was observed following serum-alone treatment. Also, when long-time-immune pigs were given injections of virus, leucopenia did not occur. White cell counts were made at intervals for many years, and no reason was found to change the original conclusions.

Leucocyte counts were made of a number of pigs affected with necrotic enteritis. White cell counts of 9 of these pigs ranged from 27,800 to 37,800 per cubic millimeter, with an average of 30,311.

Since leucocytosis was found quite regularly in pigs affected with necrotic enteritis, it is considered that the leucocytosis frequently found in pigs known to be infected with cholera might be due to the presence of secondary infection with necrotic enteritis or some other bacterial disease.

Lesions After Serum-Virus Treatment

When investigations are made to determine the cause of sickness following serum-virus treatment and lesions of cholera are found, the investigator frequently explains that the lesions were caused by vaccination. They are often referred to as "vaccination lesions." This statement has been made so often that many veterinarians consider it a statement of an established fact. Experimental evidence does not confirm this statement.

Two experiments were conducted to obtain information on vaccination lesions. The first was in 1916, when 9 pigs were treated with serum and virus. They remained normal for 10 days, and were killed and examined for lesions. No lesions were found in 5, and in the other 4 only a very few indistinct petechiae were observed on the mucosa of the bladder. In January 1952, as a part of another experiment, 12 pigs were treated with serum and virus and remained apparently normal. Four were killed on the fifth, 4 on the seventh, and 4 on the tenth day after treatment. No lesions were found in 8, and but slight abnormalities were found in the other 4. All lesions were very slight and could not be considered indicative of any particular disease. Cultures were made from the spleen, kidneys, liver, lungs, and heart blood. Growths occurred in only 3 of the 12 pigs. The organisms isolated were those often found in normal pigs.

Immunization by Serum and Virus of a Herd Affected With "Flu"

A farm herd of 60 pigs, weighing from 100 to 250 pounds each, was treated with serum and virus while in the acute stage of influenza. The entire herd was coughing and breathing with difficulty, and 40 percent had temperatures between 104° and 107° F. On the sixth day after treatment, symptoms of "flu" had subsided, and all pigs remained normal thereafter. Two months later 5 pigs from this herd were given injections of virus, and were immune. This treatment is not recommended, or considered good practice, but it did show that, if necessary, swine affected with "flu" can be successfully immunized with serum and virus.

PART 4. TRANSMISSION OF HOG CHOLERA

From the time hog cholera was found to be a specific disease, many experiments have been conducted to obtain definite information on the manner in which the disease is transmitted. In an early report (*56*), the following possible channels of infection were enumerated:

(1) Pigs purchased from infected herds, or coming in contact with pigs from such herds, or running over ground occupied by diseased swine, may spread infection.

(2) Infected streams may carry the disease below the source of infection.
(3) Virus may be carried on feed implements, and on the feet or clothing of persons from infected premises.
(4) Wind, insects, birds (particularly buzzards), and various animals may transport hog cholera virus.

Transmission Experiments

During the years 1916, 1917, and 1918, a great many transmission experiments were carried out, some of which are described.

On Clothing and Footwear

Attendants entered the building occupied by cholera-infected pigs to take the pigs' temperatures. They would then, without any precaution except to disinfect hands and thermometers, enter pens containing susceptible pigs. Sometimes they would intentionally step in excreta in the infected pens and step in the feeding troughs in the pens containing susceptible pigs. This procedure was continued for 15 to 32 days. The distance between the infected and the noninfected pens at first was 100 yards; later it was decreased to 50 yards. In many of these experiments all of the pigs exposed remained normal, and were later given injections of virus to test their susceptibility. About 40 susceptible pigs were used in these experiments and only 3 developed cholera. The results were contrary to expectations, although other experiments had demonstrated that hog cholera virus does not live long outside the animal body in warm weather. These experiments were conducted in both hot and cold weather.

Close Contact

Several experiments were conducted in the following manner, in both cold and hot weather:

Two experimental pens were placed facing together, entirely separated but only a few inches apart. The space between the pens was covered with tar paper or screen wire. Cholera pigs were placed in one pen and susceptible pigs in the other. The pens were never entered by attendants, feeding being done through chutes. In one of these experiments the susceptible pigs developed cholera by close contact, although they were entirely separated. In the other experiments, the disease was not transmitted to susceptible pigs.

A small pig was placed in one of the isolation pens in April and left there until November. During the 7 months, cholera-infected pigs were periodically kept in other pens, some of them within 50 feet of this pig. This pig was cared for by the same attendants who cared for both sick and well pigs in nearby pens. Flies were abundant during the summer months. The pig remained normal until November when it was given an injection of hog cholera virus, developed cholera, and was killed for virus.

Pigeons

Two pens were placed 10 feet apart; cholera pigs were kept in one pen, and susceptible pigs in the other. The space between the two pens was enclosed with 1-inch-mesh wire netting and pigeons were

placed within the enclosure. Both pens were open and the pigeons flew from one pen to the other. When sick pigs died, they were replaced with others so that the infection was constantly present. The pen was not cleaned during the course of the experiment. Susceptible pigs were exposed in this manner for 30 to 40 days. In no case was disease transmitted by pigeons. This and similar experiments indicated that the disease is probably not often carried from one farm to another by pigeons.

Recovered Pigs

Pigs that had recovered from cholera were tested by contact with, and by injections of their blood into, susceptible pigs. A large number of recovered pigs were tested in this manner, and none proved to be carriers of cholera.

Excretions and Secretions

Repeated experiments have shown that the blood of pigs inoculated with virus becomes infectious for others within 24 hours. The urine and feces contain the virus usually within 48 hours, and the secretions of the eyes and nose become infectious by the third day. Rarely do pigs show visible symptoms earlier than the fourth day. These experiments showed that infected pigs are capable of transmitting disease before they show any visible symptoms. Nevertheless, as previously stated, in many experiments susceptible pigs were kept in pens with serum-virus-treated pigs, the blood of which was found to contain active virus, and remained well unless the serum-and-virus-treated pigs became sick.

Contact at Different Stages of the Disease

Susceptible pigs were given injections of virus and placed in a clean, disinfected pen. Other susceptible pigs were left untreated and placed in the same pen with the inoculated pigs for 48 hours. The untreated contact pigs were removed to another disinfected pen and kept under observation. The inoculated pigs also were moved to another disinfected pen, and other susceptible pigs were placed in the pen with them and left for 48 hours. This procedure was repeated daily for 20 days. Hog cholera was not transmitted to susceptible pigs during the first 48 hours of exposure. After the first 48 hours the disease was transmitted to all pigs left in contact with the inoculated pigs, even though the inoculated pigs showed no visible symptoms of sickness.

Infected Premises

A number of experiments were carried out to determine the length of time that cholera infection is likely to remain on premises following an outbreak of cholera. Pens of different construction were used, some having dirt, others concrete, and others wooden floors. Sick pigs were placed in the pens and in some cases allowed to die. Susceptible pigs were then placed in these pens at different intervals after removal of the sick or dead pigs. In warm weather of late summer and early fall, healthy pigs did not contract cholera when

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placed in pens 24 hours after the removal of sick pigs. In one case, susceptible pigs did not contract cholera when put into a pasture 15 minutes after cholera-infected pigs had been removed. In colder weather the pens remained infectious for much longer periods. These experiments were repeated many times, both at the experiment station and on infected farms. In many cases, infection was not present in warm weather in pens and pastures 24 hours after sick pigs were removed. The practical conclusion was that it is comparatively safe in hot weather to restock farms soon after an outbreak of cholera, but not safe in cold weather.

Soil Experiments

Soil was taken in hot weather from beneath a hog house in which pigs having chronic hog cholera were kept, and where hogs had died at intervals for several years. One pint of surface soil and one pint of subsurface soil were mixed with feed, and fed to susceptible pigs. The pigs remained normal and were challenged later and found to be susceptible to cholera. This experiment indicated that the virus of hog cholera remains virulent only a short time outside the animal body in hot weather.

Artificially Contaminated Soil

Soil was collected from a location supposedly free from hog cholera virus and was fed to susceptible pigs to test it. Then soil was collected from the same location, and mixed with urine from a cholera-infected pig. The mixture was held for 20 minutes, and fed to susceptible pigs. The pigs remained normal and were later found to be susceptible. Since urine from cholera-infected pigs is known to contain hog cholera virus, this experiment confirmed the supposition that virus does not long survive outside the animal body in warm weather. The same urine used in this experiment, when injected subcutaneously into susceptible pigs immediately after it was drawn, produced cholera.

Flies

Experiments were conducted to determine if flies and other insects were important factors in the transmission of hog cholera. In some cases flies that were known to have fed on cholera-infected pigs were caught and placed in screened pens containing susceptible pigs. In other cases, flies that had fed on sick pigs were ground and fed to susceptible pigs. While it was possible to produce cholera by macerating and injecting into susceptible pigs flies that had sucked blood from sick pigs, in no case was evidence found that cholera was transmitted by flies under natural conditions. In many other experiments, such as the pigeon experiments previously described, flies were abundant but did not transmit disease to susceptible pigs.

Mosquitoes

Mosquitoes were allowed to feed for several days on cholera-infected pigs kept in screened pens. The mosquitoes were transferred to other screened pens containing susceptible pigs. The sus-

ceptible pigs all remained normal and their susceptibility was later determined by virus injection.

Hog Lice

Forty-five hog lice were removed from a cholera-infected pig and placed on one susceptible pig. It remained normal. In another experiment 100 lice were removed from a sick pig that was being killed for virus, and immediately transferred to a susceptible pig. It also remained normal for 23 days. The latter pig was then given an injection of hog cholera virus and found to be susceptible.

May Beetle Larvae

Twenty-five larvae of the May beetle were taken from the ground at the edge of a pit that contained manure from a barn in which many virus pigs were kept. The larvae were mixed with bran mash and fed to susceptible pigs. All the pigs remained free from disease, but developed cholera when given an injection of virus.

In Cured Meats

Experiments were conducted to determine whether hog cholera could be transmitted to susceptible pigs by feeding them meat from cholera-infected pigs after the curing and smoking process. Hams and shoulders from cholera-infected pigs were placed in brine, and others were placed in dry salt cure. After 52 days in the cure and 3 days in hickory smoke, portions of the meat were finely chopped, mixed with bran mash, and fed to susceptible pigs. In many instances the pigs developed cholera after eating the meat that had been cured, by either the brine or dry-salt method, and smoked.

Carcasses of Cholera-Infected Pigs

Carcasses of cholera-infected pigs were exposed in a metal wheelbarrow where they were protected from direct sunlight, and others were buried 2 feet under the ground. At intervals portions of these carcasses were fed to susceptible pigs. In summer, the infectiousness disappeared from both the buried and unburied carcasses—as a rule, within 7 days. In winter, the virus remained alive and active for several months. The natural conclusion was that the processes of putrefaction and decay destroy the hog cholera virus, while freezing preserves it.

Feed

Experiments were conducted to compare the effects of injecting virus subcutaneously and by mixing virus with feed. The only difference observed in these experiments was that the pigs infected by subcutaneous injection showed visible symptoms of sickness from 1 to 2 days earlier than the pigs exposed by feeding.

Crows

Six young crows were taken from a nest and placed in a chicken-wire enclosure, 20 by 10 feet and 8 feet high. In one corner of

this enclosure a space 5 by 7 feet was fenced off, and susceptible pigs were placed therein. In the opposite corner opened carcasses of cholera pigs were placed for short intervals. A perch was built above the enclosure where the susceptible pigs were kept. The crows were seen to feed upon the carcasses and fly to the perch above the susceptible pigs. The pigs developed cholera in a comparatively short time.

An investigator went to a farm in an isolated community to investigate the transmission of cholera. As he and the farmer approached the hog lot, a large flock of crows flew from the trees. An object was seen to drop, and was found to be a small bone. Since the farmer could not think of any other way his hogs could have been infected, the crows were blamed for the outbreak.

Scarification

In these experiments pigs were inoculated through small scarification wounds on the back of the ears. The skin of the ear was shaved, disinfected, dried, and scraped lightly with a sterile scalpel in a manner similar to that followed in smallpox vaccination. Six pigs were inoculated in this manner, and all developed cholera. Four died and two recovered.

Dogs

Two experiments were conducted in which a dog was fed for 5 days exclusively on meat from cholera-infected pigs. Feces from the dog were mixed with bran mash and fed to susceptible pigs. Hog cholera was not transmitted in that manner. However, it is reasonably certain that dogs do carry hog cholera by bringing bones from pigs that have died of cholera to farms not previously infected.

Rat-Feeding Experiment

Two gray rats were fed for 5 days with meat from cholera-infected pigs. The rats were killed, and entire carcasses were chopped up, mixed with bran mash, and fed to susceptible pigs. The pigs remained normal for 2 weeks, but were found to be susceptible when later given virus injections.

Dust

Dust was collected from the top of concrete partitions between pens in the building that housed pigs affected with hog cholera. The dust was mixed with normal salt solution in the proportion of 0.6 gram of dust to 50 cc. of salt solution. The mixture was filtered through filter paper and injected into susceptible pigs in 20-cc. doses. The pigs remained normal. In other experiments, dust was blown into the eyes and nostrils of susceptible pigs daily for as long as 14 days. In no case was disease transmitted to susceptible pigs in this manner. All pigs in these experiments were proved later to be susceptible by virus injection.

Syringes

In connection with investigations to determine the cause of unfavorable results following treatment with serum and virus, it was

considered possible that uncleaned syringes might be one of the causes, particularly where farmers had done their own vaccinating. An experiment was therefore carried out to obtain data on the time hog cholera virus would remain virulent in syringes that had been rinsed, cleansed, or sterilized after using.

Syringes and test tubes were filled with hog cholera virus known to be virulent, and after approximately 3 minutes the virus was expelled; the syringes and test tubes were held at room temperature (72° to 74° F.) for future tests without being rinsed, cleansed, or sterilized. Twenty-six days later the syringes and test tubes were filled with sterile water and agitated until the dried blood was suspended in the water. The contents of each syringe and tube were then injected into susceptible pigs in doses of 2½ and 7 cc. All pigs remained normal. They later were found to be susceptible. The results indicated that unsterilized syringes held at room temperature (72° to 74°) for 26 days or more are not likely to transmit hog cholera.

Infected Pens

A pen that contained cholera-infected pigs was closed on December 17. On June 5 the following year susceptible pigs were placed in the pen and did not contract cholera.

Conclusions

The close contact, the pigeon, the infected premises, the insect, the carcass feeding, and the dust experiments did not confirm many of the popular theories as to how cholera is transmitted. It was found that the sick pig, infected meat scraps, and meat-eating birds were responsible for many outbreaks of cholera. The investigations did not, of course, eliminate or confirm all of the possible ways by which hog cholera might be transmitted.

PART 5. VACCINES

Early Hog Cholera Vaccines

The first attempt to produce a vaccine for hog cholera, after discovery of the filtrable virus, was made in 1903. Glycerine was added to defibrinated hog cholera blood, and the mixture was heated for varying lengths of time to a temperature somewhat above fever temperature of animals. Immunity was produced in some cases, but in other cases the injection produced cholera. Lack of uniformity in results might have been studied further, and possibly solved, if the simultaneous treatment had not been discovered almost contemporaneously. After that, all research was concentrated on perfecting the treatment and serum production.

Horse Serum

The next attempt to produce vaccine was made in 1910, when the results of an experiment by the Kansas State Agricultural Experiment Station indicated that hogs had been successfully immunized with blood obtained from a horse hyperimmunized with

hog-cholera-virus blood. A horse was immediately hyperimmunized by the Bureau, and serum was prepared from the horse blood, but it proved to be impotent.

Heated-Blood Vaccine

An unidentified investigator stated that hog cholera blood heated for 30 minutes at 60° C. could be used as a vaccine. This method of producing immunity was tried, but all 14 pigs used in the test developed cholera and died.

Ammoniated-Blood Vaccines

Ammonia in varying concentrations was added to hog cholera blood. The ammoniated blood was stored at different temperatures for varying lengths of time, and then tested. A low concentration of ammonia (0.125 percent) destroyed the disease-producing properties in the blood in a very short time. Two hundred and seventy-five pigs were used in testing ammoniated viruses. All pigs remained normal and were later tested for immunity by virus injection. Many of the pigs were found to be immune, but some developed cholera, and so the method was considered impractical.

Formalized Vaccines

A solution containing about 0.31 percent of formaldehyde or 1 percent of formalin was added to hog cholera blood to make a vaccine. In 1924, three lots of formalized blood were prepared and tested. Lot 1 contained 9 parts of blood to 1 part of a 1-percent formalin solution; lot 2 contained 8½ parts of blood to 1½ parts of 1-percent formalin solution; lot 3 contained 8 parts of blood to 2 parts of 1-percent formalin solution. The three mixtures were held for 14, 30, and 55 days in a cave where the temperature was 40° to 50° F. and then tested, with irregular results. At times results appeared very favorable, but because of their irregularity, the work was abandoned.

Formalized-Tissue Vaccines

Vaccines were prepared by the Staub method from formalized tissues, mostly spleens from cholera-sick pigs. As in other attempts to produce vaccine, excellent results were obtained at times, but after a long period of investigation, it was concluded that results were too irregular for practical purposes.

Glycerine Vaccines

During the fiscal year 1933–34, 22 lots of glycerine vaccine were prepared, of which 16 were 40-percent glycerine and 6 were 30-percent glycerine. They were incubated for from 1 to 14 days. In all lots incubated 14 days the virulence of the virus was completely destroyed. At the end of incubation the vaccines were tested in doses of 5 and 10 cc. The potencies of the different lots varied considerably, owing probably to the fact that each lot was prepared from the blood of one or two pigs, and pigs vary in their

ability to produce satisfactory vaccines. The physical condition of the vaccine was not changed by incubation, and the finished product was of good consistency.

During the next fiscal year, 18 lots of vaccine were again prepared, all of which were 40-percent glycerine. Portions were removed and tested after 7 days of incubation, and the remaining portions were removed and tested after 14 days of incubation. Seventy-two pigs were used in these tests. In one test, 2 pigs died before exposure, indicating attenuation was not complete. In all of the other 35 tests the pigs remained normal, and were exposed to cholera 3 weeks later. Forty-four of the exposed pigs remained normal, 9 had slight reactions, 4 had severe reactions, and 13 died. In these experiments, 75.7 percent of the pigs were adequately protected.

Very good results were obtained with glycerol vaccines. Such results before discovery of serum and virus would have been considered miraculous.

Glycerine-Tissue Vaccines

Six lots of vaccine were prepared from tissues of cholera-infected pigs. Spleens, kidneys, livers, and lymph glands were finely ground; glycerine in the amount of half their volume was added, and the mixtures were incubated for from 7 to 10 days. Three of these lots afforded good protection when tested; three were of low potency. In the work with glycerine vaccine it was found that the disease-producing property of the blood was invariably destroyed after 2 weeks' incubation. It was found also that vaccines, each made with blood from a different pig, varied considerably in potency. When made from the mixed blood of 8 or 10 pigs, the potency was usually satisfactory.

Orthocresol Vaccines

Twelve lots of vaccine were prepared by adding 10 cc. of a 3-percent aqueous solution of orthocresol to 90 cc. of defibrinated-blood virus, and incubating the mixture at 37° to 38° C. for 2 weeks. Satisfactory protection was afforded in 66.6 percent of the 24 pigs used in the tests. These vaccines became semisolid when incubated, and the finished product was not of satisfactory consistency.

Five additional lots of orthocresol vaccine were prepared by diluting the defibrinated virus blood with an equal amount of sterile salt solution before adding the orthocresol solution. The protection was 90 percent with this diluted vaccine.

An attempt was made to overcome gelatinization by combining orthocresol and glycerine. While vaccines made in this manner afforded 100-percent protection, the gelatinization of the finished product was not overcome.

Phenolized Vaccines

A lot of old phenolized virus was tested for virulence in 1933, and was found to have lost its ability to produce disease. Injections of 10 cc. were given to two susceptible pigs to determine if

it would produce immunity. The two pigs were exposed to cholera 3 weeks later and were found to be immune. This finding resulted in an extensive study of the use of phenolized virus vaccines.

In the first experiments the blood was prepared in the same manner that simultaneous virus is prepared; that is, by the addition of 1 part of a 5-percent solution of phenol to 9 parts of blood, which gives 0.5 percent of phenol to the finished product. The phenolized mixture was then incubated at 37.5° C. Lots were tested after 1 and 2 weeks of incubation. Three of six lots incubated for 1 week were still virulent, but all lots incubated for 2 weeks had lost virulence. They were then tested for potency by injection into susceptible pigs, followed in 3 weeks by injection of virulent hog cholera virus.

As a result of these preliminary experiments it was concluded that—

(1) Potent vaccines could be prepared from defibrinated virus blood by the addition of 0.5 percent of phenol, followed by a proper period of incubation.

(2) An incubation period of 2 weeks is necessary to destroy the disease-producing element of the virus, or to attenuate it to a point of safety.

(3) Properly attenuated, phenolized vaccines, administered in 10-cc. doses, yielded 100-percent protection in a limited number of susceptible pigs.

(4) The results obtained with phenolized vaccines warranted continuation of the experiments.

