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SOME OF THE PHYSICAL-CHEMICAL PROPERTIES OF HOG CHOLERA VIRUS

- I Filterability of Hog Cholera Virus as Affected
by the Hydrogen Ion Concentration**
- II The Migration of Hog Cholera Virus when Subjected
to Electrophoresis**
- III Experiments on the Attenuation of Virus and the
Production of Immunity to Hog Cholera**

By

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GENERAL INTRODUCTION

Hog cholera is a highly infectious disease of swine which was first reported to have appeared in the United States in 1833. It has become a disease of great economic importance because of its extremely infectious nature and the resulting high mortality among susceptible swine. It is estimated that ninety per cent of the total losses from all swine diseases is caused by hog cholera. The early investigations which were carried on for the purpose of determining the etiologic agent indicated that an organism which was isolated from some field cases, now known as *S. suispestifer*, was responsible for this highly infectious disease. Later, however, the real etiologic factor was found to be a filterable virus. Following this discovery, attempts were made to produce a protective serum in order to check the spread and to reduce the heavy losses caused by this disease. The principles employed in producing a protective serum against rinderpest, a disease of cattle which is prevalent in Europe and caused by a filterable virus, were applied to the production of anti-hog-cholera serum. In 1908 the practical efficacy of anti-hog-cholera serum for protection against this disease was fully established. Both the serum and simultaneous treatment have been successfully used up to the present time. Since improved methods of production have been established under government supervision, the quality of both protective serum and virus has been standardized so as to produce reliable products for immunization against hog cholera. Private laboratories which have been established under government supervision have insured in average times an adequate

supply of potent products at a reasonable cost, making it economically possible to reduce the losses from this disease considerably.

Recently many investigations have been made on various phases of this problem and have supplied us with much information, both of practical and scientific value. Further studies are necessary to increase our knowledge of the physical and chemical properties of this virus, and particularly at this time to develop an efficient and more economic method of protection against this disease. With these objects in mind studies were conducted under carefully controlled conditions in order to contribute to the solution of the phases of this problem which are still not well understood.

PART I. FILTERABILITY OF HOG CHOLERA VIRUS AS AFFECTED
BY HYDROGEN ION CONCENTRATION

INTRODUCTION

De Schweinitz and Dorset (6) demonstrated that the true cause of hog cholera is a filterable virus. Their experimental findings were confirmed by Huttyra (10) and other workers in Europe, and by Dorset, Bolton and McBryde (7) in this country. Later, Rivers (26) included hog cholera in his classification of diseases caused by filterable viruses.

In the studies made by investigators on the properties of hog cholera virus, the standard laboratory filters such as the Berkfeld, Mandler and Chamberland were used. Later, special filters were constructed in an attempt to secure one which was capable of retaining the virus. Kramer (14,15,16) and Mudd (23) in their studies on bacterial filters and filterable viruses contributed much to our knowledge of filters, filter construction and the physical properties of certain viruses.

The filterability of a substance is dependent on the size of the particles incorporated in the medium to be filtered in relation to the size of the pores of the membrane, and the electric charge of both the membrane and the medium, as well as the viscosity of the medium. The relation between filterable media and filter membranes has been summarized in the most concise and complete manner by Buchanan and Fulmer (5) who state:- "The factors which determine whether a material will pass through

a given membrane are the size of the particles, their electric charge with reference to that of the membrane and the deformability of the particle. In the latter case (when deformable particles such as liquids of various degrees of viscosity are involved) pressure becomes another controlling factor.

The relation of the charge on a membrane (Berkfeld filter) and the filterability of the viruses has been recently pointed out by Kramer (1927) and Alexander (1927). By varying the charge on the filter, viruses previously considered filterable may become non-filterable and vice versa. This comes about from the fact that a particle smaller than the pores may not pass through a membrane of opposite charge, but is held by electrical attraction. Assume that the isoelectric point of a membrane is at $\text{pH} = 6.0$ and that of the virus whose particles are smaller than the pores, at $\text{pH} = 8.0$. At a $\text{pH} < 6$ both membrane and virus will be positively charged and at $\text{pH} > 8$ both will be negative, hence the virus will filter through. However, at a pH between 6 and 8 the membrane will be negatively charged and the virus positively charged and hence may be retained."