During 1935, phenolized vaccines were prepared in the same manner as in the preceding year, and will be referred to as "ordinary phenolized vaccines." Later, variations were tried with a view to improving the vaccines. An incubation period of 2 weeks was not always sufficient to attenuate the virus to the point of safety, and the incubation time was extended to 3 weeks.

Of 42 pigs treated with vaccine incubated 1 week, 3 became sick; of 100 pigs treated with vaccine incubated 2 weeks, 7 became sick; all of the 32 pigs treated with vaccine incubated 3 weeks remained well. The percentages of pigs found to be adequately protected when exposed 3 weeks after vaccine treatment, were as follows: With vaccine incubated 1 week, 76.9 percent; 2 weeks, 86.02 percent; 3 weeks, 96.8 percent.

TIME REQUIRED FOR IMMUNITY TO DEVELOP

To determine the time required for immunity to develop with phenolized vaccine, a number of pigs were given injections of 10 and 40 cc., respectively, with simultaneous injection of virus; other pigs received 10 cc. of vaccine, followed by virus in 1, 2, and 3 weeks, and in 4 months. The pigs given vaccine and virus at the same time, and those given virus 1 week after vaccine, developed cholera. The pigs given virus 2 and 3 weeks and 4 months after the vaccine, remained normal.

CLEAR PHENOLIZED VACCINE

Clear serum virus vaccines and defibrinated-blood vaccines were prepared from the same lots of defibrinated-blood virus. They were

phenolized, incubated, and tested at the same time with the same lots of pigs. The results of potency tests were almost identical. Approximately 75-percent protection was obtained with both types of vaccine.

Although these experiments were limited in the number of lots of vaccine prepared and the number of pigs, they indicated that potent vaccine may be produced from clear serum virus.

Since clear serum-virus vaccine production required more work and other expense, and did not excel regular defibrinated-blood vaccine, this method was not continued. The information obtained in this experiment, however, might be of interest in case it becomes necessary in the future to prepare vaccines from pasteurized serum virus.

DILUTED PHENOLIZED VACCINES

These vaccines were prepared by using equal amounts of virus blood and normal salt solution. The mixture was phenolized and incubated for 2 weeks and then tested in 10-cc. doses. The results were satisfactory, but the method was not further investigated because of other work.

POTENCY OF PHENOLIZED VACCINES

Fifty-eight lots of phenolized vaccines were prepared from the blood of pigs killed on the 4th, 5th, 6th, 7th, 8th, 9th, 10th, and 11th days after inoculation. The vaccines were tested for potency; those made from blood obtained on the 6th to 9th day after inoculation were superior in potency to those made from blood drawn either earlier or later.

SUMMARY ON PHENOLIZED VACCINES

(1) Vaccines possessing high potency may be prepared from defibrinated-blood virus by the addition of 0.5 percent of phenol, followed by incubation.
(2) Between 2 and 3 weeks of incubation is required to attenuate the virus properly, and it would probably not be safe to make the period less than 3 weeks.
(3) Blood drawn earlier than the 6th or later than the 9th day failed to afford satisfactory vaccines.
(4) In tests of 87 lots of vaccine, satisfactory protection was afforded in 85.9 percent of the pigs treated.
(5) A dose of 10 cc. administered subcutaneously was sufficient to immunize pigs weighing 50 to 100 pounds, and a limited number of tests indicated that 5 cc. might be sufficient.
(6) At least 2 weeks is required to establish immunity.
(7) Immunity following vaccine treatment lasts for at least 2 months and probably longer.
(8) There was no apparent diminution in potency of vaccines held in cold storage between 6 and 7 weeks.
(9) The addition of 40 percent of glycerine after 2 weeks of incubation appeared to have no particular advantage.
(10) Vaccines prepared with clear serum virus, obtained by centrifuging defibrinated-blood virus, yielded about the same

results as corresponding vaccines prepared from defibrinated-blood virus.

(11) After storage, phenolized vaccines thickened, and sometimes the serum and the cellular elements separated.

Propylene Glycol Vaccines

Propylene glycol vaccines were produced, but they thickened after short periods of incubation and were not satisfactory for potency.

Chloroform Vaccines

Three lots of vaccines were made from tissues of cholera pigs to which small amounts (1.5 percent or less) of chloroform were added. Portions of liver, spleen, kidney, lymphatic glands, and sometimes testicle tissues were used. The tissues were suspended in sterile salt solution, chloroform was added, and the mixture was incubated for 48 hours.

Vaccines were also made from spleen tissue only. These vaccines were of higher potency than those mentioned above. Like other vaccines, those made with chloroform at times yielded promising results but were not dependable.

In later experiments, an attempt was made to produce vaccine from the defibrinated blood of cholera pigs with chloroform as the attenuating agent. Two lots of virus blood were divided into two portions; 1.5 percent of chloroform was added to one portion, and 2 percent to the other portion of each lot. The lots were further subdivided and stored at room and refrigerator temperatures, and tested after varying periods of storage. The portions stored at room temperature completely lost disease-producing properties after 3 weeks, but those stored at refrigerator temperature were still virulent after 9 weeks. The pigs treated with blood stored at room temperature remained well, but all developed cholera when later exposed.

Brilliant Green Vaccine

This vaccine was prepared by Weichlein's formula. Pigs given injections of mixtures of brilliant green and defibrinated virus blood incubated for 24, 48, 72, and 96 hours developed swellings at the point of injection and symptoms of cholera. Pigs given injections of 10 cc. after 120 hours of incubation developed severe swellings and slight sickness, but recovered. They were given injections of virus 21 days after treatment, and remained normal. The results indicated that incubation for 4 or 5 days was required to attenuate the virus, but after sufficient attenuation, it produced immunity in two pigs. The irritating effect of this vaccine rendered it unsuitable and therefore further investigation was abandoned.

General Summary of Early Vaccines

Vaccines were produced by the methods described that were apparently incapable of causing disease but were capable of protecting up to 100 percent of the vaccinated pigs against hog cholera. The processes almost invariably involved incubation or storage of virus blood or tissues to which a bacteriostatic or bactericidal agent was

added. However, some of the most effective of these vaccines had undesirable properties, such as a tendency to thicken or separate during the incubation period, and in some cases the vaccines were not sterile.

It is probable that investigations of some of the better of these vaccines would have been continued had it not been for preliminary work suggested by Dr. Dorset involving the use of crystal violet in vaccine preparation. These preliminary experiments proved so successful that most of the experimental work thereafter had to do with the production of crystal violet vaccine.

Crystal Violet Vaccines

Dr. Marion Dorset, who for many years had sought to develop a vaccine that would afford a cheaper and safer method of immunizing swine against cholera, decided in 1934 to try crystal violet as an attenuating agent. The results obtained in the preliminary experiments with this dye were very gratifying to Dr. Dorset, but soon thereafter he was stricken with a brief illness and on July 14, 1935, he died. After his death, research work with crystal violet was carried on by his coworkers in the Bureau of Animal Industry. Results of these early experiments with crystal violet were reported in 1936 (*88*).

Crystal Violet Bacterial Vaccines

In preliminary experiments, guinea pigs were immunized against a virulent strain of *Salmonella suipestifer* by crystal violet vaccine prepared from a broth culture of that strain. Heated, killed cultures also produced immunity against *S. suipestifer*. Crystal violet vaccine prepared from cultures of both *Brucella abortus* and bovine-type tubercle bacilli failed to establish immunity. Filtrates from cultures of *Bacillus botulinus*, types A and B, were found to be detoxified by crystal violet, but the resulting mixture did immunize guinea pigs against a subsequent injection of the toxin.

First Crystal Violet Hog Cholera Vaccine Production

In preparing the first lots of crystal violet hog cholera vaccine in 1934, a 1-percent aqueous solution of the dye, made by adding 1 gram of crystal violet to 99 cc. of distilled water, was added to defibrinated-blood virus in sufficient quantities to give crystal violet concentrations of 1 to 2,500 in some cases, and 1 to 5,000 in others; the mixtures were then incubated for 2 weeks. Thirty-four lots of vaccine were prepared during 1934–35, and 22 lots were tested. Twenty of these 22 lots were found satisfactory for potency; 98.8 percent of the pigs used in the tests were adequately protected when exposed to cholera by virus injection 3 weeks after treatment.

During 1935–36, the formula for producing crystal violet vaccine was modified slightly by adding 1 part of a 1-percent phenol solution to 9 parts of blood, and then adding 5 parts of a 1-percent crystal violet solution to 100 parts of the previously phenolized blood. The phenol was added to increase the bactericidal power of the mixture. The mixture was then incubated for 2 weeks at 37.5° C. Eighteen lots of this vaccine were tested for potency. Of 80 pigs

used in these tests, 96.2 percent were adequately protected. There were no deaths, but three pigs had severe reactions.

In one experiment, 18 lots of glycerine vaccine, 87 lots of phenolized vaccine, and 20 lots of crystal violet vaccine were compared for potency. Protection up to 76 percent was obtained with glycerine vaccine, 86 percent with phenolized vaccine, and 98.8 percent with crystal violet vaccine.

TIME REQUIRED FOR DEVELOPMENT OF IMMUNITY

A number of pigs were treated with vaccine and virus simultaneously. Others were given virus 1, 2, and 3 weeks after vaccine was administered. The pigs treated with vaccine and virus simultaneously, and those given virus 1 week after treatment, developed cholera. Those treated with vaccine and exposed 2 or 3 weeks later remained normal. In this experiment the time required for immunity to develop was 2 weeks.

Three additional experiments were conducted in which 44 pigs were treated with vaccine and 28 were left as controls. Exposure was made both by contact with cholera pigs and by virus injection at 7, 10, 14, and 21 days. Pigs exposed at 7, 10, and 14 days showed some reaction but no deaths occurred; those exposed at 21 days remained normal. Of the 28 controls, 14 died and 14 were killed for virus. These results confirmed the previous experiments, and indicated that the time required to produce immunity would be between 2 and 3 weeks. The results also showed that all vaccine-treated pigs were partially protected when exposed at 7 and 10 days after treatment.

DURATION OF IMMUNITY

In testing crystal violet-phenol vaccines for potency, a number of pigs were exposed to cholera from 4 to 6 months after treatment and were found by virus injection to be immune. Duration of immunity is discussed further under crystal violet-glycerol vaccines.

FIRST FARM HERD TREATED WITH CRYSTAL VIOLET VACCINE

A farm herd in the vicinity of the Ames experiment station was treated on November 5, 1935. Before treatment, 2 pigs were purchased from this herd, tested, and found to be susceptible to cholera. All of the pigs treated remained normal. Four months after treatment, 5 hogs were purchased and tested for immunity by virus injection. This procedure was repeated at intervals of 15 days until 20 hogs had been exposed, the last ones 5½ months after treatment. Seventeen remained normal and 3 had slight reactions. All were considered adequately protected.

CONTAMINANTS

During the process of producing crystal violet vaccines, some evidence of contamination was discovered, and numerous modifications were made in production methods in an effort to obtain sterile vaccines. Two of the more common contaminants were *Salmonella suipestifer* and *Escherichia coli*, which were often found in pigs killed for virus in the later stages of the disease. Some vaccines,

when plated at the end of the incubation period, were found to be contaminated and were discarded.

Crystal violet was known to be germicidal for gram-positive members of the colon typhoid group, but not for the gram-negative members of this group.

Investigations conducted by Dr. Frank Tilley in Washington had shown that a mixture of orthocresol and crystal violet was strongly germicidal against *E. coli*. A vaccine was therefore prepared by adding 10 parts of a 2-percent orthocresol solution and 5 parts of a 1-percent crystal violet solution to 90 parts of defibrinated hog cholera blood, and incubating as usual. Eleven lots were prepared by this formula, and tests indicated that the vaccine was of high potency. It did not, however, constitute a desirable product because of marked gelatinization produced by the orthocresol solution when the blood was incubated.

Further experiments carried out by Tilley seemed to demonstrate that the irregular results in germicidal action of the attenuating agents, crystal violet plus phenol, may have been due to the varying pH values of different lots of vaccines, and that a slight change in pH might be sufficient to enhance the germicidal action. The use of phenol in the formula was abandoned because the combination of phenol and incubation caused thickening, which made the finished product unsatisfactory. However, a great many lots of this type of vaccine had been made, and many farm herds had been treated, with satisfactory results, before the formula was changed.

NEW FORMULA

The new formula consisted of adding 10 parts of Sorensen's phosphate (which contained 3.6 percent of disodium phosphate) plus 10 parts of a 0.5-percent solution of crystal violet to 80 parts of defibrinated virus blood. The incubation period remained at 2 weeks. In 1938 this formula was changed slightly by substituting 3 percent of disodium phosphate solution for the Sorensen's phosphate. The results of experiments with this type of vaccine, and the experimental treatment of many farm herds, were satisfactory. However, it was necessary to destroy a considerable amount of the new vaccine because of impotency or contamination, and it was not considered fully satisfactory.

By June 30, 1942, a total of 262 herds containing 14,068 pigs, had been vaccinated experimentally on farms with crystal violet vaccine.

METHOD OF CONDUCTING FARM EXPERIMENTS

In carrying out farm experiments with crystal violet vaccine, the owners were given all available information regarding the advantages and disadvantages of the vaccine. Because of the slow development of immunity in vaccine-treated pigs, herds were selected for treatment in communities where no hog cholera was known to exist. Infected or exposed herds were carefully avoided. The owners were required to sell to the Government at market price as many pigs as were needed to conduct immunity tests when the herd was ready for market. Pigs weighing up to 75 pounds were given 5-cc. doses and those over 75 pounds were given 10-cc. doses. The owners were requested to notify the experiment station at Ames in case sickness

of any kind developed after treatment. At the beginning of these experiments, 151 pigs were left untreated in 79 herds to determine whether any cholera would be transmitted by the vaccine treatment. These control pigs all remained normal, and the practice of leaving untreated controls in herds was discontinued.

In only 2 of the 262 herds did any sickness develop which showed symptoms similar to cholera. Two pigs from each of these two herds were purchased and left in the herds without further treatment; the remaining pigs were treated with serum. The pigs purchased were later exposed to cholera and remained normal. From these results it was concluded that either the reaction was not due to cholera infection or the vaccine provided adequate protection and the serum treatment, previously administered as a precautionary measure, would not have been necessary (*15*).

IMMUNITY TESTS OF HOGS FROM FARM HERDS

Usually four or five hogs were purchased from each herd when ready for market and given injections of virus to test their immunity.

A summary, made in 1943, of results of exposure of farm hogs showed that 1,028 had been tested for immunity (*9*). Eighty percent were found to be adequately protected, 13 percent showed severe reactions but recovered, and 7 percent died. Some of these hogs were treated before weaning, and in some of the experiments the results indicated that pigs farrowed by serum-virus-treated sows should not be treated with crystal violet vaccine until they are 8 to 10 weeks old. If the above summary had been confined to pigs treated after weaning, the results would have shown that 84 percent were protected, 11 percent suffered severe reaction, and 5 percent died.

The results of these immunity tests indicated that the injection of crystal violet vaccine constitutes a milder antigenic stimulus than the injection of virulent virus in the serum-virus treatment, and that immunity is not as solid in crystal-violet-vaccine-treated hogs as in serum-virus-treated hogs. In vaccine-treated hogs subsequently given virus injections many reactions with recoveries occur. On the other hand, hogs immunized by the serum-virus treatment are seldom affected by a subsequent injection of virus. However, many serum-virus-treated hogs are not immune when attempts are made to use them in serum production. It is not known whether this is due to loss of immunity or a failure to produce immunity. It is usually attributed to the latter. No experiments have been conducted to determine the percentage of serum-virus-treated hogs that are immune at market age as has been done with crystal-violet-vaccine-treated hogs.

TREATMENT OF PIGS FARROWED BY IMMUNE AND SUSCEPTIBLE SOWS

An experiment was conducted to determine the response to crystal-violet-vaccine treatment of young pigs farrowed by serum-virus-treated and susceptible sows.

Twenty-six pigs farrowed by serum-virus-treated sows and 13 pigs farrowed by untreated sows were vaccinated with crystal violet vaccine before weaning. These pigs were held approximately 6 months and were exposed to cholera by virus injection. The pigs from sus-

ceptible sows were found to be 100-percent protected; the pigs farrowed by immune sows were only 61.5-percent protected.

This experiment indicated that the temporary immunity of pigs farrowed by serum-virus-treated sows interfered with immunization by vaccine treatment.

TREATMENT OF PROBLEM HERDS

Several herds of hogs were treated with crystal violet vaccine on farms where heavy losses had occurred in previous years following serum and virus treatment. The most frequent cause of losses was necrotic enteritis, which developed 6 to 10 days after treatment. In no case did this disease develop after treatment with crystal violet vaccine.

KEEPING QUALITIES OF CRYSTAL VIOLET-PHOSPHATE VACCINES

Four lots of crystal violet-phosphate vaccine held in cold storage for 1, 2, 3, and 4 years were tested for potency. All four lots still gave satisfactory protection when tested in standard doses of 5 cc.

WHITE CELL COUNTS OF VACCINE-TREATED PIGS

One hundred and seven white cell counts were made on 11 vaccine-treated pigs. Counts were made on 2 successive days prior to treatment to establish the normal counts of each pig, and counts were made daily thereafter for 10 to 13 days. Two of the 11 pigs showed a slight decrease in leucocyte count—one on the 7th, and one on the 12th day. Neither of these counts was considered low enough to be classed as a leucopenia. The other nine pigs failed to show any significant change in leucocyte count.

EFFECT OF HEAT ON VACCINE

In one experiment, a vaccine was divided into three portions and incubated for 2 weeks—one portion at 35° C., one at 37.5°, and one at 39.5°. In another experiment, a portion of vaccine was held in an incubator at 37.5° for 6 weeks, and another portion was held for 8 weeks. Subsequent potency tests showed that neither variations in temperature between 35° and 39.5° nor prolonged incubation at 37.5° had any apparent effect on the potency of the vaccine. In another experiment, a sample of vaccine was held for 2 weeks at 50° without impairment of potency.

EXPOSURE OF TREATED PIGS TO DIFFERENT VIRUSES

Commercial viruses were obtained from six sources and used to expose pigs treated with crystal violet-disodium phosphate vaccine prepared by the Bureau of Animal Industry. In addition to these viruses, a sample of BAI virus was used for comparison. A lot of 70 pigs was divided into seven groups. Each group contained 3 pigs treated with 5 cc. of vaccine, 3 pigs treated with 10 cc. of vaccine, 2 treated with serum and virus, and 2 untreated controls. Four weeks after treatment all pigs were exposed by virus injection, each group being exposed to a different virus. The vaccine protected

against all seven viruses. The controls all developed cholera. Vaccines were made from the bloods of the control pigs inoculated with different commercial viruses. These bloods apparently produced vaccines of varying potency. Three of the six vaccines were high in potency, one was medium, and two were low. These and other experiments have shown that vaccine made from the blood of individual pigs inoculated with the same virus may vary in potency, and consequently it cannot be stated definitely that the differences in potency shown in this experiment were due to variations in the pigs or to the difference in the immunogenic properties of the commercial viruses used.

DOSAGE OF CRYSTAL VIOLET-DISODIUM PHOSPHATE VACCINES

Comparisons were made in exposure tests on farms of pigs treated with 5 cc. and 10 cc. of vaccine. The results in some years showed the smaller dose was superior to the larger dose, and in other years the larger dose seemed to be superior. In some years two doses of vaccine were administered at 1-week intervals to part of the pigs, and the results were compared with results following single doses in the same herds. There were no significant differences between 5-cc. and 10-cc. doses, or between single doses and two doses administered 1 week apart.

CLEAR VACCINES FROM DIFFERENT FRACTIONS OF BLOOD

Previously, an experiment was described in which clear serum virus was used to prepare phenolized vaccine. It was shown that, with the doses used, there was no difference in potency between vaccines prepared from defibrinated-blood virus and clear serum virus.

After the successful production of crystal violet vaccine from defibrinated-blood virus, the question arose whether or not potent vaccine could be produced from clear serum virus. To obtain an answer to that question a series of experiments was carried out.

Blood was drawn from four pigs previously inoculated with virus, the bloods were mixed and used to prepared vaccines. Four different vaccines were prepared as follows:

No. 1.—400 cc. of defibrinated-blood virus.
No. 2.—400 cc. of clear serum virus.
No. 3.—400 cc. composed of 196 cc. of clear serum virus and 204 cc. of sterile water.
No. 4.—400 cc. composed of 204 cc. of cellular material, which had been removed from No. 3, and 196 cc. of sterile water.

To each of these vaccines 50 cc. of a 3-percent solution of disodium phosphate and 50 cc. of a 0.5-percent solution of crystal violet were added. The vaccines were incubated 2 weeks at 37.5° C., and tested for potency in doses of 2.5, 5, and 10 cc. Six pigs were used to test each vaccine and four pigs were used for controls. All pigs remained normal for 3 weeks and were exposed to cholera by virus injection.

All vaccine-treated pigs remained normal except the four that received 2.5 and 5-cc. doses of No. 3. The two that received 10 cc. were immunized. One control recovered in poor condition, and

three controls were killed for virus. The results indicated that vaccine made from the cellular part of defibrinated blood was more potent than vaccine made from the clear part. It also confirmed a previous experiment which indicated that potent vaccine could be made from clear serum virus.

In another experiment, four types of vaccine were prepared from the same lot of mixed virus blood:

No. 1.—Defibrinated blood, crystal violet-phosphate vaccine.
No. 2.—Clear serum virus, crystal violet-phosphate vaccine.
No. 3.—Defibrinated blood, crystal violet-glycerine vaccine.
No. 4.—Clear serum virus, crystal violet-glycerine vaccine.

These vaccines were prepared and incubated in the same manner and tested for potency. No. 1 gave protection to 84 percent, No. 2, to 64 percent; No. 3, to 92 percent; and No. 4, to 93 percent. The clear serum-phosphate vaccine was definitely lower in potency than the defibrinated-blood-phosphate vaccine. The glycerol vaccines appeared more potent than the companion phosphate vaccines. However, more experiments would be required to justify final conclusions.

In another series of tests the following mixtures were prepared from the same lot of blood:

No. 1.—Clear-filtered phenolized vaccine.
No. 2.—Clear-filtered vaccine without chemical preservative.
No. 3.—Defibrinated blood plus crystal violet and disodium phosphate.

These vaccines were incubated for 2 weeks and tested in 5-cc. doses. All vaccine-treated pigs remained normal after exposure, and all controls died.

This series of experiments indicated that (1) a potent crystal violet vaccine can be prepared from clear serum virus; (2) a vaccine derived from the cellular portion of virus blood appeared to be more potent than one derived from the clear portion of the same blood; (3) potent vaccine can be prepared from clear, filtered, serum virus by incubation alone, without the addition of a chemical; (4) the principal function of chemical agents, previously referred to as attenuating agents, may be only to afford protection against contamination during and after preparation. Further, viruses have been converted to vaccines at room and refrigerator temperatures without heat, but heat accelerates the conversion of virus to vaccine.

GENERAL CONCLUSIONS ON CRYSTAL VIOLET-PHENOL AND CRYSTAL VIOLET-PHOSPHATE VACCINES

Following numerous experiments in the practical application of crystal violet-phenol and crystal violet-disodium phosphate vaccines, the following conclusions were drawn:

(1) The vaccines are safe to use in healthy herds as there was no evidence of disease in the treated pigs following vaccination or in numerous untreated controls kept in contact with treated pigs.

(2) Ten cc. of the phenolized crystal violet vaccine afforded somewhat better protection than 5 cc. No difference was noted in the immunity conferred by 5-cc. and 10-cc. doses of crystal violet-disodium phosphate vaccine.

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(3) In a limited number of tests, disodium phosphate vaccine appeared to surpass the phenolized vaccines in immunizing properties.

(4) Immunity conferred by both kinds of vaccine persisted for at least 4 months.

(5) In physical appearance the crystal violet-disodium phosphate vaccine was distinctly superior to the crystal violet-phenol vaccine.

Vaccine Prepared Without Heat

Heating hog cholera blood (preserved with a chemical agent) at 37.5° C. greatly reduces the time required to kill or attenuate the virus to a point at which it no longer produces disease. This method is generally used in vaccine production. However, vaccines have been produced without incubation or other methods of heating.

Crystal violet-disodium phosphate vaccines were prepared and placed in a refrigerator instead of the incubator. These products were tested at intervals and were found to be still virulent after up to 6 months' storage. After 8 months of storage they were found, by testing with susceptible pigs, to have lost virulence. The pigs remained normal for 3 weeks and were then given injections of known virulent virus and found to be immune.

A phenolized virus kept in a refrigerator until it would no longer produce disease was tested for immunizing properties by injecting it into susceptible pigs. When the pigs were challenged 3 weeks later they were found to be immune.

Ammonia was added to hog cholera virus in concentrations of 0.125 and 0.5 percent. The mixtures were tested after 35 days of storage in a refrigerator and were found to have lost virulence. They produced immunity when administered to susceptible pigs.

Formaldehyde was added to virus in concentrations of 0.2 and 0.15 percent. The mixtures were held at room temperature for 32 days when they were found to have lost virulence, and produced immunity in susceptible pigs.

Five different kinds of vaccine were prepared without the application of heat. However, heat greatly reduces the time required to attenuate the virus in hog cholera blood, and is used in practically all crystal violet vaccine production. It was also found that vaccine could be prepared by heating, without chemical agents, but these are necessary for preservation of the finished product and to destroy or reduce the number of contaminants originally present in the virus blood.

Crystal Violet-Glycerine Vaccines

Very good results were obtained in experiments and field tests of the previously described vaccines, but they were not considered entirely satisfactory because the methods of producing them, in too many cases, failed to render the finished product sterile. Many of the contaminated lots of vaccine, although satisfactory for potency, were not used or distributed for use by others, which, of course, materially increased the cost of the vaccine. Also, there seemed to be a possibility that losses in potency that at times had been observed could be due to the growth of contaminants. Search for an

additive that would insure sterile vaccines was therefore begun. As a result of this search, crystal violet-glycerol vaccines were developed and are the type now in use. The first lot of crystal violet-glycerol (CVG) vaccine was produced on May 28, 1942. Tilley (*116*) obtained a patent on this method of producing vaccine, and assigned it to the Secretary of Agriculture.

TABLE 1.—*Farm herds treated with CV (phenolized), CVF (phosphated), and CVG (glycerol) vaccines in the vicinity of the Ames station* [1]

Vaccine and date of treatment	Herds treated	Pigs treated with—				Total
		5 cc. (Single)	10 cc. (Single)	5 cc. (Double)	10 cc. (Double)	
CV:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
3/5/1936–6/30/36	1	0	47	0	0	47
7/1/36–9/25/36	15	150	410	0	0	560
Total	16	150	457	0	0	607
CVF:						
10/23/36–6/30/37	5	48	186	0	0	234
7/1/37–6/30/38	33	375	1, 131	22	25	1, 553
7/1/38–6/30/39	87	2, 822	669	967	29	4, 487
7/1/39–6/30/40	83	4, 198	480	143	0	4, 821
7/1/40–6/30/41	20	846	409	0	0	1, 255
7/1/41–6/30/42	24	345	807	0	0	1, 152
7/1/42–6/30/43	23	493	532	0	0	1, 025
7/1/43–6/30/44	13	281	279	0	0	560
7/1/44–8/5/44	4	61	79	0	0	140
Total	292	9, 469	4, 572	1, 132	54	15, 227
CVG:						
7/1/42–6/30/43	4	35	19			54
7/1/43–6/30/44	7	83	103			186
7/1/44–6/30/45	15	327	283			610
7/1/45–6/30/46	18	768	441			1, 209
7/1/46–6/30/47	11	810	263			1, 073
7/1/47–6/30/48	9	493	292			785
7/1/48–6/30/49	14	1, 155	423			1, 578
7/1/49–6/30/50	12	726	321			1, 047
7/1/50–6/30/51	8	506	0			506
7/1/51–6/30/52	4	288	5			293
7/1/52–6/30/53	18	1, 343	0			1, 343
7/1/53–6/30/54	32	2, 910	169			3, 079
7/1/54–6/30/55	29	2, 138	376			2, 514
Total	181	11, 582	2, 695			14, 277
All vaccines:						
CV	16	150	457	0	0	607
CVF	292	9, 469	4, 572	1, 132	54	15, 227
CVG	181	11, 582	2, 695	0	0	14, 277
Total	[1] 489	21, 201	7, 724	1, 132	54	30, 111

[1] No hog cholera occurred in any of the 489 treated herds during the 19-year period.

This vaccine is prepared in the following manner: First, a solution of 1 part of crystal violet to 400 parts of glycerine is prepared. One part of this solution is added to 4 parts of defibrinated hog cholera blood, and the mixture is incubated for 2 weeks at 37.5° C. After incubation, the vaccine is stored in a refrigerator. The formula for preparation of this type of vaccine (designated CVG vaccine) was released in 1944, and commercial production was started.

For some time after the first lot of CVG vaccine was prepared a portion of the same defibrinated blood was converted to crystal violet-disodium phosphate vaccines. Many comparative tests were conducted using the two types of vaccine. The tests indicated that the CVG vaccine was equal to, or somewhat superior to, the phosphate vaccines.

Tests were also conducted in 20 farm herds. Part of each herd was treated with CVG vaccine and part with phosphate vaccine. When these hogs were ready for market 6 or 8 months later, 60 head treated with CVG vaccine and 65 head treated with phosphate vaccine were purchased and tested for immunity at the experiment station. The results of exposure of these 125 head showed that 93 percent of the CVG vaccine-treated pigs and 83 percent of the crystal violet-disodium phosphate vaccine-treated pigs were adequately protected against cholera. The hogs that remained normal, or had only a slight reaction, were considered adequately protected. Eleven hogs in these groups suffered severe reactions and recovered, but were considered inadequately protected, in addition to those that died.

After 48 lots of each type of vaccine had been produced, they were tested for sterility. All lots of CVG vaccine were sterile, while 15 percent of the phosphate vaccines were contaminated. As production of CVG vaccines continued, more than 500 samples were tested and all were sterile. After long periods of storage the CVG vaccines were decidedly superior in consistency and physical appearance. The CVG vaccine gradually replaced the phosphate vaccine, and after June 1944 was the only kind produced.

Attenuation by CVG Vaccine

Experiments were conducted to determine the time required to attenuate the virus used in preparing CVG vaccine. Incubation for 3 days decidedly decreased virulence and incubation for 5 days destroyed the ability of the virus to produce disease. Comparative tests showed that attenuation to the point of nonvirulence is reached about 1 day sooner with crystal violet-glycerine vaccine than with phosphate vaccine. Portions of these vaccines were left in the incubator for 31 days. Tests indicated no loss of potency.

Time Required To Produce Immunity

Numerous experiments, similar to the one previously described, were conducted to determine the time required to produce immunity in vaccine-treated pigs. Both CVG and phosphate vaccines were used, as well as CVG vaccine alone. The results were very much the same. Some protection was noted in pigs exposed 7 to 10 days after vaccine treatment, but adequate protection was not obtained until 2 to 3 weeks after treatment.

Duration of Immunity in CVG-Vaccinated Pigs

In the first test to determine the duration of immunity, both CVG and phosphate-vaccine-treated pigs were used. The pigs were divided into groups; one group was treated with CVG, and one with phosphate vaccine. Half of the pigs in each group, with one control, were exposed at 8 months after treatment, and the other half, with one control, at 12 months. All the pigs treated, with either vaccine, were found to be immune and both controls developed cholera.

In the second experiment, CVG vaccine only was used. Twenty-eight pigs were treated with vaccine in 5-cc. doses, and 13 pigs from the same lot were left untreated and kept in the pen with the treated pigs. At 9-week intervals after treatment, four pigs and one or two controls were exposed to hog cholera. The results of this experiment were as follows: One vaccinated pig in the group exposed 18 weeks after treatment died, but all other vaccine-treated pigs remained normal. The last group was exposed 13 months and 11 days after treatment. All vaccinated pigs were adequately protected, and all controls developed cholera. This experiment was repeated with similar results.

The pigs in these experiments were kept in a large barn used for susceptible pigs only. In addition to the untreated controls from the same lots, several hundred other susceptible pigs were kept in the same barn for short intervals throughout the experiment. It is therefore certain that the pigs did not receive exposure to strengthen their immunity, and that immunity, which persisted for 13 months and 11 days, was produced by the vaccine alone.

Virus Content of Blood Used in Vaccine Production

Experiments were designed to determine the cause of variations in the potency of crystal violet vaccine. In the first of this series, an attempt was made to determine whether or not there was a relation between the infectivity titer of the blood used for vaccine production and the potency of the vaccine. The amount of virus used to inoculate pigs for vaccine production was measured by determining the minimum lethal dose of different viruses. Some vaccines were prepared from blood of high virus content, and others were made from blood of low virus content. The vaccines produced from each of these two kinds of virus were in some cases of high potency and in other cases of low potency. One pig's blood that produced disease in a dose of 1/5,000,000 cc. was converted to vaccine, which was found to be very low in potency. Thus, no definite relation was found between the infectivity titer of the virus and the potency of the derived vaccine.

Amount of Virus Given Donor

Vaccines were prepared from the blood of pigs inoculated with doses of virus ranging from 1/1,000,000 cc. up to 10 cc. In some instances vaccines made from the blood of pigs that received the largest dose of virus were more potent than vaccines from the blood of pigs that received the smallest dose, and sometimes the reverse was true. No relation was found between the amount of virus administered to the donor and the potency of the derived vaccine.

Vaccine Prepared From the Blood of Low-Temperature Pigs

Two lots of CVG vaccine were prepared from the bloods of two pigs—one with a temperature of 106.2° on the third day, which dropped to 102° on the sixth day, and one with a temperature of 106.2° on the fourth day which dropped to 100° on the sixth day. The latter pig died while being prepared for blood drawing. The vaccines prepared from the bloods of these two pigs were of good potency.

Vaccine Shipments in Hot Weather

Samples of two lots of crystal violet vaccine were shipped from Ames, Iowa, to Washington, D.C., in hot weather and arrived 4 days later. They were shipped back to Ames, and were received 3 days later and placed in a refrigerator. After storage for 10 months they were tested for potency in 3- and 5-cc. doses, along with samples of the same vaccines that had been held in a refrigerator constantly since they were prepared. All were of satisfactory potency.

Intradermic Administration of Vaccine

Eight pigs were treated with 1 cc. of crystal violet vaccine injected intradermally, eight with 1 cc. injected subcutaneously, and eight with 5 cc. injected subcutaneously. After 3 weeks they were exposed to cholera. Somewhat better protection was afforded by 1 cc. of vaccine injected intradermally than by 1 cc. injected subcutaneously, but protection was not equal to that provided by 5 cc. injected subcutaneously. Intradermal injections were made on the inner surface of the thigh.

South American investigators (*20*, *21*) reported excellent results by injecting vaccine intradermally on the dorsal surface of the ear. After these reports were received, a limited experiment was conducted to obtain additional data on this method. Pigs given injections of ½ cc. of vaccine intradermally on the dorsal surface of the ear were better protected than pigs receiving subcutaneous injections of ½ cc., 1 cc., 2 cc., or 5 cc.

Immunity of Offspring of Vaccine-Treated Sows and Response to Treatment

Pigs farrowed by serum-virus-treated sows have considerable immunity to hog cholera during the suckling period, but such pigs do not respond to vaccination with crystal violet vaccine as well as pigs from susceptible sows.

Experiments were conducted at Beltsville, Md., and at Ames, Iowa, to obtain information regarding the immunity of pigs farrowed by crystal violet vaccine-treated sows, and their response to vaccination. The results of these experiments, although not conclusive, suggested that pigs farrowed by vaccine-treated sows are susceptible to cholera when 4 weeks old, and that such pigs develop considerable immunity when treated with vaccine at 4 weeks of age or older.

Several farm herds of pigs farrowed by vaccine-treated sows were treated when 4 to 6 weeks old with crystal violet-glycerine vaccine.

Later exposure tests indicated that they developed lasting immunity similar to young pigs farrowed by susceptible sows.

Glass Containers for Storage of Crystal Violet Vaccine

Five lots of crystal violet vaccine were prepared. Each lot was divided into three portions and stored in three different kinds of glass containers—Pyrex, Illinois Glass Company–1845, and ordinary milk bottles. One potency test was made of each portion of four lots, and two tests were made of one lot. In the first of the latter tests, made after 418 days of storage, very little difference was observed in the potency of the three portions, but in the second test, after 760 days of storage, the portion stored in a milk bottle was of lower potency. In three of the six tests, the portions stored in milk bottles were of lower potency than the portions stored in Pyrex or 1845-glass containers.

While definite conclusions could not be made from this limited investigation, the indications were that ordinary glass milk bottles were not satisfactory for long-time storage of crystal violet vaccine, and conversely, the Pyrex and 1845-glass containers were satisfactory.

Effect of Agitation of Vaccine During Incubation

It had been routine practice in vaccine production to agitate by shaking the vaccine in the containers each day during the time it was incubated. Experiments were designed to determine whether or not agitation was a necessary step in the production method. In each of two experiments, two 250-cc. bottles were filled with vaccine and two were half-filled. One full bottle and one half-filled bottle were left undisturbed and the other two were shaken for 5 minutes each day during the 2-week incubation period. Potency tests were made of the contents of each bottle by giving injections of 1 cc. to two pigs and 5 cc. to two other pigs. The results indicated no difference in potency between the vaccines shaken and not shaken.

Treatment of Pigs With Serum and Vaccine

The principal disadvantage of crystal violet vaccine for the prevention of hog cholera is the slow development of immunity. Since serum alone confers an immediate but short immunity and crystal violet vaccine confers a delayed but relatively lasting immunity, it was considered that a combination of the two treatments might retain the advantages and eliminate the disadvantages of the two methods of preventing hog cholera. Early small-scale experiments indicated that serum administered simultaneously or within 7 days after vaccine treatment interfered with the normal action of the vaccine. However, the use of serum and vaccine, either simultaneously or first one and shortly afterwards the other, has been advocated and practiced since the vaccine was first developed. Doyle and Wright (*59*), in reporting the results of an experiment, suggested that serum could be administered 5 days before, or 5 days after, vaccine treatment without inhibiting the antigenic action of the vaccine.

Because of the discrepancy of results obtained by different investigators regarding the effectiveness of vaccine when used with serum, more extensive experiments were conducted to obtain additional information. Altogether, seven experiments involving 251 pigs, were conducted by the Bureau of Animal Industry. Detailed reports of these experiments were published (*12*).

In one of these experiments 60 pigs were divided into 12 groups of 5 pigs each, and each group was given a different treatment. The treatment and the results following exposure 92 days after treatment are shown in table 2.

TABLE 2.—*Protection afforded pigs exposed to hog cholera 92 days after various treatments with serum and vaccine*

Group No.[1]	Treatment	Percent protected
1	35 cc. of serum alone	0
2	8 cc. of vaccine alone	80
3	35 cc. of serum and 8 cc. of vaccine simultaneously	0
4	35 cc. of serum and 8 cc. of vaccine 7 days later	0
5	35 cc. of serum and 8 cc. of vaccine 13 days later	0
6	35 cc. of serum and 8 cc. of vaccine 21 days later	40
7	35 cc. of serum and 8 cc. of vaccine 28 days later	0
8	8 cc. of vaccine and 35 cc. of serum 7 days later	0
9	8 cc. of vaccine and 35 cc. of serum 14 days later	80
10	8 cc. of vaccine and 35 cc. of serum 21 days later	100
11	8 cc. of vaccine and 35 cc. of serum 28 days later	100
12	Controls [2]	----------

[1] 5 pigs in each group.
[2] 4 died; 1 recovered.

The results of this experiment show clearly that serum in protective doses cannot be successfully administered subcutaneously with regular doses of vaccine, either simultaneously or within 4 weeks before or 1 week after vaccine treatment. Results were satisfactory in groups 9, 10, and 11 because the normal action of the vaccine was partly or entirely completed before serum was administered. This experiment confirmed the results of three previous experiments in which 55 pigs were used.

Three other experiments involving 136 pigs were conducted later. The results consistently indicated that serum interfered with the action of vaccine. The interference varied from slight when small doses (10 cc.) of serum and large doses (20 to 30 cc.) of vaccine were used, to 100 percent when 5 cc. of vaccine was given with 15 to 35 cc. of serum. In these experiments the serum and vaccine were injected subcutaneously, in most cases in comparatively large doses.

D'Apice and Penha (*20*) reported successful experiments in Brazil wherein serum was injected intramuscularly in 10-, 20-, or 30-cc. doses, and vaccine was injected intradermally in 1-cc. doses at the point of the ear.

In experiments conducted subsequently by the Bureau of Animal Industry, a technique similar to the one reported by the Brazilian investigators was used. In these experiments pigs were treated with ½ cc. or 1 cc. of vaccine injected intradermally, and 15 cc. or 35 cc.

of serum injected subcutaneously. One test showed satisfatory immunity, but another test showed practically no immunity. Similar discrepancies were obtained in later tests of the same type.

Distribution of Crystal Violet Vaccine

After the experimental use of vaccine on thousands of farm pigs by Bureau veterinarians, vaccine was distributed free of charge to be used for field tests by State, Federal, and practicing veterinarians. A total of 485,250 cc. was distributed for this purpose in Alabama, Illinois, Iowa, Maryland, Mississippi, North Carolina, Oklahoma, Tennessee, and Virginia. The cooperating veterinarians were required to supervise and report results from all herds treated. A total of 1,974 herds containing 49,950 pigs were treated in these cooperative experiments, and 175 reports were received, 164 of which reported no losses. It is assumed that those who did not report had satisfactory results.

Only 11 reported that cholera had occurred in herds under their supervision. Over half of the breaks reported occurred during the first 2 years the vaccine was distributed. Deliberate exposure was not made, but many reports stated that pigs remained normal when cholera was present in neighboring herds and, in some cases, where it appeared in unvaccinated pigs on the same premises.

The overall conclusion from field tests, which were discontinued when commercial vaccines became available, was that good results were obtained when the vaccine was properly used.

Field tests in cooperation with State, Federal, and practicing veterinarians were carried out from 1941 to 1951, inclusive. In this work and in the field work by the force at the Ames station, more than 70,000 pigs were treated with crystal violet vaccine.