Some of the investigators who made careful studies of filters were able to classify them according to their electric charge. Olitsky and Boez (24) classified various types of Berkfeld and Chamberland filters as negatively charged. The charge of filters can easily be demonstrated by filtering certain colloidal suspensions possessing either electro-positive or electro-negative color ions. The ordinary siliceous filters being negatively charged will allow a solution of Congo

red which has negatively charged color ions to pass through the filter pores, while a solution of Victoria blue which possesses positively charged ions will be retained. Kramer devised a positively charged filter constructed of calcium sulphate, calcium carbonate and magnesium oxide.

Among the early investigators who conducted filtration experiments with hog cholera virus were Dorset and his associates, Butyra, Ostertag (25) and McClintock, Boxmeyer and Siffer (20). Ultra-filtration defined by Zinsser and Tang (29) as filtration through graded collodion membranes and defined by Beechhold (2) as filtration through jelly filters was used in hog cholera studies by Mayer (21), von Betagh (3) and Roderick and Schalk (28). More recently Kernkamp (11) carried out investigations on hog cholera virus using siliceous filters as well as graded collodion membranes.

In these earlier reports of the studies made on hog cholera virus there was no report on the possible effect a change in hydrogen ion concentration of hog cholera virus might have on its filterability. For this study the siliceous filters were selected because of their uniformity. Several special filters were also constructed in an attempt to secure one which would be impermeable to the virus of hog cholera.

TECHNIQUE

The first serious problem which presented itself in these studies was a suitable means of accurately determining the hydrogen ion concentration in biologic fluids, such as blood serum and tissue extracts which are the most convenient and reliable sources of hog cholera virus. The quinhydrone measurements are materially affected by oxidizing and reducing substances resulting in drifting potentials and unreliable results. The hydrogen electrode was found to be poisoned readily by the protein constituents of the blood serum, so could not be used for this purpose. The use of the glass electrode seemed to offer the only possibilities for this particular purpose. Kerridge (12,13) in her early work on the glass electrode pointed out its advantages. MacInnes and Dole (17,18,19), Mirsky and Anson (22), Brown (4) as well as Anson and Mirsky (1), demonstrated the value of the glass electrode in the fields of biology and chemistry.

Before the development of electron tubes, electrometers were used as null instruments. The great sensitivity of the electrometers was essential because of the high resistance of the glass membranes. This necessitated expensive equipment and elaborate shielding for proper operation. An electron tube potentiometer seemed to be the desirable type for this purpose. A simple, inexpensive electron tube potentiometer which could be used with a glass electrode was made. The wiring diagram was that of Harrison (9) modified to use an inexpensive electron tube which could be obtained readily. The details of construction are described by Goodhue et al (8) including the type of glass electrode

used in these studies. The electrodes were standardized with buffer solutions of known hydrogen ion concentration, as determined by the hydrogen electrode. The hydrogen ion concentration of the serum virus or tissue extracts could be determined accurately and quickly.

The filters selected for this study were thoroughly cleansed by passing approximately a liter of distilled water through them before they were tested. Besides the air test, they were tested with suspensions of either *Brucella abortus* or *Serratia marcescens* which were made in physiologic saline solution from cultures approximately 36 hours old. This precaution seemed necessary to avoid the use of filters having imperfections, several of which proved to be defective. The filters were cleaned by reversing and passing a liter of distilled water through them. The filters and filter flasks were placed in an Arnold sterilizer for one hour. When partially cooled the stopper and terminals of the rubber tubing were sealed with paraffin. A filter flask containing cotton, tightly fitted with a rubber stopper having suitable outlets, was sterilized for the purpose of admitting only sterile air when the vacuum in the filter flask was released. This unit was connected with rubber tubing between the vacuum pump and the filter flask. After the filtration was completed the filters were again tested with bacterial cultures to test their efficiency.

The experimental animals were placed in segregated units where accidental infection was highly improbable. These units consisted of individual portable houses six feet square, the floors of which were constructed of concrete. The concrete extended up the side walls for seven inches above the floor level, so constructed that the drainage was directed into a small metal container outside of the house. Three sides of the houses were constructed of tightly matched lumber. The front was