The annual report of the Ames station for 1939–40 gives the setup of proposed field tests, and station reports from 1942 through 1951 give amounts of vaccine shipped from Ames for the project. Reports were sent to Washington by the practicing veterinarians. The only published reports are those included in the 1941 and 1942 annual reports of the Chief of Bureau. The 1941 report states 43,200 cc. of vaccine was distributed, 250 herds (5,081 pigs) were treated, and of 27 herds reported exposed, all pigs in 17 herds remained well. The 1942 report states that 100,000 cc. of vaccine was distributed in eight States, 257 herds (8,578) pigs) were treated, and of 29 herds containing 1,120 pigs exposed, 85 percent were adequately protected. Vaccine shipments from Ames for use in this project are shown in table 3.

Survival of Virus in Vaccine-Treated Pigs

A number of pigs were treated with crystal violet vaccine and 3 weeks later were given injections of hog cholera virus. These pigs were bled on the 2d, 4th, 8th, 10th, and 15th day after virus injection and their blood was injected into susceptible pigs. The pigs that received injections of blood drawn on the 2d or 4th day developed cholera. Those that were given injections of blood drawn on the 8th and 15th days did not develop cholera. The pigs that received 10th-day blood sickened—one on the 9th, and one on the

TABLE 3.—*Distribution of crystal violet vaccine in field tests, in cooperation with practicing veterinarians, 1941–51*

Fiscal year	Iowa	Pathological Division, Washington, D.C.	Illinois	Oklahoma	Alabama	Other States	Total
	Cubic centimeters	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*	*Cubic centimeters*
1941	10, 800	40, 500	4, 000				55, 300
1942	17, 900	66, 500				23, 500	107, 900
1943	34, 075	68, 000				12, 500	114, 575
1944	16, 275	36, 500	15, 000				67, 775
1945	16, 700	29, 750	13, 000	3, 000			62, 450
1946	15, 550	25, 500	15, 500	2, 500			59, 050
1947	18, 100	23, 000		6, 500	4, 000		51, 600
1948	8, 000	15, 000		2, 000			25, 000
1949	8, 500	10, 000					18, 500
1950	11, 250	16, 000				1, 000	28, 250
1951	4, 500						4, 500
Total	161, 650	330, 750	47, 500	14, 000	4, 000	37, 000	594, 900

14th day after virus injection. The fact that the 8th-day blood did not produce cholera, and that the pigs given 10th-day blood did not sicken until the 9th and the 14th days, led to the conclusion that the latter group sickened by accidental infection. It was therefore concluded that virus persisted for 4 days, but not for 8 days, in the blood of vaccine-treated pigs after exposure to hog cholera. For comparison, it should be remembered that virus persisted in the blood of serum-virus-treated pigs for approximately 14 days, and that no virus was found in the blood of long-time serum-virus-treated pigs on any of the days they were bled.

Vaccine From Blood Containing Secondary Infection

Studies were made to determine what effect the presence of *Salmonella choleraesuis* and *Erysipelothrix rhusiopathiae* in the blood of cholera pigs had on the potency of the derived vaccine. Two vaccines were prepared from the blood of pigs that had been given injections of *S. choleraesuis* and hog cholera virus. Three vaccines were made from pigs that had received hog cholera virus only. When these vaccines were subjected to potency tests, the results indicated that the most potent was made from the blood of a pig that did not receive *S. choleraesuis*. However, the two next highest in potency were from pigs that received both virus and *S. choleraesuis*. For this reason, the possibility that the presence of *S. choleraesuis* in virus has some influence on the potency of vaccine was not considered to have been definitely eliminated.

Potency tests were carried out with 11 vaccines that were made from the blood of erysipelas-reacting pigs that had been given injections of hog cholera virus, and from two pigs that had been exposed to hog cholera virus only. The most potent vaccine was from the blood of an erysipelas-reacting pig. However, similar

vaccines were classed as poor and unsatisfactory. The results indicated that there was no advantage in using erysipelas-reacting pigs for vaccine production.

Experiments To Determine if Virus in Vaccine Multiplies

In many publications crystal violet vaccine has been referred to as a "killed" or "dead" vaccine. In Bureau of Animal Industry publications it was referred to as a "killed-" or "attenuated-" virus vaccine. Evidence indicates that it is incapable of producing disease; for this reason it has been considered a "killed-virus" vaccine.

Experiments were conducted to furnish information on this subject, based on the supposition that if the virus in a vaccine does not multiply in the animal body, for practical purposes the vaccine would be considered a dead-virus vaccine, and conversely, if the virus did multiply, it would be considered an attenuated-virus vaccine. Theoretically, a virus could be biologically alive without having the power to reproduce.

In two experiments pigs were treated with crystal violet vaccine, bled at intervals thereafter, and their blood was used in attempts to immunize susceptible pigs. Some evidence was obtained that at certain stages after treatment the virus in the vaccine had multiplied.

Genetic Relation of Virus Donor Pigs to Vaccine Potency

Bureau workers at Beltsville and at Ames observed for years a marked variation in the potency of CVG vaccines prepared from virus from different pigs receiving the same virus exposure, and to overcome this difficulty, CVG vaccines were usually prepared from virus pools from a number of pigs.

The Beltsville workers in 1944–45 investigated the genetic relation of the virus-donor pig to vaccine potency. The work showed definite evidence of a genetic relation between the pig and virus used for CVG vaccine production, but the investigation had to be discontinued because of the foot-and-mouth disease outbreak in Mexico. CVG vaccine prepared from each of four litter mates from a crossbred sow showed the same variations in potency range (one good, two fair, and one satisfactory) as was usually obtained from any four pigs selected at random from a group, whereas similar vaccines prepared from four litter mates from a closely inbred swine strain (results from brother-sister matings for three generations) resulted in four vaccines all alike in potency.

Genetic Relation of Swine to Their Ability To Develop Immunity

Evidence was obtained that there is a heredity factor connected with a pig's ability to develop immunity. Pigs purchased from different farms, vaccinated with the same lots of vaccine and challenged with the same exposure virus, showed marked variation in the percentage of immunity for different groups. Part of this variation may have been due to their early environment. However, some Beltsville-raised control groups from different hereditary lines, raised under the same environmental conditions, also showed the same wide variation in immunity pattern.

Variations in Response of Vaccine-Treated Pigs

In the course of investigations of crystal violet vaccine, a vaccine of repeatedly proved potency occasionally failed to protect certain pigs. In some cases differences were noted in the response of pigs of the same herd. In other and more important instances, a vaccine that offered adequate protection to all pigs in one herd failed to protect any pigs in another herd.

A striking example of these differences was noted in a series of tests carried out during 1944 to 1946, in which one vaccine (CVG 159) was tested in different places, namely, Ames, Iowa; Beltsville, Md.; and Hull, Quebec, Canada (*95*). In some of these tests the vaccine used in the three places was from the same bottle. The exposure virus used was a BAI virus in all tests. In addition, at Beltsville and in Canada, some of the vaccinated pigs were also exposed to a Canadian virus. A control vaccine (CVF 164) was used at Beltsville, but not in Canada or at Ames. In all tests, unvaccinated controls developed typical cholera.

The results of these tests indicated that there were differences in—

(1) *The exposure viruses used.* Of the pigs treated at Beltsville with the control vaccine CVF 164, all exposed by Canadian virus died, whereas all exposed by BAI virus remained well. Further, in the tests as a whole, all of the 28 pigs exposed by Canadian virus died, whereas, of 30 exposed by BAI virus, only 5 died.

(2) *The vaccines.* Of the pigs exposed to Bureau virus at Beltsville, all of those vaccinated with CVG 159 died, whereas all of those vaccinated with CVF 164 remained well.

(3) *The pigs.* Of 20 pigs treated at Ames with CVG 159 and exposed there by BAI virus, all were adequately protected, whereas, of 4 similarly treated and exposed in Canada, 3 died; and of 2 similarly treated and exposed at Beltsville, both died.

Although only 2 pigs were used in this test of CVG 159 at Beltsville, the results were in general agreement with those of a number of other comparative tests at Beltsville and Ames. In practically every instance in which a difference was found, the Beltsville pigs failed to develop as satisfactory immunity as those tested at Ames.

The fact that vaccinated pigs responded differently to the BAI and Canadian viruses indicated a difference between these viruses, and a sample of virus was therefore obtained from Canada for use in further tests. This virus was used to inoculate a pig, and from this pig's blood a vaccine (Canadian 6309) was made. The remaining virus blood was retained for use as exposure virus.

Some pigs were treated with Canadian vaccine 6309. Other pigs from the same source were treated with a regular Bureau vaccine (CVF 171) prepared at Ames from BAI virus and there found to be satisfactory for potency. A third group consisted of serum-virus-treated pigs. Part of the pigs of each group were exposed to BAI virus, and part to the Canadian virus. Unvaccinated controls were also exposed to each virus. The results were striking. All pigs vaccinated with the Canadian vaccine remained well whether exposed to the Canadian or to the BAI virus. Of the pigs vaccinated with BAI vaccine CVF 171, those exposed to BAI virus remained well but those exposed to Canadian virus died. Regardless of the exposure virus used, all serum-virus-treated pigs remained well, whereas all controls died.

Only a few pigs were used in the above tests. It was decided to carry out further tests with a larger number of pigs. Five vaccines were used. Two of these were Canadian vaccines (Nos. 1 and 2) prepared from pigs that had been inoculated with Canadian virus derived after one or two passages in pigs at Beltsville of the original Canadian virus. Two Bureau vaccines were also prepared, and vaccine CVF 171 was also used as a control vaccine. Thus, there were five groups of vaccinated pigs—BAI Nos. 1 and 2, Canadian Nos. 1 and 2, and CVF 171.

It was necessary, because of the large number of pigs needed, to obtain pigs from six different sources. They were not uniform in size. The pigs of two groups weighed from 100 to 150 pounds, and were treated with 10 cc. of vaccine, whereas the others were treated with 5 cc. Four pigs from each source were given injections of one of the vaccines. As far as possible, pigs from each source were distributed equally in the different vaccinated groups. Half of the vaccinated pigs in each group were exposed to BAI virus and half to Canadian virus. Two methods—virus injection and contact—were used to expose corresponding pigs of each group. Although a relatively large number of pigs were used, the facilities available for housing did not permit the use at one time of as many pigs as were required to control all the variables that were found to be present. Post-mortem or bacteriological tests were made on all pigs, whether they died or survived virus exposure.

Unlike the results of the previous test, no sharp differences were found in the potencies of the Canadian and BAI vaccines. All the vaccines appeared to be of relatively low potency. The presence of unavoidable variables also complicated conclusions. Thus, based on the results as a whole, the BAI vaccines appeared to be twice as potent as the Canadian vaccines against BAI virus, whereas against Canadian virus, the Canadian vaccines were as potent as the BAI vaccines. However, if the groups of pigs were considered separately according to source, pigs from four sources were better protected against Canadian virus by the BAI vaccines than were the pigs vaccinated with Canadian vaccines. Results are shown in table 4.

TABLE 4.—*Results of tests of different vaccines against different viruses*

Vaccine and virus	Pigs dead		Pigs living	
	Number	*Percent*	*Number*	*Percent*
BAI vaccine vs. BAI virus	9	30	21	70
Canadian vaccine vs. BAI virus	17	70	7	30
BAI vaccine vs. Canadian virus	18	60	12	40
Canadian vaccine vs. Canadian virus	14	60	10	40
Vaccine 171 vs. BAI virus	1	16	5	84
Vaccine 171 vs. Canadian virus	4	67	2	33

That the Canadian vaccines (Nos. 1 and 2) were of relatively low potency was shown in a test carried out later, when facilities became available, with pigs from the same sources mentioned above. In this later test, 7 pigs were treated with Canadian vaccine No. 1, and 7 were treated with Canadian vaccine 6309; all were later exposed to Canadian virus. Of the 7 treated with Canadian vaccine No. 1,

only one remained well. The others died or were sick from 2 to 15 days, whereas all 7 treated with vaccine 6309 remained well after exposure. In this test, vaccine CVF 171 was again more effective against BAI virus than against Canadian virus.

Although no marked difference was evident between Canadian vaccines Nos. 1 and 2 and BAI vaccines Nos. 1 and 2 against Canadian and BAI viruses, rather marked differences were observed in the degree of immunity developed by the pigs from different sources. The different sources were identified as AH, B, C, ES 2, ES 3, and ES 4. Thus, in the tests as a whole, 83 percent of the C group and 72 percent of the B group survived, whereas only 28 percent or less of the pigs in the AH, ES 3, and ES 4 groups survived. However, since the dose of vaccine used was based on the recommended dose, the pigs in the B and C groups, being large, received a 10-cc. dose, whereas the small pigs in the other groups received only 5 cc.

No marked differences were found between pigs exposed by virus injection and pigs exposed by contact. In 32 of 42 pairs, one each of which was exposed by contact and one by injection, both lived or both died.

A number of supplemental potency tests of these vaccines were made, but they did not aid in permitting definite conclusions. However, in tests made at Ames of the BAI and Canadian vaccines against BAI virus, the BAI vaccine adequately protected all pigs whereas the Canadian vaccines adequately protected only 3 out of 8 pigs. These results are quite different from those obtained at Beltsville, which is another example of the difference in results that frequently occurred when the same vaccine was tested at two different places.

The post-mortem examinations made at Beltsville of the pigs used in the tests of the two BAI and two Canadian vaccines suggested that complicating factors were present. Unusually severe abscess formation and necrosis of the tonsils, pneumonia involvement of the anterior lobes of the lungs similar in location and extent to that occurring in swine influenza, and an enteritis and lesions of necrosis of the colon and cecum—not characteristic "button" ulcers—were present in pigs that died, but not in those that survived.

On bacteriological examination, growth was obtained from the heart blood of 83 pigs, from the spleens of 52, and the lungs of 119. *Pasteurella* was obtained from 69 of the 140 pigs, and *S. choleraesuis* from only 3.

Although the results of the experiment with the two Canadian and two BAI vaccines were disappointing, the results of this series of tests as a whole strongly indicated (1) that pigs at Beltsville, and more particularly in Canada, responded differently from pigs at Ames to treatment with crystal violet vaccine; (2) that the Canadian virus, as originally obtained, was different from the regular Bureau virus; and (3) that this difference apparently decreased after about two passages through pigs.

Foreign Reports on the Use of Crystal Violet Vaccine

Schellner (*107*) made the following statements concerning crystal violet vaccine in 1953:

In the respective contaminated area 8,000 pigs were inoculated with crystal violet vaccine, produced at Schleisshein, and the epizootic was eradicated. . . .

Following the good results observed by us in 1946, other experiments were carried out from 1946–1952 with crystal violet vaccine, produced according to the method of Cole and Henley (*11*) in 1946. . . . Under the control of official veterinarians, 50,000 other pigs were vaccinated up to December 15, 1952. The result of these vaccinations was not only encouraging but satisfactory. When about 20,000 pigs were vaccinated in England with the same vaccine, no harmful effects or spreading caused through vaccination were observed there either. . . .

Based on the success obtained from more than 800,000 vaccinations, Cury, Penha, and d'Apice (*17*) recommended in 1947, the use of vaccines. In 1949, Laiset (*74*) reported good vaccination results in France. In the course of the same year, these successes were confirmed by Dalling (*19*) in the Polish veterinary review Medyc, Wet. and in 1951 they were emphasized in the same review by Manninger (*92*). In 1951 Kilchsperger (*71*) reported that in Switzerland the vaccine was used on a large scale in various places, and the vaccination with crystal violet vaccine "is the easiest, the safest, and the least expensive way to get rid of hog cholera gradually in big areas." Zeller (*121*) was also able to observe good results in 1951 through the use of crystal violet vaccine in Württenberg.

The vaccine is harmless and if one takes into consideration the practical conditions of raising pigs, it guarantees sufficient immunity in regard to degree and duration, and, what is most important, it does not contain active virus.

Summary

On farms exposed to swine fever, about 80,000 pigs were preventively vaccinated with a crystal violet vaccine made according to Dorset's method. The author reports satisfactory results achieved in that way. This method of vaccination is recommended.

At the same meeting where the preceding report was presented, another report by Zeljko (*120*) included a section on the use of crystal violet vaccine in Yugoslavia. The following statements were translated from Zeljko's report:

Immunization tests of pigs with formalized vaccines did not give practical results. Immediately after the discovery of crystal violet vaccine in the United States, we carried out the first tests with the latter in our country in 1937. They were encouraging and the vaccine was used up to 1940, although with certain modifications in regard to its production. In addition to the laboratory tests, we have employed it here and there, and on a small scale in the field. All these tests were satisfactory but they had to be interrupted on account of the war. . . . In 1946, the number of pigs vaccinated with this vaccine, in areas where hog cholera existed, amounted to 1,411. According to verified data, not one vaccinated pig of more than 2 months of age, succumbed to hog cholera, although the noninoculated pigs which lived in the same area were affected with the disease. . . .

. . . . With the vaccine produced in Kalinovica, the number of vaccinated pigs in Yugoslavia was as follows:

In 1948	32,000
1949	350,000
1950	1,048,000
1951	940,000
1952	506,000
Total	2,876,000

As a conclusion we might say that as far as the crystal violet vaccine is concerned, it possesses indisputable advantages over the simultaneous treatment, sharing in this respect Ramon's opinion that the dead* vaccines, which give solid immunity, are the most desired vaccines. On the other hand, we

* Editor's note: Whether crystal violet vaccine is a dead or an attenuated virus vaccine has not been definitely proved.

face the negative phase which can easily compromise the vaccination itself in a country with numerous sources of hog cholera. Nevertheless, we have proven that even in such areas one can utilize with precaution the crystal violet vaccine, thus refuting the opinion of those who persist in believing that the crystal violet vaccine cannot be used in countries where hog cholera exists. . . .

Modified Live-Virus Vaccines

The first license for the production and distribution of modified live-virus vaccines was issued by the U.S. Department of Agriculture in 1951. Issuance of other licenses soon followed, and during the 6-month period from July 1, 1953, to December 31, 1953, 11,500,500 doses were sold.

This type of vaccine is prepared by the modification of virulent hog cholera virus so that its disease-producing ability is gradually decreased but it still retains its viability. Some viruses are so modified that they may be used without anti-hog-cholera serum, while others are administered simultaneously with serum. These modified live-virus vaccines are prepared either by multiple passages in rabbits or multiple passage in special kinds of tissue-culture media. Their development, accomplished largely by commercial establishments, stems from research work with rabbits, conducted in 1946, by Baker (*1*) and by Koprowski and associates (*73*), and with tissue culture by Boynton (*3*) in 1946.

No research was carried on by the Pathological Division of the Bureau on the production of modified live-virus vaccines, but many tests were conducted in cooperation with other Divisions. Some potency tests were conducted at the request of the Virus-Serum-Control Division in support of applications by commercial firms for production licenses. Other tests were made in cooperation with the Interstate Inspection Division in efforts to determine the cause of losses following administration of the vaccines in the field. A few investigations were made to obtain information as to the efficiency of the vaccines, and reactions following their administration.

Field Investigations

In making field investigations, the first step was to make an accurate diagnosis of the disease with which the animals were affected. The history, symptoms, and lesions were carefully noted, and specimens of tissues and blood were obtained for bacteriological examination. If the examination indicated the presence of cholera, the blood was filtered and injected into susceptible pigs. When, by these methods, it was determined that cholera was present, an attempt was made to obtain samples of the vaccine used to vaccinate the herd under investigation, and test it for potency and purity. In some of these tests, the vaccine, when used as directed, not only did not produce disease, but did produce solid immunity. In other tests the vaccines were low in potency. Some veterinarians who administered the vaccines were firmly of the opinion that the vaccine had produced the disease. However, in the tests made in these investigations, it was found that none of the vaccines produced disease.

Potency Tests of Modified Live-Virus Vaccines

Eighteen samples of modified live-virus vaccines produced by six different firms were tested. Eight of the 18 afforded 100 percent protection, four afforded an average of 83.75 percent, and the six lots lowest in potency afforded an average of 35.8 percent protection, with a range of from 20 to 50 percent.

The use of any immunizing agent against hog cholera has always been followed by more or less trouble, and the modified live-virus vaccines were not different. It is possible that they are as good as, or better than, other methods of immunization; only time will develop the answer to that question.

Comparison of Leucocyte Counts[5]

An experiment was conducted in January 1952 to study the reactions of pigs to immunizing treatment with modified live-virus vaccines, crystal violet vaccine, and serum and virus.

Thirty susceptible pigs were obtained from two farms for this experiment. On the fourth and second day before treatment, and on the day of treatment but before treatment was administered, total and differential leucocyte counts were made and the temperatures were recorded. On the day of treatment the 30 pigs were divided into five groups of 6 pigs each, and were treated as follows:

Group A—2 cc. of a modified live-virus vaccine of rabbit origin.
Group B—2 cc. of a modified live-virus vaccine of porcine origin.
Group C—5 or 10 cc. of crystal violet-glycerol vaccine.
Group D—2 cc. of regular virus and a normal dose of commercial serum.
Group E—2 cc. of regular virus and double the normal dose of serum.

On the fourth and fifth days after treatment, total and differential leucocyte counts were made on all 30 pigs. On the fifth day, 2 pigs from each group were sacrificed, necropsies were performed, and bacteriological cultures were made from the heart-blood, lung, liver, kidney, and spleen. On the seventh day after treatment, total and differential leucocyte counts were made on the remaining 20 pigs, and 2 pigs from each group were sacrificed for necropsy and bacteriological cultures. On the 10th day after treatment, the same procedure was followed for the 10 remaining pigs. Temperatures were taken daily through the test, and clinical observation indicated that all pigs remained normal.

In 13 of the 30 pigs, no lesions were found. Slight abnormalities were found in 17. Most of the lesions were slight and were not considered as indicative of any particular disease. Lesions were distributed as follows: 11 in bladders, 5 in one or more lymph glands, 5 in the stomach causing inflammation or congestion, 4 in the epiglottis, 1 in the spleen, and 1 in the lung. The most common lesions found were petechiae in the bladder. Lesions were found in pigs from all five groups almost equally distributed. In groups B, D, and E, 3 pigs showed lesions and 3 did not. In groups A and C, 4 showed lesions and 2 did not. There was an increase in the number of pigs in which lesions were found from the first

[5] Leucocyte counts are also discussed on pp. 39, 40, 57, and 87.

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to the last day on which they were sacrificed. Three, or 30 percent, showed lesions when examined on the 5th day; 6, or 60 percent, showed lesions on the 7th day; and 8, or 80 percent, on the 10th day.

BACTERIA FOUND

Streptococci, various bacilli, and *Pasteurella suisepticus* were found in one or more cultures from 10 pigs following all treatments. Five cultures were obtained from pigs that had lesions, and five from pigs that had no lesions.

TOTAL WHITE CELL COUNT

The average leucocyte count before treatment was 20,467; on the 4th day after treatment, 21,981; on the 5th day, 24,383; on the 7th day, 20,618; and on the 10th day, 15,948.

The total leucocyte counts on the 10th day after treatment may serve to distinguish between the treatments in which serum was used and those in which it was not used. Thus, on the 10th day the total leucocyte counts following treatments which did include serum (B, D, and E) were below pretreatment range, while the counts following treatment that did not include serum (A and C) were within pretreatment range on the 10th day.

In these experiments it was found that after treatment D (virus and normal dose of serum) there was a drop of about 4,000 leucocytes per cubic millimeter on the fourth day, and that after treatment E (virus and two times the normal dose of serum) there was an increase of about 1,000 leucocytes per cubic millimeter on the fourth day.

It is noteworthy that on the fourth day an increase of leucocytes was recorded following treatments in which modified live-virus vaccines were used (A, B, and C), while following treatments in which regular virus was used, a slight decrease occurred (treatment D), or no essential change occurred (treatment E).

DIFFERENTIAL COUNT

In making differential counts, 200 cells were counted in the blood smear of each pig from each group, and the standard values expressed in percent, together with the standard error. The cells differentiated and counted were basophiles, eosinophiles, juvenile neutrophiles, band neutrophiles, segmented neutrophiles, lymphocytes, and monocytes.

It is highly significant that the lymphocytes following treatments with crystal violet and serum and virus (C, D, and E) increased 12.4 percent, 16.9 percent, and 17.3 percent, respectively, whereas the total neutrophiles following the same treatments decreased 17.1 percent, 31.2 percent, and 33.1 percent, respectively; this inverse correlation did not occur following treatments in which modified live-virus vaccines were used (A and B).

This correlation of lymphocytes and neutrophiles indicates that there is a difference in the leucocytological reaction to treatments with modified live-virus vaccines, and treatment with serum and virus, or crystal violet vaccine.

There was a significant variation in the reactions of the types of

neutrophiles according to age, as a result of the various treatments. Following treatments, A, B, C, and D, the juvenile neutrophiles increased 29.4, 64.3, 70.4, and 48 percent, respectively; the band neutrophiles, following treatments C, D, and E, decreased 44, 43.1, and 61.1 percent, respectively; and the segmented neutrophiles, following treatments D and E, decreased 35.2 and 36.5 percent, respectively.

The results are of a preliminary nature and are only suggestive of possible leucocyte variation for the following reasons:

(1) The counts made before treatment and on the 4th and 5th days after treatment represented 6 pigs with each treatment. Since the counts made on the 7th day represented only 4 pigs and the counts made on the 10th day represented only 2 pigs for each treatment, these values are proportionally less significant. However, it should be noted that the smaller numbers of pigs used on the 7th and 10th days after treatment were considered in determining the standard deviation, which in turn was used to establish the standard error.

(2) The values on the 10th day for several leucocyte-treatment combinations and for the total leucocytes had not returned to pretreatment range, and thus had not established comparative stability.

These results suggest that for investigations of this question, a larger number of pigs should be used and the experiments should be extended over a longer period of time.

PART 6. VARIANT VIRUS

History of Breaks

From the time the simultaneous treatment was discovered and put into extensive use to immunize swine, unfavorable and sometimes unexplained results have occasionally occurred following treatment. When compared with the large number of successful immunizations of swine, these occurrences have been of minor importance; nevertheless, they have been a matter of concern in isolated cases, and have been the subject of study for many years.

Various reasons have been advanced for these adverse reactions, commonly known as "breaks," such as impotent serum, underdosage, secondary infection, unthriftiness, nutritional disturbances, and parasitism. All these conditions have, no doubt, at times been responsible for unfavorable results following simultaneous treatment, but in some cases, none of these conditions existed, and the cause of breaks could not be determined.

Events Leading to Investigations

In the early summer of 1949 reports began to come from farmers that recently vaccinated hogs were sick. Preliminary investigations by practicing veterinarians failed to reveal the cause. Representatives of serum companies were called to investigate, but still no acceptable explanation could be made for the unfavorable results. In July 1949, at the request of serum producers, the Bureau of Animal Industry began an investigation.

Bureau Investigations

A survey showed that losses followed the use of virus of certain serial numbers. Only a few viruses were involved, and one in particular was followed by losses in practically every herd in which it was used. In some herds practically all animals were sick and many had died; in others, fewer became sick; and in still others, no losses were noted or reported. Serum of the same serial numbers used in this outbreak, when used with other serial numbers of virus in other herds, had been completely successful.

The seriousness of the situation is indicated by the fact that more than 4,900 pigs died, most of which had been in normal condition when treated with serum virus.

Similar breaks occurred in the summer of 1950. Investigations carried on in both years, and continued to the present time, are herein described.

Autopsies

Autopsies were conducted in the field and specimens of blood and tissues were obtained for laboratory studies. Samples of the serum and virus used were tested for purity and potency. The predominating lesions were such as are usually found in cases known to be hog cholera. Atypical symptoms and lesions due to complications were observed in some herds, but the overall conditions were typical of hog cholera.

Samples of the blood and tissues, as well as the serums and viruses, were cultured and portions were injected into mice, rabbits, and guinea pigs. These tests failed to reveal the presence of extraneous viruses or pathogenic bacteria that could account for the trouble. Tests of the viruses associated with the losses were made against the serums used, and against other brands and serial numbers not associated with the trouble. A brief description of these tests follows.

Experiment To Reproduce Field Conditions

FIRST TEST

The object of the first test was to find out whether conditions similar to those seen in the field could be reproduced experimentally. In this experiment, carried out at Beltsville, virus A and serum C–1, both of which had been used in the field, were tested. As controls, commercial serum C–2 (produced by another company) and virus B (a regular commercial virus) were used. No trouble had been reported in the field from their use. The amounts of serum were as recommended for the weights of the hogs; and the dose of virus was 2 cc. in all cases. The results were as follows: The field serum and the control serum each gave 100-percent protection against commercial virus B, but failed to protect adequately against virus A, associated with trouble in the field. Four pigs, serum-virus immunes, remained normal after injection of 10-cc. doses of virus A.

SECOND TEST

In the second test, conducted at Ames, three pigs were treated with 35 cc. of an unrelated serum, C–3, and 2 cc. of virus A. Two controls were given virus A only. In the first test (at Beltsville) neither serum protected adequately against virus A, but in the second test (at Ames) serum C–3, a commercial product, gave adequate protection against virus A. This might be interpreted as indicating that field serum C–1 was of low potency, but this serum gave adequate protection against virus B in the first test, and against other viruses in later tests.

The results in the second test showed that serums C–1 and C–3 were different in potency and the results of the first test showed that viruses A and B were immunogenically different. Two serums that failed to protect against virus A did protect against virus B. A slight difference in potency between lots of serum is not uncommon and could not be considered the cause of the trouble. On the other hand, such radical difference between two viruses had not previously been encountered. From the results of these experiments it became reasonably certain that the cause of the extremely severe losses in the field was the virus used. The question naturally arose as to whether one simultaneous virus contained more actual hog cholera virus than the other, or whether there was a difference in quality. The following experiment was carried out to obtain information on this subject.

The first step in this experiment was to titrate virus A, the field virus, and a Bureau virus (No. 276) that had been regularly used in experimental work at Ames. The doses were 1 cc., 1/8,000 cc., 1/10,000 cc., 1/1,000,000 cc., and 1/5,000,000 cc. In this test all pigs given injections of virus A remained normal except the two pigs that received 1 cc., and one of the two pigs that received 1/8,000 cc. This latter pig was sick for a few days but recovered. All of the pigs that did not get sick and the one that recovered were later given injections of regular virus, and all developed cholera. This would indicate that the reaction in the recovered pig was due to some cause other than the 1/8,000 cc. of virus A that it received. Therefore, the smallest dose of virus A that produced cholera in this test was 1 cc. In the test of BAI virus 276, the pigs that received from 1/1,000,000 cc. to 1 cc. developed cholera.

The second step in this experiment was to determine the comparative amount of serum that would be required to protect against these two viruses.

Test of Serums Used in the Field

Two kinds of serum, C–1 and C–3, were both used against 2-cc. doses of virus A and BAI virus No. 276. The doses of serum administered were 5 cc., 15 cc., and 45 cc. The results were as follows: Five cc. of serums C–1 and C–3 afforded adequate protection against BAI virus 276, which contained at least 1,000,000 minimum lethal doses per cc., whereas 45 cc. of serum C–1 and 15 cc. of serum C–3 were required to protect against virus A, which contained less than 8,000 minimum lethal doses per cc. The results of this experiment indicated that the difference between virus A and BAI virus No. 276 was a difference in the kind of virus and not in the amount

of active virus per dose administered. Virus A was therefore designated "variant virus." This finding was announced by the Department on November 8, 1949 (*122*), and a full report was published in 1951 (*18*).

Identification of Variant Virus

After exhaustive tests, a commercial serum was found that afforded, in certain doses, 100-percent protection against BAI virus, and just as regularly failed to protect against variant virus. The serum was identified as BAI experimental serum No. 1. This serum was used thereafter as a standard serum to detect variant viruses.

Doses of Serum With Variant Virus

In the field work that led to the recognition of variant virus, it was observed that large doses of serum appeared to have afforded protection, whereas the recommended and usually employed doses failed to protect adequately. This point was investigated in a series of experiments, which confirmed the field observations.

In one experiment, pigs were separated into groups and given doses of 5, 15, 20, 25, 30, and 45 cc., respectively, of BAI experimental serum No. 1, and 2 cc. of a variant virus. Other groups were given 5, 15, 20, and 25 cc., respectively, of the same serum and 2 cc. of regular BAI virus. Each group contained 6 to 9 pigs. The doses of serum and the results in percentage protected are shown in table 5.

As a result of these studies, the recommended dosage specified on the labels of serum containers was increased by 25 percent.

TABLE 5.—*Protection afforded by various doses of serum against variant and BAI viruses*

Serum dosage (cc.)	Pigs protected against—	
	Variant virus	BAI virus
	Percent	*Percent*
5	0	78
15	12½	100
20	11	100
25	55½	100
30	67	----------
45	100	----------

Tests of Commercial Sera Against Variant Virus

A number of commercial serums were tested for antibodies against a variant virus that had caused trouble in the field in 1950. As controls, the same number of pigs used in tests against variant virus were treated with the same doses of the same serum and a regular BAI virus. The protection afforded by commercial serums when used in small doses was, in all cases, better when used against BAI virus than when used against variant virus. In this experiment all

pigs that received doses of 30 cc. or more were adequately protected against variant virus.

It was observed in the field that all herds, or all pigs in a herd, did not react in the same way to the same serum and virus. For example, losses in some herds were heavy among the larger pigs and very light among the smaller ones. In other herds some pigs of the same weight were protected by a certain dose of serum while others died. From these experiments it was concluded that losses due to variant virus can be reduced or entirely eliminated by vaccinating pigs before weaning and by increasing the dose of serum.

Tests at Commercial Serum Laboratories

As soon as recognition of a variant virus was announced, inquiries were received regarding the possible presence of variant virus in establishments not involved in losses occurring in the field. To obtain information on this subject, BAI experimental serum No. 1 was furnished to practically all licensed establishments, and they were advised how to test their products for variant characteristics. Tests were made in nearly all licensed establishments, but no definite evidence of variant virus was found. Since all serums tested, when used in sufficiently large doses, protected against variant viruses, it was considered logical to produce serums and vaccines from the blood of pigs inoculated with variant virus. Efforts were made at Beltsville and at Ames to find or develop a variant strain of virus having satisfactory immunogenic properties. To date, neither serums nor vaccines of satisfactory potency have been produced consistently with variant virus.

Titre

The titres of most variant viruses studied were considerably lower than those of regular viruses, but relatively high titres were found in the blood of pigs that developed cholera following injection of small doses of serum and variant virus.

Tests of Commercial Viruses for Variant Characteristics

Two experiments were conducted in which 175 pigs were used to compare results of treatment with variant virus and with commercial virus plus minimum doses of BAI experimental serum No. 1. In these tests 10 commercial viruses, a regular BAI virus, and a known variant virus identified as 1–B were tested. The pigs were divided into groups and each group received 2 cc. of a different virus; half of each group received 10 cc. of serum, and half received 15 cc. Most of the pigs weighed between 50 and 90 pounds. The results showed that all the groups in which BAI virus or a commercial virus was used were better protected than the groups in which the variant virus was used. The two groups that received BAI virus and six of the groups that received commercial virus were 100-percent protected.

In four other groups in which commercial viruses were used 80, 90, 90, and 60 percent of the pigs were protected. In the two groups that received variant virus 1–B, 30 and 50 percent were protected. The results from the group that received commercial virus, in which

only 60 percent of the pigs were protected, led to the conclusion that one of the ten commercial viruses might have variant characteristics. An attempt was made to obtain another sample of this virus for further study but none was available. The producer reported that a large quantity of the virus had been distributed and no unfavorable results had been reported. Since the dosage of serum was low for the weight of the pigs used, some unfavorable results were expected. The results did show that virus 1–B was a variant. Virus 1–B was the eighth passage with serum of variant virus A, which had been associated with heavy losses in the field in 1949.

Diluted Variant Virus

Two experiments were conducted to determine whether the addition of variant virus to a regular virus would transmit variant characteristics to the mixture. In these experiments pigs were given 10 and 15 cc. of BAI experimental serum No. 1. Some of the pigs were given simultaneous injections of regular BAI virus 286; some, variant virus 1; some, a mixture of 5 parts of virus 1–B and 95 parts of virus 286; and others, a mixture of 10 parts of virus 1–B and 90 parts of virus 286. The results were—

(1) The group that received virus 286 remained normal.
(2) The group that received virus 1–B was only 33⅓-percent protected.
(3) The group that received a mixture of 5 percent of virus 1–B and 95 percent of virus 286 was 83⅓-percent protected.
(4) The group that received a mixture of 10 percent of virus 1–B and 90 percent of virus 286 was 100-percent protected.

Thus, no evidence was obtained to show that the addition of 5 or 10 percent of variant virus 1–B to regular virus 286 transmitted variant characteristics to the mixture.

Effects of Small Doses of Variant Virus

In the first part of this experiment a number of pigs were given BAI experimental serum No. 1 in doses of 10, 15, 20, and 30 cc. At the same time, half of each lot received variant virus 1–B and half received variant virus H in doses of 2 cc. Of the pigs that received virus 1–B, 37½ percent were protected, but none of the pigs that received virus H were protected.

In the second part of the experiment, pigs were treated with 15 cc. and 20 cc. of BAI experimental serum No. 1. With these doses of serum, one group received variant virus 1–B, and one received variant virus H, in doses of $\frac{1}{10}$, ½, 1, and 2 cc., respectively. Of 4 pigs that received $\frac{1}{10}$ cc., 100 percent were protected; of 4 that received ½ cc., 25 percent; of the 4 that received 1 cc., 50 percent; and of those that received 2 cc., 75 percent were protected. Although the group that received the smallest dose ($\frac{1}{10}$ cc.) was the best protected, the results were not consistent since the pigs that received 2 cc. were better protected than the group that received ½ cc. This work was not sufficiently pursued to reach definite conclusions.

Homologous Serum

Serum, said to be homologous to variant virus, was obtained from a commercial plant experiencing variant virus trouble. This serum and BAI experimental serum No. 1 were given in 5- and 15-cc. doses to a group of pigs, half of which received one, and half the other serum. Half of each group received simultaneously 2 cc. of variant virus H, and half received 2 cc. of regular virus 286. The protection against variant virus H was 25 percent, and against virus 286, 100 percent with both serums. Since this was not a controlled experiment the results did not reveal any reliable information. However, this and many similar experiments helped to confirm the evidence that had been accumulated to show the existence of a variant strain of hog cholera virus.

Effects of Variant Virus on Immune Hogs

In 1950, doses from 12 serial numbers of virus produced at one commercial serum plant were injected into immune pigs without causing any sickness. Four of these viruses were selected for a test with BAI experimental serum No. 1 in doses of 5, 15, 30, and 45 cc., respectively, for variant characteristics. In this test only one virus was shown to be variant. This was confirmed by subsequent tests.

Maintaining Variant Characteristics

In the early work with variant virus some difficulty was experienced in maintaining variant virus. That is, variant characteristics seemed to be reduced or lost after a few direct passages through susceptible pigs. It was found that by passage with nonprotective doses of serum the variant characteristics were maintained through nine passages. In the hope that variant characteristics had been fixed, the virus was passed seven successive times without serum and was found to still retain the original characteristics. A large supply of this virus, identified as variant virus 9096, was collected and frozen for experimental use. It was passed an additional 7 times, or a total of 14 times, without serum and appeared to retain its pathogenicity, but when tested with serum its variant characteristics appeared to be reduced. In former tests of this virus, 15-cc. doses of serum would not protect, but the same doses did protect against the virus of the seventh passage without serum.

Variant Virus Vaccines

Eleven pigs were inoculated with variant virus 9096, and 11 with BAI virus 298. The two groups were penned and handled separately. On the seventh day after inoculation the pigs were killed for virus. Blood amounting to approximately 100 cc. from each pig was frozen for future use, and the remainder was converted to crystal violet-glycerine vaccine by standard methods. The blood from each pig was processed and stored separately. After incubation was completed, samples were taken from each vaccine and a composite of each kind of vaccine (variant and regular) was tested for potency. Eighteen pigs were treated with 5 or 10 cc. of variant

virus vaccine, and 18 were treated with the same doses of regular virus vaccine. Twenty-two days later they were divided into groups and exposed to hog cholera. The treatment, the method of exposure, and the results are shown in table 6. The only group adequately protected was group 4, which was treated with regular virus vaccine and exposed to regular virus.

TABLE 6.—*Protection afforded by variant and regular vaccines against variant and regular viruses*

Group	Composite vaccine	Exposure virus [1]	Percent protected
1	9096	C298	33⅓
2		C9096	50
3		O9096	0
4	298	C298	100
5		C9096	0
6		O9096	16⅔

[1] C—composite; O—original.

Variant Serum

Two kinds of serum were prepared, one made from hogs hyperimmunized with variant virus and one made from hogs hyperimmunized with regular virus. These sera were tested for potency against variant virus in doses of 5 cc., 15 cc., and 30 cc. of serum and 2 cc. of virus. When variant virus anti-serum was used simultaneously with variant virus, the results were as follows: In 5-cc. doses, no protection; in 15-cc. doses, 50-percent protection; and in 30-cc. doses, 75-percent protection. When regular anti-serum was used against variant virus the results were: In 5-cc. doses, no protection; in 15-cc. doses, 50-percent protection; and in 30-cc. doses, 100-percent protection. Both variant and regular anti-serum protected 100 percent in 5-cc. doses when used with regular BAI virus.

A summary of these results indicated that regular serum protected somewhat better against variant virus than did the variant virus serum, and both variant serum and regular serum protected very much better against regular virus than against variant virus. The differences may have been due to the amount of actual virus in the defibrinated blood used to produce the regular and variant serums.

Conclusions of Investigations

The conclusions reached as a result of the study of the cause of heavy losses in the field following vaccination in 1949 and 1950 were as follows:

(1) A variant strain of hog cholera virus was discovered.
(2) Because of the general lack of knowledge at the time the losses occurred, they could not have been prevented.
(3) Veterinarians, serum producers, and most farmers performed their various functions properly.
(4) Variant virus was low in virulence as measured by titration.
(5) The trouble in the field was reproduced in the laboratory.

(6) The trouble can be prevented by vaccinating pigs when they are small, and by increasing the dosage of serum.

(7) Hogs immunized with serum and virus are not visibly affected by injections of variant virus.