of solid construction to a height of two and one-half feet above the floor. A door suspended from the top by hinges provided adequate ventilation. It was found necessary to use a wooden frame constructed of three inch strips of wood placed at intervals of four inches, suspended from the top of the opening in order to prevent the experimental animals from climbing out through the door. These units could be easily cleaned and thoroughly disinfected. They proved very satisfactory in this work as it was possible for the attendant to care for the animals and take temperatures without entering the house. Metal cages were also used as isolation units for the experimental animals. These consisted of woven wire cages five feet square and three feet in height, with removable wooden floors and solid metal tops. A base support of solid metal seven inches high reinforced the woven wire sides and doors. The two doors when open made it possible to remove the wooden floors while cleaning and disinfecting. These cages were supported by metal legs which elevated them a foot from the floor. The cages were placed in separate pens in one of the experimental buildings in which no other animals were kept. Several isolated pens in this building were used when additional units were required. Each unit was provided with separate metal receptacles for feed and water which could be cleaned and disinfected easily. At the termination of each experiment these units were cleaned and disinfected before being used again. These units proved to be efficient as there was no accidental infection of experimental animals in the course of these experiments as shown by control animals. As each animal was placed in these segregated units, a series of preincubation temperatures was taken to insure the use of experimental animals which were in a good condition of health, thus avoiding the complications of other disease conditions so commonly encountered in field cases of hog cholera.

THE VIRUS

One of the most important factors in conducting experimental investigations with virus diseases is the source of a potent virus. Virus frequently loses its virulence and new virus has to be employed from time to time. The most convenient source for these studies was the blood serum taken from pigs infected with hog cholera virus. The animals were bled from the carotid artery, care being taken to prevent bacterial contamination of the blood. A small portion of the blood was defibrinated in a sterile container. This was phenolized, sealed in sterile brown glass bottles and kept in a refrigerator at 5 to 6° C. as a reserve virus supply. The bulk of the blood was collected in a sterile flask, allowed to clot after which the serum was drawn off in a sterile flask. This serum being clear and free from red blood cells made a suitable source of virus for these investigations. All virus obtained from field cases was filtered through a Berkfeld "W" filter and tested on culture media in order to avoid the use of contaminated serum. The serum was diluted before being subjected to filtration experiments. Twenty-five cc. of serum and 75 cc. physiologic saline solution were used in each case. The dilution permitted a more rapid filtration and provided a product of suitable concentration. The serum virus was adjusted to the desired hydrogen ion concentration by using N/10 hydrochloric acid and N/10 sodium hydroxide solutions. A pH range from 5.0 to 9.0 was selected because the potency of the serum virus was not materially affected within

this range as indicated by preliminary tests. The quantity of filtrate used in the inoculation of experimental animals was 2 cc. This volume was selected since 2 to 3 cc. has for some time been considered a standard dose of hog cholera virus sufficient to cause a typical case of hog cholera in a susceptible animal. Roderick and Schalk, as the result of their experimental work, concluded that .00002 cc. of serum virus was sufficient to cause hog cholera infection in susceptible animals. Therefore there must be a considerable concentration of virus in the blood serum of cholera infected swine and considerable latitude may be taken in the concentration of serum virus used in experimental work.

EXPERIMENTAL

All inoculations of filtrates were made intramuscularly. Daily temperatures were recorded and symptoms observed. The animals were destroyed several days after symptoms appeared. The serum virus of these animals was used in succeeding experiments.

Experiments with Siliceous Filters

In this series of experiments the filters selected included the regular Mandler filter, the Berkfeld No. 3 "F" and the Chamberland-Pasteur "F" filters. The serum virus and the experimental animals used are indicated in the summary of the following experiments. The cultures made from the filtrates of the bacterial suspensions used to test the efficiency of the filters after filtering the serum virus were negative. The summary of this series of experiments is shown in Table I.

TABLE I
Experiments with Siliceous Filters

Filter	Serum Virus	pH	Volume Delivered	Time	Temperature	Test Pig	Symptoms	Diagnosis
Mandler (regular)	3903	5.0	45 cc.	3 min.	22°C.	3928	6th day	H. C.
Mandler (regular)	3903	6.0	40.2 cc.	3 min.	22°C.	3929	5th day	H. C.
Mandler (regular)	3903	7.0	47.0 cc.	3 min.	22°C.	3930	5th day	H. C.
Mandler (regular)	3903	8.0	52.2 cc.	3 min.	22°C.	3931	5th day	H. C.
Mandler (regular)	3931	9.0	45.0 cc.	3 min.	24°C.	3901	4th day	H. C.
Berkfeld "H"	3931	5.0	67.5 cc.	3 min.	24°C.	3904	6th day	H. C.
Berkfeld "H"	3930	6.0	124.8 cc.	3 min.	21°C.	3934	4th day	H. C.
Berkfeld "H"	3934	7.0	102.3 cc.	3 min.	24°C.	3949	4th day	H. C.
Berkfeld "H"	3942	8.0	86.4 cc.	3 min.	29°C.	3905	5th day	H. C.
Berkfeld "H"	3942	9.0	101.6 cc.	3 min.	29°C.	3951	4th day	H. C.
Chamberland-Pasteur "F"	3942	5.0	43.5 cc.	3 min.	31°C.	3950	5th day	H. C.
Chamberland-Pasteur "F"	3909	6.0	51.2 cc.	3 min.	23°C.	3957	4th day	H. C.
Chamberland-Pasteur "F"	3909	7.0	47.6 cc.	3 min.	24°C.	3958	6th day	H. C.
Chamberland-Pasteur "F"	3971	8.0	42.0 cc.	3 min.	23°C.	3972	8th day	H. C.
Chamberland-Pasteur "F"	3971	9.0	54.1 cc.	3 min.	23°C.	3979	8th day	H. C.

Vacuum Maintained at 350 mm. Hg.
H. C. = Positive Diagnosis of Hog Cholera

Experiments with Gypsum Filters

These gypsum filters were made according to the formula Kramer used in the construction of his filters having basoid adsorptive properties as described by Rivers (27). A paste was made in the proportion of 75 gms. gypsum ($\text{CaSO}_4 + 2.5$ per cent CaCO_3) to 26 gms. MgO in 75 gms. water. The paste was cast in sections of thin brass tubing 15 mm. in diameter and 7 mm. in height and allowed to harden. This made a small cylindrical filter block which could be accommodated in a rubber gasket of a Jenkins filter. The gasket containing this filter block could be tightly compressed in the base of the filter after sterilization making an efficient means of filtering small volumes of liquids. As these filter blocks were of a rather fragile nature, a new one was used in each experiment and carefully tested with bacterial suspensions. The cultures made from the filtrate of bacterial suspensions passed after filtering the serum virus were negative.

The results of the experiments with the gypsum filters are summarized in Table II.

TABLE II
Experiments with Gypsum Filters

Filter	Serum Virus	pH	Volume Delivered	Time	Temperature	Test Pig	Symptoms	Diagnosis
Gypsum	3971	8.0	14.1 cc.	20 min.	23°C.	3978	9th day	H. C.
Gypsum	3979	8.0	16.5 cc.	20 min.	22°C.	3967	5th day	H. C.
Gypsum	3971	7.0	12.2 cc.	20 min.	23°C.	3976	7th day	H. C.
Gypsum	3971	8.0	18.2 cc.	20 min.	22°C.	3977	12th day	H. C.
Gypsum	3977	9.0	15.4 cc.	20 min.	20°C.	3986	5th day	H. C.

Vacuum Maintained at 350 mm. Hg.

H. C. = Positive Diagnosis of Hog Cholera

Experiments with Compound Filters

Compound filters were made by casting a cortex of the same composition described under gypsum filters around a Berkfeld No. 3 "W" filter candle. This gypsum layer was 7.5 mm. thick and was extended down over the metallic base of the Berkfeld filter on a thin rubber gasket which was compressed tightly against the mantle making a bacteria-proof joint. Theoretically the negatively charged particles would be removed by the cortex and the positively charged particles would be removed by the Berkfeld filter. The results of the experiments with this type of filter are summarized in Table III. The cultures made from the filtrate of the bacterial suspension used to test the filters were negative.

TABLE III

Experiments with Compound Filters

Filter	Serum Virus	pH	Volume Delivered	Time	Temperature	Test Pig	Symptoms	Diagnosis
Compound	3996	5.0	36.5 cc.	20 min.	21°C.	3998	5th day	H. C.
Compound	3996	6.0	41.3 cc.	20 min.	21°C.	3999	5th day	H. C.
Compound	3999	7.0	34.8 cc.	20 min.	22°C.	4000	6th day	H. C.
Compound	4708	8.0	44.2 cc.	20 min.	20°C.	4003	10th day	H. C.
Compound	4708	9.0	40.1 cc.	20 min.	22°C.	4010	4th day	H. C.

Vacuum Maintained at 350 mm. Hg.