PART 7. MISCELLANEOUS

Virus and Serum Control

By 1912 the demand for serum and virus had increased to the point where it became apparent that some method of Federal control over commercial serum-producing plants was necessary. Congress, on March 4, 1913, passed a law requiring that all producers of virus, serum, toxins, and analogous products intended for the treatment of diseases of domestic animals, if shipped interstate or imported into the United States, must comply with regulations prescribed by the Secretary of Agriculture. The task of drawing up and enforcing these regulations was assigned to the staff engaged in hog cholera research. As a consequence, there was a marked diminution in research activities between 1912 and 1915.

The Virus-Serum-Toxin Act became effective July 1, 1913, and a section was set up in the Biochemic Division, under the direction of Dr. Howard J. Shore, to inspect serum plants. This section was designated the Office of Virus-Serum Control. It was later made a separate Division of the Bureau, known as the Virus-Serum-Control Division. During 1915, 208,571,232 cubic centimeters of anti-hog-cholera serum were produced by 89 plants.

Hog Cholera "Cures"

From time to time persons claimed that they had discovered cures for hog cholera. Some of these claims had no scientific basis, but the Bureau was compelled to test a number of them. Other claims, made by commercial laboratories, were investigated in connection with regulatory work. A few of these claims are discussed briefly.

A claim that 1 tablespoonful of sulfuric acid to a gallon of drinking water given regularly would cure and prevent cholera was tested. Two pigs were given the treatment for 16 days and exposed to cholera. Both promptly developed cholera. Four pigs were given injections of virus and treated in the same manner, treatment beginning on the first day they became sick. All developed typical cholera.

Another product which was claimed to be a cure for hog cholera was Lugal's Solution. Five pigs were treated in the early stages of cholera, some with a single, and some with a double, subcutaneous injection of sufficient Lugal's Solution to equal a 0.5-gram dose. Four other pigs were given a 0.025-gram dose intravenously. All died of cholera.

Another recommended remedy, consisting of calomel, ipecac, and soda, was tested on a farm where cholera prevailed and at the experiment station. Six pigs were treated, and all died.

A claim that tomatoes in the feed was considered a remedy for hog cholera was investigated. Pigs were fed liberal quantities of

tomatoes for 6 days, after which they were exposed to hog cholera by virus injection, and were fed tomatoes daily thereafter. No mitigation of the incidence, progress, or outcome of the disease was observed.

A preparation called D. O. D., widely advertised as a preventative and treatment for hog cholera, was tested, and proved to be of no value in either the prevention or treatment of cholera.

In 1932 a commercial preparation known as hog cholera antigen, produced by a laboratory in Kansas City, was tested. Four pigs were treated according to the directions of the producer and placed in a pen with three untreated controls. They remained normal for 30 days. At the end of 30 days the treated pigs and the untreated controls were given injections of virus and all died.

Many other products sold as cures or immunizing agents were tested by the Bureau and by State agencies, but none except serum and vaccines proved to be of any value.

Breed Immunity

Three sows, crosses of the wild hog of Germany with native American hogs, were considered by the owner to be immune to hog cholera. These hogs were shipped to the Ames experiment station and tested for immunity. They all developed typical hog cholera. Similar results were obtained in tests of mule-foot hogs, for which claims of natural immunity had been made.

Effects of Sodium Ricinoleate on Hog Cholera Virus

Sodium ricinoleate was reported to be an active detoxifying agent for bacterial toxins. In order to determine the effect of this soap on hog cholera virus, a 1-percent solution of pure sodium ricinoleate was prepared and mixed in equal proportions with a solution of the laked red blood cells obtained from a pig sick of hog cholera. After contact for 3½ hours, pigs were given injections of the treated laked cells. All pigs developed hog cholera in typical form. There was no evidence of attentuation by the ricinoleate.

Effects of Formaldehyde on Blood Serum

Following adverse effects attending the use of formaldehyde as a serum preservative, a number of chemical studies were made to determine the effects of formaldehyde on the immunizing fraction (globulins) of serum. It was found that even small amounts of formaldehyde produced profound changes over a period of time (*64*, *65*, *67*).

Spirochaeta suis

In 1914, it was announced by W. E. King that an organism, which he named *Spirochaeta suis*, existed in the blood and intestines of cholera pigs. King and Hoffmann (*72*), from the results of their studies, claimed that this organism was more clearly established as

the specific cause of hog cholera than any other known organism. Studies made by the Bureau showed that spirochaetes were found in considerable numbers in the intestinal contents, particularly in and around intestinal ulcers, of cholera pigs. In no case were they found in the blood. Extended examination of the intestinal contents of healthy pigs showed that the same spirochaetes were present in considerable numbers. This research led to the belief that the spirochaete referred to by King was a saprophytic inhabitant of hogs' intestines and is in no way responsible for the production of hog cholera.

Diagnostic Methods

One of the outstanding needs of the veterinary profession was a method of quickly and accurately diagnosing hog cholera. Many hogs were lost by delays in treatment pending a diagnosis. The Bureau endeavored to discover a better method and also investigated methods suggested by other research workers.

Leucocyte Counts

Dinwiddie (*31*) was perhaps the first to report that leucopenia was observed in pigs affected with cholera, even in the early stages of the disease. He stated that leucopenia was sometimes so pronounced that it was discernible in stained smears. The Bureau's work on leucocytes (p. 40) indicated that leucopenia was a usual, but not constant, feature of hog cholera.

Capillary Fragility

Certain diseases of humans, including avitaminosis, weaken the walls of the capillaries, and when suction is applied to the skin, leakage of blood occurs. It was considered that this test might be applicable in the diagnosis of hog cholera. An improvised suction apparatus was assembled, and tests were made on the skin of normal and cholera-affected pigs. Suction was applied for varying lengths of time and with varying amounts of vacuum. Diffuse reddening was produced, but no petechiae or leakage was observed in either normal or cholera pigs. It was not determined by this test whether or not cholera causes a weakening of the capillary walls since failure to obtain a reaction might have been due to the thickness of the skin of pigs.

Boynton Method of Diagnosis

The method of diagnosing hog cholera, described by Boynton (*4*), by examination of gall bladder cells was investigated. Gall bladders from 8 normal, mature hogs were examined, and all were negative. Gall bladders of 66 pigs killed for virus and of 14 that had died of cholera were examined. All were positive except those of two of the pigs that were killed for virus. The blood from one of the negative pigs was filtered and injected into two susceptible pigs. Both developed cholera. This investigation did not include any pigs in the early stages of cholera.

Blood Clotting

Preliminary studies on the possibility of basing a diagnostic test on the diminished clotting power of the blood of pigs affected with hog cholera were not encouraging.

Passage of Virus in Guinea Pigs

It has been claimed that by intracerebral inoculation, hog cholera can be serially propagated in guinea pigs, though in a form that is nonpathogenic for swine. Such experiments initiated in the Biochemic Division resulted in only slight lesions of questionable significance. It was concluded that proof of growth of the virus in guinea pigs was inadequate.

Complement

Titrations of complement in the sera of two pigs, before and after artificial infection, confirmed previous observations that complement content definitely increased within a few days after infection with hog cholera.

Precipitin and Agglutinin Tests

Efforts to find a satisfactory precipitin or agglutinin reaction of service in the diagnosis of hog cholera were unsuccessful.

Conclusions

Although some of the above-described methods were helpful in making a diagnosis of cholera, the only one recognized as accurate in all stages of cholera was the filtration method. This method is complicated, expensive, and requires considerable time to complete. Another objection to the filtration method is that it cannot be applied to the blood of pigs recently treated by the simultaneous method because the virus is known to circulate in the blood of vaccinated pigs for 2 to 3 weeks after treatment.

Combating Contamination

Sterilamp Efficiency

An experiment was conducted to determine the efficiency of a sterilamp in combating air-borne contamination. The lamp used in this experiment was a Westinghouse sterilamp, Type SB, Fixture for W1–782 lamp, Style No. 1122593, 115 volt, 60 cycle, 0.50 normal line amperes. The lamp was situated 6 feet 4 inches above a worktable in a room 9 feet high, 9 feet 7 inches long, and 5 feet wide. All plates were placed on the worktable and exposed for 5 minutes by removing the lid of the petri dishes.

The first plate was exposed before the light was turned on, a second plate was exposed at the time the light was turned on, and a plate was exposed each 5 minutes thereafter for 60 minutes. The lamp was then turned off and a plate was exposed each 5 minutes for another hour. Eight control plates were left covered in the

same room and approximately in the same location on the table. All exposed and control plates were then placed in an incubator. They were examined 48 hours later and the following results were noted:

All plates exposed 25 minutes or less after the lamp was turned on were contaminated except plate No. 6, exposed at 20 minutes. All plates exposed from 30 to 60 minutes after the lamp was turned on were sterile. Three plates exposed 5, 10, and 15 minutes after the lamp was turned off were sterile. Of the plates exposed 20 to 45 minutes after the lamp was turned off, three were sterile and three were contaminated. All plates exposed 50, 55, and 60 minutes after the lamp was turned off were contaminated. The eight plates left covered during the exposure of the other plates were sterile. It would appear that this lamp, which had been used approximately 165 hours, will have to be turned on 30 minutes before the danger of airborne contamination is eliminated.

In another experiment conducted in the same room with the same lamp, which had been turned on 30 minutes, 2 cc. of tested hog cholera virus was poured into a petri dish and exposed for 30 minutes. The virus was then injected into a susceptible pig and it produced hog cholera. The rays of the lamp apparently had no effect on the phenolized hog cholera virus. Later, many experiments were carried out with ultraviolet light in attempts to destroy virulence but to retain the antigenicity of clear serum hog cholera virus. All were unsuccessful.

Mixtures of Crystal Violet and Other Agents

The original crystal violet vaccine was prepared with crystal violet alone. Such vaccines were frequently found to be contaminated. In order to increase the bactericidal effectiveness of the dye, Dr. F. W. Tilley suggested the addition of disodium phosphate. Vaccines so prepared (that is, with 1–2,000 crystal violet and M/50 dibasic sodium phosphate) were used for several years with good results. Occasionally, however, contaminated lots were found. As a means of further increasing the bactericidal effectiveness of the dye, Dr. Tilley proposed the use of a mixture of crystal violet and glycerol instead of the crystal violet-phosphate mixture.

In order to determine the relative effectiveness of the crystal violet-glycerol mixtures, several experiments were carried out. The general plan of these experiments was to add to packing-house, defibrinated, hog blood, mixtures of various bacteria to determine the number present, to add the different bactericidal agents to different portions of the contaminated bloods, to incubate the portions, and to make counts at various intervals on the incubated portions.

The contaminants added were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella suipestifer*, and three cultures of contaminating organisms Nos. 136, 318, and 364, isolated from different samples of hog cholera vaccines. Subcultures of *E. coli* added in the first three experiments were derived from the same source, but a different culture was used in experiment 4.

The blood was collected in the slaughterhouse at Beltsville. In the various experiments, different intervals of time elapsed between the collection of the blood and the addition of the contaminants and bactericidal agents. In the first experiment, the agents were added

the day the blood was collected; in the second, the day after the blood was collected. In experiments 3 and 4, the same lot of blood was used. The agents were added 2 days after the blood was collected in experiment 3, and 8 days after collection in experiment 4. The results are shown in table 7.

The results of all tests were in substantial accord: In all tests in which differences appeared, the crystal violet plus 20-percent glycerol mixture was most effective bactericidally, and the phosphate mixture the least effective. Although not all of the organisms were killed by the 20-percent glycerol mixtures in experiments 3 and 4, about 99.7 percent were killed, and multiplication was prevented in those that survived, whereas in the portions treated respectively with 15-percent glycerol and phosphate mixtures, the organisms survived and multiplied enormously. In experiment 3, the organisms appeared to be gradually dying out in the 20-percent glycerol portion kept at room temperature. In experiment 4, the organisms remaining in the 20-percent glycerol portion after 4 weeks at room temperature were apparently spores.

The greater resistance shown by the organisms in experiments 3 and 4 was perhaps attributable to the greater interval of time between collection and use of blood. In the first two experiments the blood, which in no case was sterile when collected, was used within 24 hours after its collection, whereas 2 days elapsed in experiment 3, and 9 days in experiment 4. Following these experiments the process was patented (*116*), and the patent assigned to the Secretary of Agriculture. From that time, the process was the only one used at Ames to produce vaccines for field use, and all lots so prepared were sterile.

Breaks in Immunity

Many investigations were made to determine the cause of unfavorable results following the vaccination of pigs by the serum-virus treatment. The sickness was referred to as a break. When it occurred soon after vaccination it was called a serum break, and when it occurred some time later, it was referred to as a virus break. The tendency, usually, was to place the blame on the products used—either impotent serum or nonvirulent virus. In such cases, samples of the products were, when possible, obtained and tested at the experiment station. Only on rare occasions was it found that a virus was not virulent or that a serum was entirely lacking in potency.

In the very early stages of the use of anti-hog-cholera serum, the dosage recommended was not sufficient to protect adequately in all herds. It was therefore considered that during that period insufficient dosage was the cause of some unfavorable results.

It was also discovered that a leucopenia, which is equivalent to lowered natural resistance, followed the use of serum and virus. During the period of lowered resistance, pigs were susceptible to bacterial infection and other debilitating conditions. Breaks occurred under these conditions when the serum and virus were found by test to be satisfactory.

After the discovery of variant virus and the knowledge that at times it quickly reverted to normal virus, it was recognized that quite probably variant virus was the cause of unexplainable early

TABLE 7.—*The bactericidal effectiveness of crystal violet-glycerol mixtures in comparison with the effectiveness of crystal violet-phosphate mixtures*

Experiment	Organisms present per cubic centimeter [1]	Attenuating agents used with crystal violet (1–2000)	Bacteria per cubic centimeter remaining—					
			After incubation				After additional standing at room temperature	
			3 days	7 days	14 days	21 days	2 weeks	4 weeks
	Number							
1	190, 000	15% glycerol	Sterile					
		20% glycerol	do					
		M/50 Na_2HPO_4	do					
2	910, 000	15% glycerol	19,000	500	Sterile			
		20% glycerol	Sterile					
		M/50 Na_2HPO_4	29,000	130, 000	30,000,000			
3	[2] 600, 000	15% glycerol	10,000,000					
		20% glycerol	6,000	2, 300	3,500	1, 200	1, 700	900
		M/50 Na_2HPO_4	1,000	240, 000	30,000,000			
4	[3] 550, 000	15% glycerol		230, 000, 000				
		20% glycerol		2, 000	1,800		2, 000	1, 900
		M/50 Na_2HPO_4	430,000,000					

[1] Cultures in all experiments: *Staphylococcus aureus, Salmonella suipestifer, Escherichia coli,* and contaminants isolated from vaccines.

[2] Blood used in experiment 3 kept in a refrigerator for 48 hours before being used.

[3] Blood used in experiment 4 the same as for experiment 3 after being held in a refrigerator for 7 days.

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breaks. Since large doses of serum not only protected against variant virus but also prevented severe reaction after vaccination, it was logical that breaks of this kind could be prevented by administering large doses of serum, and that was the recommendation.

Late breaks may be caused by improper handling of virus or inactive or attenuated virus. In one investigation, 1 of 6 commercial viruses did not produce disease. In later tests, 1 of 10 did not produce disease using 2-cc. doses.

Many supposedly immune hogs, when challenged with virus prior to hyperimmunization, develop cholera. For this reason, serum producers prefer to obtain hogs for hyperimmunizing from garbage feeders or other sources where cholera infection is more prevalent than it is on ordinary farms.

While some of the causes of failure to produce immunity with serum and virus are well known, other causes will no doubt be determined in the future.

Relation of *Salmonella Choleraesuis* to Hog Cholera

Despite the finding of a filtrable virus as the cause of hog cholera, *Salmonella choleraesuis* continued to receive attention because of its close association with hog cholera. It was often found when investigations were made of hog cholera outbreaks. Frequently these investigations involved pigs that had been serum-virus vaccinated. Some of these studies were made prior to 1937, before it was possible to preserve cultures and maintain their pathogenicity by lyophilization.

Experiments Prior to 1937

The 1921 Report of the Chief of the Bureau mentioned the occurrence of disease in hogs that had previously been treated by the simultaneous method. This information led to commercial production, on a large scale, of bacterins, which were recommended by the producers for preventing and overcoming "breaks," the theory being that the disease in the treated pigs was due not to lack of immunity against hog cholera, but to invasion by certain micro-organisms. Experiments carried out with *S. choleraesuis* indicated that bacterins made from that organism were of very doubtful value. It was well demonstrated in this experimental work, however, that the filtrable virus of hog cholera exerted a remarkably favorable influence on *S. choleraesuis*, and it appeared that the latter was an important factor in hog diseases, but its exact relation to hog cholera or other diseases of swine was not defined.

The studies were continued in 1922, during which time bacteriological examination of a large number of samples of commercial hog cholera virus failed to reveal the presence of *S. choleraesuis* in any case. Apparently there was no need to fear bad results from its presence in properly prepared virus. The results of the experiments did indicate that a severe *choleraesuis* infection, occurring simultaneously with the administration of serum and virus, may be a cause of trouble. It was found also that ill effects resulting from this organism at the time of simultaneous inoculation could, in large part, be overcome by increased doses of anti-hog-cholera serum.

In 1927, studies were made of the effect of *S. choleraesuis* on immunization of pigs against cholera. In these experiments the object was to determine if the presence of *S. choleraesuis* in the infecting virus would cause pigs previously immunized with virus not containing *S. choleraesuis* to contract cholera, and at the same time to determine whether or not immunization with virus containing *choleraesuis* would prevent such breaks in immunity. For this study, 49 pigs were immunized by the simultaneous method, 24 of them with virus containing *S. choleraesuis*, and 25 with virus free of *S. choleraesuis*. The 49 pigs thus immunized were held on pasture for 2 to 4 months, and then subjected to an immunity test by the injection of virus containing *S. choleraesuis*. Both groups of pigs remained well, while nonimmune controls promptly contracted the disease.

Studies were made of the survival of *S. choleraesuis* in virus after the addition of 0.5 percent of phenol to the defibrinated virus blood. After the phenol was added, the mixtures were held in a refrigerator at a temperature varying from 45° to 50° F. Daily bacteriological counts were made. The results agreed with those previously reported—that is, *S. choleraesuis* had lost its viability after 10 days.

While these experiments were not conclusive with respect to the part played by *S. choleraesuis* in so-called breaks in hog-cholera immunity, they afforded no indication that the micro-organism is an important factor in such cases.

Again in 1928, studies were made to determine the effect of *Salmonella choleraesuis* and *Pasteurella suisepticus* in breaks at the time of treatment—in other words, "early breaks." Susceptible pigs were given injections simultaneously of serum, virus, and cultures of the two organisms named. In two out of four experiments with cultures of *S. choleraesuis*, the investigators produced very definite early breaks in immunity; that is to say, the serum failed to protect completely against the virus and cultures. In the third experiment there was a slight break—that is, slight illness of the pigs given injections. In the fourth experiment, no break occurred. In two experiments in which *P. suisepticus* was used, there was no evidence of a break in immunity.

Late breaks were studied in much the same way, except that no serum was injected at the time the culture was used. In these cases, a number of pigs were immunized in the regular way with serum and virus and, after an interval of 1 to 6 months, were tested by injections of a mixture of hog cholera virus and *S. choleraesuis* cultures. Out of three experiments, one very definite break was produced; in one experiment there was a slight reaction, whereas in the other there was no effect. Attempts to produce late breaks with *P. suisepticus* cultures were unsuccessful.

While the experimental work was not extensive, it indicated that both early and late breaks apparently may occur in supposedly immune shoats that are exposed simultaneously to virulent strains of *S. choleraesuis* and hog cholera virus.

The effect of *S. choleraesuis* with respect to a number of field cases of necrotic enteritis was investigated. It was evident that *S. choleraesuis*, as it occurs in nature, varies greatly in its pathogenic properties. Some of the strains isolated had comparatively little effect upon pigs, whereas others possessed great virulence.

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There is no doubt that certain members of this *suipestifer* group are capable of causing very severe enteritis in pigs.

Experiments After 1937

Studies were made of various strains of *Salmonella choleraesuis* and other paratyphoid organisms recovered from swine. A number of outbreaks of disease in swine herds were observed, and seven of these, which occurred in herds previously immunized against hog cholera, were selected for further study. In each of these seven cases, animal-inoculation tests demonstrated the presence of hog cholera virus in the blood of the affected animals examined. In two cases *S. choleraesuis* was also present, and in one case a paratyphoid organism other than *S. choleraesuis* was found; *Pasteurella suisepticus* was associated with one case; and in the other three cases, none of these pathogens were found. Efforts to recover swine influenza virus from some of these outbreaks were unsuccessful.

In studies of paratyphoid infection, when *Salmonella choleraesuis* organisms were fed to normal swine the result in each case was either an acute disease terminating in septicemia and death, usually in 3 days to a week, or a chronic form characterized by unthriftiness and loss of weight, often terminating in necrotic enteritis. In one test, in which *S. choleraesuis* was fed to normal shoats the day before treatment with hog cholera serum and virus, an acute disease developed, followed by death within a week. Pigs fed a culture of *S. choleraesuis* but not treated with serum and virus developed only a chronic form of the disease. Results indicated that serum and virus vaccination of animals carrying *S. choleraesuis* may result in severe losses.

In further experiments to obtain information on the virulence of *S. choleraesuis*, and the effect of vaccination with hog cholera serum and virus on animals infected with the organism, 20 hogs were fed *S. choleraesuis*, and 12 of them died from paratyphoid infection. Of 50 pigs fed the culture and vaccinated with serum and virus the following day, 29 died. Of 30 hogs simultaneously vaccinated with hog cholera serum and virus to which large numbers of *S. choleraesuis* organisms had been added, 17 died during the 10 weeks following treatment. The results of these experiments indicated that *S. choleraesuis* may cause losses among unvaccinated hogs, and that similar losses may occur following vaccination with hog cholera serum and virus when the animals are already infected with the *S. choleraesuis* organism.

In another experiment, 10 pigs were vaccinated with crystal violet vaccine, and a second group of 10 pigs, with serum and virus. One-half of each group was also treated with a bacterin prepared from *S. choleraesuis*. Three weeks after this treatment, all pigs were placed in a large barn and exposed to pigs that were carriers of *S. choleraesuis* and had been infected with hog cholera virus by inoculation. All the latter pigs died, and at post mortem showed typical lesions of hog cholera and also the presence of *S. choleraesuis* in their tissues. All the vaccinated pigs survived this exposure to hog cholera virus and *S. choleraesuis*, but 40 percent of those not treated with bacterin became seriously ill, were off feed for some time, and did not thrive during the course of the experiment. The

bacterin-treated pigs showed little or no reaction and were never off feed. Although the number of animals used was too small to draw more than general conclusions, the bacterin treatment showed promise of immunizing value.

Diagnosis of Hog Cholera and Swine Erysipelas

During the fiscal year ending June 30, 1933, when swine erysipelas was prevalent in some States, veterinarians experienced considerable difficulty in differentiating between that disease and acute hog cholera. This difficulty was increased by the coexistence of the two diseases in some herds. Samples of blood from 10 herds suspected of having erysipelas were shipped in thermos bottles to the experiment station at Ames, and were tested for hog cholera virus by injecting the blood into susceptible pigs. Four of the 10 samples were found to contain the virus of hog cholera.

In some of these cases, and in many others, a diagnosis of erysipelas was made, the herd was treated for that disease, and many pigs died of hog cholera. The important information gained from these tests was that in doubtful cases anti-hog-cholera serum should always be administered.

EVALUATION OF THE WORK OF THE BUREAU OF ANIMAL INDUSTRY ON HOG CHOLERA

To evaluate the research work on hog cholera by the Bureau of Animal Industry, the condition of the swine industry before the Bureau was established must be compared with conditions at present.

Before research work on hog cholera was begun, extensive losses in swine had occurred in all parts of the country. Indeed, it was the enormous losses that led to the first appropriation by Congress for the study of animal diseases, and to the establishment of the Bureau of Animal Industry. Much confusion existed as to the number of diseases affecting swine, and their causes. Some veterinarians believed there was only one disease, while others believed that several different diseases were involved.

Various estimates have been made of the number of hogs lost and their value. A bulletin issued in 1917 (*34*) contained a statement that the monetary loss in a single year due to cholera was $64 million, and that direct losses for the preceding 40 years had averaged $40 million per year. The death rate due to cholera was estimated to have been between 40 and 103 per thousand pigs raised. These estimates were based on reports from regular correspondents in the various States, and from county officials. It is evident that losses were enormous, and very little was known about hog diseases.

The first discovery of importance was the recognition of two distinct micro-organisms—one the hog cholera bacillus and the other the swine plague bacillus. A bacterium, then called the hog cholera bacillus, was erroneously identified as the cause of hog cholera. However, this conclusion resulted in successful attempts to produce vaccines from killed cultures of these bacteria. It was found that such vaccines would produce immunity against subsequent infection by the organism. This important finding was utilized in producing

various bacterins and vaccines to protect man and animals against serious diseases.

The next important discovery was that a filtrable virus was the actual cause of hog cholera. This finding was quickly followed by the most important discovery of all—that an anti-serum could be produced that would temporarily protect pigs against cholera when administered alone, and permanently when given simultaneously with virus. These discoveries ended the formerly uncontrollable menace of hog cholera, furnished farmers with methods of protecting their swine against the disease, and stabilized (or possibly preserved) the swine industry.

Investigations of the modes of transmission and the effect of various disinfectants against the hog cholera virus made it possible to institute sound sanitary practices. Investigations also resulted in the development of improved serums, and a sterile vaccine incapable of producing disease.

The knowledge of hog cholera was greatly increased, and dependable methods were developed for its control and prevention. While control is accomplished at considerable cost,[6] it is now comparatively safe to produce swine on a large scale. The swine raiser, the veterinarian, and the economy of the Nation have been greatly benefited by the research work of the Bureau of Animal Industry. Full benefit will be realized only when the results of the research are utilized to eradicate the disease.

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PUBLICATIONS ON HOG CHOLERA BY BUREAU WORKERS[7]

[7] Numbers refer to publications in the list of selected references, pp. 96 to 103.

PATENTS RELATING TO HOG CHOLERA [8]

(1) Patent No. 823,110. M. Dorset. Manufacture of hog cholera antitoxin. U.S. Patent Office Off. Gaz. 122: 2163. June 12, 1906.
(2) Patent No. 1,264,285. M. Dorset and R. R. Henley. Process for separating serum from corpuscles of mammalian blood. U.S. Patent Office Off. Gaz. 249: 1034. April 30, 1918.
(3) Patent No. 1,270,270. M. Dorset and R. R. Henley. Process of refining defibrinated blood antitoxin. U.S. Patent Office Off. Gaz. 251: 685. June 25, 1918.
(4) Patent No. 1,270,271. M. Dorset and R. R. Henley. Process for separation of blood serum. U.S. Patent Office Off. Gaz. 251: 685–686. June 25, 1918.
(5) Patent No. 1,475,580. R. R. Henley. Clarified serum and antitoxin and process of making the same. U.S. Patent Office Off. Gaz. 316: 855.
(6) Patent No. 1,784,928. M. Dorset. Vaccines for hog cholera and processes for manufacturing the same. U.S. Patent Office Off. Gaz. 485: 408. Dec. 16, 1930.
(7) Patent No. 2,102,235. M. Dorset, deceased. By Virgil Jackson Dorset. Vaccines and process for manufacturing vaccines. U.S. Patent Office Off. Gaz. 485: 408. Dec. 14, 1937.
(8) Patent No. 2,369,267. Frank W. Tilley. Method of preparing hog cholera vaccine. U.S. Patent Office Off. Gaz. 571: 240. June 1, 1943.

[8] All of the above patents were dedicated to the public or assigned to the U.S. Department of Agriculture.

ADDENDUM

Hog Cholera Research in the U.S. Department of Agriculture, 1954–60

In the reorganization of the U.S. Department of Agriculture in January 1954, the Bureau of Animal Industry, Agricultural Research Administration, became the Animal Disease and Parasite Research Branch, Agricultural Research Service; recently the name was changed to the Animal Disease and Parasite Research Division. However, hog cholera research has been a continuing function.

Dr. B. T. Simms, who had been Chief of the Bureau (1945–53) continued as Chief of the Branch until January 29, 1956. He was succeeded by Dr. H. W. Johnson, who was Chief of the Branch until February 21, 1957, when he became Director of the Division.

Most of the research since the reorganization has been directed toward obtaining information needed for developing and conducting an effective national hog cholera eradication program. Hog cholera research activities were expanded in 1955 to include (1) establishment of a pilot plant hog cholera eradication program and testing station at Live Oak, Fla., for cooperative work with that State; (2) re-establishment at Beltsville, Md., of research to investigate variant viruses, tissue culture-virus adaptation studies, and crystal violet vaccine; and (3) expansion at the Ames, Iowa, station, of research on fundamental and field problems relating to hog cholera viruses, immunity of swine, the environmental and hereditary factors that affect the pig itself, and the interrelationship of these factors. Research work at the three stations was closely coordinated through the central office of the Viral and Rickettsial Diseases Section of the Animal Disease and Parasite Research Division.

FLORIDA HOG CHOLERA RESEARCH STATION

The Florida Hog Cholera Research Station was established at Live Oak, Suwannee County, Fla., on January 1, 1956. This was in accordance with a Memorandum of Understanding entered into by the U.S. Department of Agriculture and the Florida Livestock Board relative to establishing a pilot hog cholera eradication test area in Suwannee County as recommended by the U.S. Livestock Sanitary Association.

One objective of the investigations at the Florida station is to devise and evaluate effective and practical methods adaptable to a program for nationwide eradication of hog cholera and to determine the efficacy and safety of certain biological products presently available from commercial sources. The biologics being tested in Suwan-

nee County are modified live virus vaccines of lapine origin, porcine origin, and tissue culture. Also being considered are the killed virus vaccines (crystal violet and Boynton's Tissue Vaccine). No other products are permitted within the test area.

Suwannee County has approximately 60,000 swine on 1,500 farms. The county is bounded on three sides by the Suwannee and Santa Fe Rivers, and contains 25 points of access by roads, only 12 of which are main arteries of traffic. Swine movements are controlled by 24-hour truck inspection stations at 5 of the 12 major entrance points. The inspection stations require veterinary certification of vaccination of all swine entering Suwannee County. Also, all swine returning to farms from public markets must be vaccinated.

All swine vaccinations in the area are made by practicing veterinarians with modified live virus vaccines and serum in minimum dosages of 15 ml. The Florida Livestock Board supplies these products free of charge to practicing veterinarians in the county. Vaccination coverage in the county is 48.3 percent of the herds and 48.8 percent of the swine. Two percent or more of the swine in a herd (but not less than two swine) are purchased from the owners at the time of marketing, and immunity is challenged by exposing the hogs to virulent hog cholera virus. The challenge virus has been a single large lot of frozen virus prepared from a passage of regular BAI virus lyophilized in 1946. Its minimum lethal dose at the latest 1959 titration was 2×10^{-6} ml.

During 1957, 1958, and 1959 the percentage of adequately protected hogs gradually declined, but in the first 9 months of 1960 the percentage increased for hogs inoculated with vaccine of lapine origin but continued to decline for hogs inoculated with the other two vaccine types, more sharply for those inoculated with the vaccine of porcine origin than for those inoculated with the tissue culture type (table 1).

TABLE 1.—*Hogs challenged and protected by various vaccines, 1957–60*

Type of vaccine	1957			1958		
	Challenged	Protected		Challenged	Protected	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Lapine origin	67	65	97. 0	415	389	93. 7
Porcine origin	83	79	95. 2	388	377	97. 2
Tissue culture	47	46	97. 9	450	422	93. 8
Total	197	190	96. 4	1, 253	1, 188	94. 3
	1959			1960 [1]		
Lapine origin	168	137	81. 5	295	271	91. 9
Porcine origin	215	176	81. 9	161	112	69. 6
Tissue culture	257	223	86. 8	38	28	84. 8
Total	640	536	83. 8	489	411	84. 0

[1] First 9 months.

An investigation has revealed that the decline in immunity of farm-vaccinated pigs is directly associated with the storage age of the vaccine at the time it is used. There was a direct correlation between age of vaccine at time of use and drop in immunity, as measured by comparing challenge results obtained with vaccines used less than 6 months with those used more than 6 months before the expiration date (table 2).

TABLE 2.—*Hogs challenged and protected by various vaccines, as related to age of vaccine at time of use*

Type of vaccine	Vaccine used less than 6 months before expiration date			Vaccine used more than 6 months before expiration date		
	Hogs challenged	Hogs protected	Days till expiration date [1]	Hogs challenged	Hogs protected	Days till expiration date [1]
	Number	*Percent*	*Number*	*Number*	*Percent*	*Number*
Lapine origin	100	75	114. 2	100	89	355. 4
Porcine origin	100	65	137. 3	100	80	341. 2
Tissue culture	100	82	66. 6	100	92	303. 4
Total	300	74	104. 6	300	87	333. 3

[1] Average.

Another objective of the pilot-plant eradication study is to identify the virus and determine the source of infection for suspected outbreaks of the disease in the test area. Practicing veterinarians have reported suspected outbreaks of hog cholera in the test area after changing from virulent virus and serum to modified live virus and serum vaccination as follows: 13 in 1956, 18 in 1957, and 14 during the first half of 1958. Most of these suspected outbreaks were reported to have been associated with purchases of unvaccinated swine through auction markets both in and outside of the test area; however, none were verified by virus isolation or laboratory study.

After July 1, 1958, all suspected hog cholera outbreaks were investigated by animal inoculation tests of specimens from suspected outbreak in both vaccinated and unvaccinated herds. Hog cholera virus was found in 22 of 56 specimens from suspected outbreaks in the area between July 1, 1958, and January 1, 1960. During this period all unvaccinated pigs at public markets were vaccinated before being returned to farms. However, table 3 shows there was still a marked correlation between confirmed hog cholera cases and the public market purchases of swine—72.7 percent of confirmed cases of hog cholera were associated with public market purchases. These results suggest that most confirmed cases of hog cholera involved animals that were probably in the incubative stage of the disease when presented to the public market for sale as unvaccinated pigs. They were then vaccinated before being returned to farms.

These findings demonstrate one of the major problems in any proposed national eradication program.

TABLE 3.—*Relation between public market purchases and confirmed cases of hog cholera, 1959 and 1960*

Year	Confirmed cases	Cases associated with public market purchases	
	Number	*Number*	*Percent*
1959	8	7	87. 5
1960	14	9	64. 3
Total	22	16	72. 7

An additional analysis of confirmed hog cholera among unvaccinated swine in the outbreak area (table 4) showed that 85 percent of the cases occurred on farms in 6 of the 24 townships in the county, or one-fourth of the pilot test area; the remaining 15 percent occurred on farms in 18 townships, or three-fourths of the pilot test area. Also, 47.6 percent of the swine in the heavy outbreak area (6 townships) were unvaccinated, as compared with 40.0 percent in the remaining area.

TABLE 4.—*Location of farms on which hog cholera was confirmed as related to farms marketing vaccinated and nonvaccinated pigs*

Contiguous townships (number)	Farms on which hog cholera was confirmed		Farms marketing pigs					
			Vaccinated		Nonvaccinated		Total	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
6	17	85. 0	140	52. 4	127	47. 6	267	100. 0
18	3	15. 0	275	60. 0	183	40. 0	458	100. 0

WORK AT BELTSVILLE, 1955–59

Hog cholera research was reactivated at the Animal Disease Station, Beltsville, Md., for a 4-year period (July 1955 to July 1959). The three principal lines of investigation were (1) variant virus studies, (2) tissue culture adaptation studies, and (3) crystal violet hog cholera virus immunization studies. These studies were made at Beltsville rather than at the Ames station because the trained workers, the equipment, and the isolation space needed for tissue culture studies were available at Beltsville but not at Ames. Also, uniform cholera-susceptible swine were available from the Animal Husbandry Research Division's Swine Research Branch at Beltsville. On July 1, 1959, the tissue culture work was transferred to the Ames station, and the other studies were discontinued because of the transfer of technical personnel to other projects.

Variant Virus Studies

Variant virus investigations in pigs included 10 serial passages of the variant virus isolated from a commercial source in 1949 and described by Dale and coworkers (*1–5*).[1] The first 7 passages were made simultaneously with small dosages of antiserum. The 10th passage retained the variant characteristics; that is, it was not counteracted by BAI Experimental Serum No. 1, which counteracted regular hog cholera virus. Like most variant virus isolates, this variant virus had poor antigenic properties. Crystal violet vaccine prepared from the virus failed to immunize pigs when administered in 10-cc. amounts. Serum obtained from pigs receiving 30-cc. dosages of crystal violet glycerol (CVG) vaccine prepared from the variant virus failed to protect when injected in 45-cc. amounts or less into cholera-susceptible pigs. Hyperimmune antiserum prepared from immune swine administered 5 cc. of tissue culture-variant virus per pound protected only one of two pigs receiving 15 cc. of serum and 2 cc. of regular virus, and both of two other pigs receiving 30 cc. of serum and 2 cc. of regular virus. Hyperimmune antiserum prepared from immune swine administered 5 cc. of blood-variant virus per pound adequately protected pigs receiving 15 cc. Hyperimmune antiserum prepared from immune swine administered 0.5 cc. of the same blood-variant virus per pound failed to protect pigs receiving 15 cc. but did protect those receiving 30 cc.

Variant virus, after 28 passages in swine (9 with serum, 7 without serum, 7 with serum, and 5 without serum), was successfully adapted to tissue culture by use of minced primary spleen tissue in the Maitland system. Variant virus had retained its variant characteristics for swine after 10 serial passages in tissue culture. These studies indicate that the variant form of hog cholera virus may be perpetuated by tissue culture propagation, whereas serial passage of the virus in swine without the administration of serum results in a loss of the variant characteristics and a reversion toward the characteristics of regular virus.

Tissue Culture Adaptation Studies

Three methods of virus adaptation to tissue culture were investigated: (1) the Maitland system of primary swine spleen cells in liquid medium, (2) monolayers of primary swine kidney cells on glass, and (3) established lines of tissue culture cells from commercial sources and from cell lines developed by our workers. In most instances, a minimum of 10 serial passages of virus was made for evidence of virus multiplication. Cholera-susceptible swine were inoculated to test virus-tissue culture series for live virus because no virus has produced cytopathogenic effect (CPE) in the cells. Also, it was necessary to make a post-inoculation regular virulent virus challenge of all pigs inoculated with tissue-culture virus that did not become sick. This was done to test for virus growth where virulence of the virus had been lost yet the virus could immunize without making pigs sick.

[1] Italic numbers in parentheses refer to Selected References, p. 119.

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Numerous adaptations of virus in the Maitland system in primary swine spleen were successful. In most instances, these virus cultures were successfully grown on primary swine kidney monolayer tissue culture cells. However, attempts to passage this virus in 16 different cell lines from a commercial source were unsuccessful.

Some initial adaptations of virus in primary swine kidney monolayer of tissue culture cells were successful, but the data indicate that the most successful propagation of virus in tissue culture is associated with initial adaptation of the virus in primary spleen tissue in the Maitland system.

Four lines of swine cells were established in tissue culture and are identified as follows:

Tissue	Date established	Passages as of April 1960
		Number
Swine kidney (SK)	Jan. 22, 1958	97
Swine testes (STJ)	Aug. 26, 1958	74
Swine bone marrow (S2D)	Mar. 18, 1958	94
Swine epithelium (D19)	Apr. 16, 1959	59

Attempts to propagate hog cholera viruses in series of 10 passages in these established cell lines have resulted in no gross CPE in the cells or evidence of virus multiplication in inoculated pigs, whereas the same virus after 10 passages in primary bone marrow cells, used as controls, infected pigs but produced no CPE in the cells.

The adaptation and growth of hog cholera virus in primary tissue culture cells of swine origin have resulted in some fundamental and important findings associated with the *in vitro* attenuation or modification of virus in serial passages. Parallel serial passage of many different viruses—that is, passage at approximately 48-hour intervals as compared with passage at approximately 96-hour intervals—has consistently resulted in a more rapid loss of virulence in the viruses passed at 48-hour intervals. Nineteen viruses from 10 tissue culture serial lines passaged about 10 times at 48-hour intervals resulted in 11 positive cultures when tested in pigs. Of these 11 positives, 8 decreased in virulence and 3 were still virulent. One of the 3 virulent cultures lost its virulency after 30 short-interval passages.

Twenty-seven viruses from 21 lines passaged about 10 times at 96-hour intervals resulted in 21 positive cultures when tested in pigs. Of these 21 positives, 20 were fully virulent and one had lost some virulence. The rate of attenuation varied considerably among the viruses tested. Variations in the attenuation rate were related to the initial virus isolate used, as well as the number of times it had been passaged in tissue culture. One virus lyophilized in 1946 was passaged 40 times at 48-hour intervals before any loss of virulence was observed.

Live hog cholera virus propagated in tissue culture has been successfully used for immunizing swine and for hyperimmunizing swine used in producing hog cholera antiserum. However, use of this virus in the production of killed virus (CVG) vaccines has

consistently failed to produce satisfactory immunity in vaccinated pigs. The reason for the differences in antigenicity between virus of swine blood origin and virus propagated in tissue culture is not understood, but it is thought to be due to some nutrient or virus fraction in swine that has not as yet been incorporated in the tissue culture system.

Crystal Violet Hog Cholera Vaccine Immunization Studies

Irregularities in results of hog cholera virus CVG vaccine immunization studies have occurred repeatedly over the years and have been primarily associated with groups of weanling pigs 8 to 16 weeks of age. Young pigs have been used for most of the experimental vaccine studies because they are the usual farm vaccination age, and use of young pigs lowered feed and experimental costs.

A 4-year study at Beltsville resulted in development of a method whereby comparative potencies of CVG vaccines could be based on quantities of vaccine required to protect 50 percent of the swine. In previous tests at the Ames station, this method of measuring vaccine potency on weanling pigs from different farms had given irregular results; however, the tests at Beltsville were successful. Tests with mature swine from selected herds (Animal Husbandry Division), repeated at yearly intervals, gave similar results when aliquots of the same vaccine and aliquots of the same challenge virus were used. For the first time the comparative potency of hog cholera CVG vaccines could be accurately measured with this test. In most tests, groups of mature swine—4 to 6 months of age and weighing 100 to 150 pounds—were vaccinated with each of three vaccine dosages (5, 2.5, and 1 cc.) followed by a 3-week post-vaccination challenge with virulent virus.

Revaccination with CVG vaccine was studied for its booster value in increasing degree and duration of immunity in valuable breeding herds and for swine in hog cholera outbreak areas. Prior to 1955, the Animal Husbandry Division swine breeding herd had been immunized against hog cholera by simultaneous virus and serum administration. During 1955, this herd was changed to CVG vaccination. The vaccination program included double vaccination each year for 2 years, with a minimum period of 4 weeks between vaccine administration, and revaccination on an annual basis. As surplus breeding animals became available they were moved to the Animal Disease Station where they were challenged with hog cholera virus to measure immunity. The results showed a marked increase in degree and duration of immunity of double-vaccinated pigs as compared with single vaccinations. The challenge results on double-vaccinated pigs showed almost a solid immunity similar to that usually obtained from the simultaneous virulent virus and serum method, whereas single CVG vaccination produced about 70-percent protection.

OTHER RESEARCH ACTIVITIES

Hog cholera research at the Ames station directed toward obtaining information needed for hog cholera eradication have included

work on laboratory diagnostic tests, disinfectant studies, virus fractionation and toxin isolation, causes of hog cholera outbreaks, vaccine immunity studies with emphasis on the inability of some swine herds to develop a satisfactory immunity, and the development and standardization of testing procedures.

Laboratory Diagnostic Test

A satisfactory, simple, rapid, accurate laboratory test for hog cholera is seriously needed in developing control and eradication programs. This problem has received almost continuous study. The standard and only reliable diagnostic test for hog cholera consists of immune and susceptible pig-inoculation studies, which require about 16 weeks to complete the identification and classification of isolated virus into virulent, immunizing, and variant viruses. Consequently, most present-day field diagnoses of hog cholera are based on an analysis of the herd history, clinical manifestations of disease, and post-mortem findings. Numerous serological, hematological, and histopathological tests have been investigated with limited success except as diagnostic aides.

In the most recently investigated test—the agar diffusion precipitation test—the pancreata from 272 known cases of hog cholera were tested against a number of hyperimmune sera made from different viruses. Incubation at room temperature for 2 to 5 days was required for positive reactions to develop. Positive reactions developed from the pancreas tissues of 77 percent of pigs that had been sick with cholera at least 6 days. However, tissues from pigs that had been sick only 4 days developed only 13.5 percent positive reactions, and positive reactions developed in 40 percent of the tissues from pigs that had been sick 5 days before being sacrificed and tested.

If the test were to be used as an aid in diagnosing hog cholera, it would be imperative to obtain an accurate history of illness in the herd, and to test several pancreata from the same herd from pigs that had been sick 5 days or more.

Virus Fractionation and Toxin Isolation Studies

The following chemical agents were tested for properties of inactivating hog cholera virus in swine blood and in fecal material: Nolvasan (bis-p-pchlorophenyl-diaguanidohexane diacetate); Wheaton's Safety Disinfectant (sodium o-phenylphenate); Roccal (Quaternary ammonium compound); sodium carbonate; sodium hydroxide; ethyl alcohol; isopropyl alcohol; phenol; formalin; sodium hypochlorite; cresol; Septisol (hexachlorophene); and beta-propiolactone. In general, the technique was to mix recommended dilutions of the chemical agents and the virus-containing material, followed by dilution of the mixture after a 30-minute exposure period. The diluted mixtures were then injected into cholera-susceptible swine. Virus was considered to be completely inactivated if the test animals showed no clinical symptoms for a period of 2 to 3 weeks and were susceptible to hog cholera when later challenged with virulent virus.

Of these 13 chemical agents, only 5 were effective in inactivating

the virus in blood. Roccal (at a concentration of 2 percent), Cresol (2 percent), and sodium hypochlorite (1 percent) were effective when virus blood was diluted 1:10 before the chemical agents were added, but were not effective when in blood diluted 1:2. Wheaton's Solution (1 percent) was effective in blood diluted 1:2 but not in undiluted blood. The most effective agent was beta-propiolactone, which inactivated virus in undiluted blood at a concentration of only 0.175 percent.

The five agents effective for inactivating the virus in blood were tested at the same concentrations for effectiveness in inactivating the virus in fecal material. All were effective and, in addition, sodium hydroxide (2 percent) inactivated virus in feces. However, this concentration of sodium hydroxide did not inactivate the virus in blood.

Except for mixtures containing sodium hydroxide, there were no extreme pH values of the virus-chemical mixtures in which virus was inactivated. Roccal, sodium hypochlorite, and beta-propiolactone all destroyed the virus at pH values near neutrality. Cresol was effective at pH 8.4, and Wheaton's Solution at pH 10.2. Thus, disinfection by these agents seems to be of a chemical rather than a physical nature.

Attempts were made to determine which fraction of virus-containing blood (red blood cells, serum, albumin, pseudoglobulin, or euglobulin), when made into crystal violet glycerol vaccine, would give the best protection against hog cholera. Each fraction of infective blood was made into CVG vaccine by the standard procedure of adding 1 part CVG to 4 parts of the blood fraction of whole virus blood.

Two pigs were each injected with 5 cc. of each CVG fraction prepared except euglobulin, which was given in 2.5-cc. doses. The fraction from each pool of virus blood was tested on a different lot of pigs. All pigs were challenged in 21 days with 2 cc. of virulent virus. There was considerable variation in the percentage of protection stimulated by the different fractions.

The three serum virus CVG fractions stimulated a greater degree of immunity than did the respective whole virus blood from which they were prepared. Two of the red blood cell CVG fractions produced no resistance to hog cholera, but the third red blood cell fraction produced protection equal to that of the whole virus blood from which it was prepared. The albumin CVG fractions stimulated considerably less protection than did the whole virus blood from which they were prepared. The globulin fractions produced protection equal to or slightly greater than that produced by the respective whole virus blood.

These limited observations indicate that no single fraction of virus blood is entirely responsible for protecting pigs vaccinated with crystal violet vaccine against cholera.

An attempt was made to isolate a toxic principle from the serum of hogs suffering from hog cholera. Virus infected blood serum was dialyzed against running tap water until the water-insoluble globulin (euglobulin) had precipitated. The precipitate was removed from the supernatant by centrifugation and resuspended in approximately 5 parts of saline.

Injection of 5 to 10 grams of this material intraperitoneally into cholera-susceptible pigs caused a severe reaction within 20 to 30

minutes. The symptoms observed were urination, defecation, and dyspnea. These symptoms intensified, with the pig lying on its sternum, until death occurred in 24 hours to 4 or 5 days. Cholera-immune pigs reacted much less severely and recovered completely within a few hours. Water-insoluble globulin prepared from normal swine serum did not produce symptoms of a toxic reaction.

A 10-fold increase in the virus content of the water-insoluble globulin was demonstrated by comparative titrations with the original virus blood. The highest virus titer of three batches of virus blood was 1:1,000,000, while the highest virus titer of each of the respective water-insoluble globulin was 1:10,000,000.

The toxic principle was lost when the virus of the water-insoluble globulin was inactivated by incubation in glycerol at 37° C. for 2 weeks. Thus, it appears that the toxic characteristics of the virus euglobulin fraction are associated with the great increase in viable virus.

A 6-year study was made of 152 specimens collected from 152 farm herds in which death losses of pigs occurred after vaccination with modified vaccines as well as with serum and virulent virus. All types of commercial vaccines were used in this study. In each case specimens were tested for hog cholera virus because a positive field diagnosis could not be made on the symptoms and post-mortem findings.

No virus was isolated from 30 (28.6 percent) of the 105 specimens from herds that had been vaccinated with modified live virus vaccines of either lapine or porcine origin. Regular hog cholera virus was isolated from 37 specimens (35.2 percent). Virus showing variant characteristics was isolated from 15 (14.3 percent), and an immunizing virus was isolated from 23 (21.9 percent).

No virus was isolated from 5 (20 percent) of the 25 specimens from herds that had been vaccinated with serum and virulent virus. Regular virus was isolated from 15 (60 percent), and variant virus was isolated from 5 (20 percent). No immunizing virus was isolated from these specimens.

No virus was isolated from 8 (61.5 percent) of the 13 specimens from herds that had been vaccinated with inactivated vaccines. Regular virus was isolated from 3 (23 percent), and variant virus was isolated from 2 (15.4 percent). No immunizing virus was isolated from these specimens.

No virus was isolated from 2 (22.2 percent) of the 9 specimens from herds that had been vaccinated with tissue culture vaccines. Regular virus was isolated from 1 (11.1 percent). A variant virus was isolated from 2 (22.2 percent), and a nonpathogenic virus with immunizing properties was isolated from 4 (44.4 percent).

The bacteria most commonly isolated from the field specimens were salmonella, pasteurella, coliforms, and corynebacterium. Staphylococci, streptococci, and pseudomonas were isolated occasionally. In many cases death occurred when salmonella and pasteurella cultures were injected with modified vaccines. These same agents were not pathogenic when injected alone.

Attempts to immunize unthrifty pigs with modified live virus of rabbit origin were not successful; 17 of 30 pigs thus vaccinated died. Of the 30 pigs, 16 also received serum and 7 of these died. Either salmonella or pasteurella was isolated from about 50 percent

of the tissues cultured in the two accession lots from which these experimental pigs were obtained. Indications are that complete immunity is not obtained in this type of animal, when the effects of the initial vaccination are survived.

During investigations of death losses of pigs in herds vaccinated with modified live virus, various organisms were isolated from the specimens submitted. Some of these organisms were tested in pigs for pathogenicity, both alone and simultaneously with modified live virus vaccine of rabbit origin. Escherichia, alcaligenes, corynebacterium, and pseudomonas were nonpathogenic when injected subcutaneously; this was true whether the bacteria were administered alone or simultaneously with modified live virus.

Some strains of *Salmonella choleraesuis* and *Pasteurella multocida* appeared to be more pathogenic when injected with modified live virus (rabbit origin) than when injected alone. This was especially true of salmonella. Reactions in pigs to field strains of salmonella varied from noticeable to severe; however, when some of these same strains were injected into pigs in combination with modified virus, the pigs usually died. Salmonella organisms from specimen 167 caused slight to severe reactions in four pigs when administered alone at two dosage levels, but no deaths occurred. Of six pigs exposed to the same organism in combination with modified virus, five died or were killed in moribund condition, and one recovered after a severe reaction. Of three pigs exposed to salmonella organisms from specimen 217, one remained normal and two had slight reactions. Both pigs exposed to these bacteria simultaneously with modified virus died. Some strains of salmonella did not show this apparent increase in pathogenicity. One strain exhibited increased pathogenicity after passage with modified live virus. Attempts to show increased virulence of modified virus after passage with bacteria have not been successful.

Five strains of pasteurella were tested. When tested alone, two had no effect on pigs, one caused reactions with subsequent recovery, and two resulted in fatal infections. One strain, from specimen 168, increased in virulence when tested in combination with modified live virus.

Death losses had occurred in herds of vaccinated pigs when only hog cholera virus and serum were used, but death was usually attributed to the poor virus or impotent serum. Deaths continued to occur after modified hog cholera vaccines had been introduced, so it became more obvious that the lack of protection was due to the pig. Certain herds of pigs failed to become immune after vaccination. This condition could not be detected before vaccination, so it was not known how prevalent it might be. There was some indication that failure to become immune after vaccination might be a genetic factor or the result of certain feeding practices.

Herd Immunity Studies

A 5-year farm-herd vaccination program was begun to find herds that might not become immunized properly, and if possible to study the causes of this condition. An attempt was made to stabilize most of the known variables that affect immunity results of vaccine tests. These known variables included selection of a tested seed virus with-

out variant virus characteristics, selection of donor pigs for the production of virus for preparation of vaccines, and production and freezing for storage of large lots of challenge virus so as to obtain a uniform virus challenge throughout the year. However, the greatest variable causing irregular results in immunity in vaccine tests is the pig itself. It was the purpose of this study to identify these pig-producing variables that might be associated with heredity, nutrition, age at vaccination, physical condition, and mixed infection of bacterial, viral, or parasitic nature which could affect immunity test results.

Crystal violet glycerol vaccine was used for vaccination, so that no live virus would be introduced into a herd. The vaccine and the challenge virus were prepared in large lots so that only one or two serials of vaccines would be used in a year. The vaccines were tested before use, and were known to give good protection in test pigs. Pigs were vaccinated with CVG vaccine 2 weeks after weaning and 6 pigs from each herd were challenged with 2 cc. of virulent virus. Two were challenged 1 month after vaccination, two were challenged 3 months, and two 6 months after vaccination. One serial lot of virus was used as challenging material during each year.

This study was begun on 93 widely scattered farms in three counties. As farmers dropped out of the program, no others were added.

Four years of this program have been completed. The number of farms dropped from 93 the first year to 69 the fourth year. The number of swine vaccinated increased from 11,911 the first year to 15,234 the third year, and 14,608 the fourth year.

Only two herds in this program were purebred hogs. The others were hybrid or crossbred herds; consequently, the accumulated data were of little value for an analysis of hereditary immunity.

Any swine losses that occurred on the farms under test were reported, and the causes of losses were investigated by bacteriological culturing and pig-inoculation tests. There was not a single case of hog cholera identified in any of the farm herds during the 4-year period.

A point system was used to indicate the reaction of each pig tested with virulent challenge viruses. The more severe the reaction, the greater the number of points.

The reactions were designated as no reaction, slight, severe, or dead. The number of pigs in each of the four groups varied in the 4 years (table 5).

TABLE 5.—*Reactions of farm pigs challenged with virulent virus after vaccination with CVG vaccine*

Year	Pigs	Reaction				Protection
		None	Slight	Severe	Died	
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1956	727	47. 73	17. 47	21. 87	12. 93	71. 30
1957	747	26. 11	23. 29	44. 04	6. 56	64. 50
1958	425	9. 82	20. 64	49. 90	19. 84	50. 75
1959	474	24. 2	31. 85	32. 06	12. 02	64. 70

Why the percentage of pigs in each group changed as it did in the different years cannot be fully explained. However, two major factors—other infection present in the pigs and variations in potency for different lots of vaccine—were the principal causes of these differences. In 1958, some herds had other infections that had not been detectable in previous years. Salmonella and pasteurella were isolated from some of the pigs that died. Atrophic rhinitis was observed in several herds. On two occasions, enteric infection characterized by a bloody diarrhea was observed in pigs after they had been challenged with virulent virus. The infection spread to other pigs in the barn, and probably accounted for the increased death loss. The causative agent could not be demonstrated bacteriologically, and was thought to be of a viral nature.

The sum of all points for each animal was expressed in percentage so that the degree of protection could be expressed in percentage.

The degree of protection was 71.30 percent in 1956; 64.50 percent in 1957; 50.75 percent in 1958; and 64.70 percent in 1959. The reaction in pigs from a particular farm in 1 year did not necessarily continue to be the reaction the following years. Death loss occurred following virus challenge of pigs from some farms for 2 or 3 years in succession. Other swine infections were present on most of these farms and apparently interfered with the immunization of the pigs.

Development and Standardization of Hog Cholera Testing Procedures

Because of the marked variation obtained with different viruses, vaccines, antiserums, pigs, and testing methods, the research worker has attempted to develop and standardize hog cholera testing procedures.

The standards for virus, vaccine, and antiserum studies usually consist of producing and storing large quantities of single lots of the biological material, which are used as standard controls in all experiments in combination with experiments on other materials being tested. Comparisons of test results of other agents are possible by obtaining measurements of their relation to the standards used.

The greatest variable in virus, vaccine, and immunity studies and the most difficult to control is the pig. No standard or uniform pig or test animal has been developed. Three known variables that affect hog cholera virus research work in pigs must be considered in evaluating results. They are (1) the effect of the donor pig on the virus, or the alteration or change in the characteristics of a virus that occurs with its passage through each different pig; (2) the pig's susceptibility to virus exposure; and (3) the pig's ability to develop an immunity against hog cholera. These three variables may or may not be related.

The pig is the only known satisfactory experimental animal susceptible to natural hog cholera virus; consequently, the testing procedures have received much attention, and the following testing procedures are used for different accession lots of purchased pigs:

The susceptibility of pigs to hog cholera virus is usually determined by inoculation challenge of pigs with a large dose containing several thousand times the minimum infecting dosage of any virulent virus. The relative susceptibility to hog cholera virus of groups of

pigs purchased from different farms is measured by inoculating representatives from each group with four or more ten-fold dilutions of a previously titrated standard virus. The resulting higher or lower virus titration titer indicates a respectively greater or lesser susceptibility index for the groups of pigs. This information is important to the scientist testing unknown field material for virus, because these field viruses are frequently too weak to infect the less susceptible test pig but will cause infection in the highly susceptible test pig.

The ability to develop immunity is measured by inoculating six or more pigs from each farm-purchase group with a previously tested standard CVG vaccine. Three groups of two or three pigs are vaccinated with 5-ml., 2.5-ml., and 1-ml. dosages, respectively, followed by challenge on the 10th post-vaccination day with a uniform dose of standard virulent hog cholera virus. The objective of the test is to obtain a measure of the immunity development rate and the 50-percent protection from the standard vaccine dosage, both of which indicate the relative immunity that can be obtained for the new group of pigs as compared with other groups tested.

Swine, as well as man, other animals, and poultry species, are born with an undeveloped immunizing mechanism. The mechanism develops at irregular rates in different individuals, but most pigs may be immunized at or near weaning age. However, groups of pigs from some farms, and individual pigs in other farm groups, still have a poorly developed immunizing mechanism at weaning time. Maximum development of the immunizing mechanism in swine appears to be reached at about the age of maturity. The Beltsville investigators, working with the Animal Husbandry swine herd, found that pigs between 4 and 6 months of age weighing from 100 to 150 pounds developed uniform immunity when a single serial of vaccine was used over a 4-year period. Swine from this herd near weaning age have always shown marked variation in vaccine immunity protection.

As a result of these findings it has been recommended that mature pigs be selected as the standard test pigs for critical comparative tests. This procedure has several disadvantages: (1) Difficulty of handling the heavier swine, (2) the increased cost of holding the swine in quarantine isolation during the growth period, and (3) the constant danger of their accidental exposure to hog cholera during the holding period.

The diagnostic-inoculation tests for hog cholera of suspected material from farms having swine losses are made by inoculating a minimum of three pigs with blood or spleen-saline suspension from sick or dead pigs. Two of the three pigs must be susceptible test pigs; one receives a standard anti-hog cholera serum and the suspicious agent; one receives only the suspicious agent. The third pig—a known hog cholera immune—also receives only the suspicious agent. On the seventh post-inoculation day, a blood sample is collected from the susceptible test pig inoculated with the agent alone, and stored frozen for future passage or seed virus, if needed. A diagnosis of virulent hog cholera may be made if the susceptible pig that received no serum sickens and the other pigs remain normal. If all three pigs remain normal, they receive a 4-week post-inoculation challenge with virulent virus. No reaction following challenge

in the two susceptible test pigs indicates that the original agent contained an immunizing hog cholera virus; if the susceptible pigs become sick it proves that the original material was negative for hog cholera virus. Sickness or death of the known immune pig indicates that the tested material contained a disease agent other than hog cholera.

Vaccine immunity tests are made by vaccinating a group of known susceptible pigs with the recommended vaccine dose, followed in 2 or 3 weeks by post-vaccination challenge with a uniform dose of standard virus. Early workers used only two classifications for measuring vaccine immunity protection to the pig: (1) unsatisfactory, if associated with death or severe reactions, or (2) satisfactory, if associated with slight or negative reactions and recovery. The research worker needed a uniform, accurate measurement, expressed in figures, which would allow a better comparison of different materials being tested or similar materials being tested by workers at different locations. These needs resulted in the development of a grading system based on the degree and duration of sickness, determined by observation of the individual test pig's appetite and activity at feeding time. Daily points are assigned each test pig, as follows: Normal pigs, no points; pigs slightly off feed, 1 point; pigs that get up when fed but return to the nest after a few bites of food, 2 points; and pigs that fail to get up or show any interest in eating, 3 points.

For example, a pig that failed to eat for 5 days and then recovered would receive 3 points per day for 5 days, or 15 points.

To convert degree and duration of sickness to lack of resistance or percentage of loss of immunity for individual pigs, 1 point equals 2 percent. In the above example, 15 points multiplied by 2 equals 30 percent loss in immunity, or, conversely, 70-percent protection.

Another pig that was only slightly off feed for 5 days and recovered would receive 1 point per day for 5 days, or a total of 5 points. To convert, 5 multiplied by 2 equals 10 percent loss in immunity, or 90-percent protection.

Pigs receiving less than 11 points (indicating more than 80-percent protection) are referred to as having slight reactions. Those receiving more than 10 points (indicating less than 80-percent protection) are referred to as having severe reactions.

The value of this grading system is in the group of test pigs referred to as having severe reactions. All of these were classified as unsatisfactory vaccine tests by our earlier workers; they are now graded between zero and 80 percent so their comparative value can be determined.

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