H. C. = Positive Diagnosis of Hog Cholera

Experiments with the Seitz-Uhlenhuth Type of Filter

This type of filter consists of thin sheets of asbestos tightly compressed with a finely meshed cloth gauze on the under side. These filter discs are 6 cm. in diameter with a thickness of 3 mm. They are placed in the filter base supported by a finely meshed wire gauze. A thin rubber gasket is fitted over the filter disc and the top section of the filter is tightly screwed to the base, forming a bacteria-tight joint. In some preliminary experiments with this type of filter difficulty was experienced in securing a tight joint between the filter and the filter disc. For this reason a thin rubber gasket was used above the filter disc and the winged nuts used to attach the base of the filter to the top were carefully tightened with pliers. When the filter discs were properly placed in the filter no difficulty was experienced in securing bacteria-free filtrates. The discs when placed in the filter left a filtering surface 35 mm. in diameter. The results of these experiments are summarized in Table IV. Cultures made from the filtrates of bacterial suspensions used to test the efficiency of these filters were negative.

TABLE IV

Experiments with Seitz-Uhlenhuth Type of Filters

Filter	Serum Virus	pH	Volume Delivered	Time	Temperature	Test Pig	Symptoms	Diagnosis
Seitz-Uhlenhuth	4010	5.0	14.0 cc.	5 min.	21°C.	4711	7th day	H. C.
Seitz-Uhlenhuth	4714	6.0	16.4 cc.	5 min.	22°C.	4715	6th day	H. C.
Seitz-Uhlenhuth	4714	7.0	18.6 cc.	5 min.	20°C.	4716	5th day	H. C.
Seitz-Uhlenhuth	4716	8.0	15.2 cc.	5 min.	21°C.	4726	6th day	H. C.
Seitz-Uhlenhuth	4716	9.0	16.5 cc.	5 min.	21°C.	4727	6th day	H. C.

Vacuum Maintained at 350 mm. Hg.

H. C. = Positive Diagnosis of Hog Cholera

SUMMARY AND DISCUSSION

In conducting experiments with hog cholera virus a supply of susceptible swine must be available. No other animals will serve as experimental animals for this purpose. Inoculation of susceptible swine is the only means we have of detecting the presence of this virus. Because of the highly infective nature of this disease isolation of experimental animals must be carefully arranged so as to prevent accidental infection. Through the use of the isolation units described and with great precaution in the feeding and care of these animals, no difficulty was experienced by accidental infection as shown by control animals.

The necessity of conducting preliminary experiments in order to determine whether or not hog cholera virus would pass through filters possessing these characteristics was eliminated by assuming that the virus under ordinary conditions would pass through these filters, as determined by Kernkamp (18). The results of this series of experiments not only confirm this work but give a basis for further study.

CONCLUSIONS

1. Serum virus of hog cholera passes readily through the regular Mandler, Berkfeld "H", Chamberland-Pasteur "F" and Seitz filters which are all standard. It also passes readily through the gypsum and compound filters which were made and tested in the laboratory.

2. These filters were efficient in removing bacteria from liquid suspensions.

3. The volume of the filtrate delivered by individual filters of the same type under identical pressures varies considerably.

4. The studies on the effect of pH upon the filterability of hog cholera serum virus showed that within the range used, pH = 5.0 to 9.0, this factor is not of practical significance.

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PART II. THE MIGRATION OF HOG CHOLERA VIRUS WHEN
SUBJECTED TO ELECTROPHORESIS

INTRODUCTION

Electrophoresis as defined by Buchanan and Fulmer (3) is the phenomenon of the migration of colloidal particles under the influence of the electric current, anaphoresis and cataphoresis designating the migration to the anode and cathode respectively. The etiologic factor of virus diseases has long been regarded as colloidal in nature or associated with the colloidal constituents present in blood serum and tissue extracts. Consequently its behavior was believed to resemble that of colloids when subjected to electrophoresis.

One of the first observations of this phenomenon was made by von Angerer (1). He observed that bacteriophage in an electric field migrated toward the anode. Later Koch (9) in his studies on the Shiga bacteriophage claimed that it moved toward the cathode. His apparatus consisted of a V tube, the upright limbs of which had two stopcocks in the middle and a lower horizontal limb with a central opening closed by a rubber stopper. Neither von Angerer nor Koch mentioned the hydrogen ion concentration at which they worked. Since protein colloids behave differently in acid and basic solutions it is difficult to interpret their results.

Charles Todd (14) carried out some careful experiments on the

nature of the electric charge carried by bacteriophage. He devised one of the most effective and conveniently constructed pieces of apparatus for this type of work. His investigations on the electrical migration of bacteriophage for B. Shiga, at a number of points over ranges between pH 3.6 and 7.6, showed that it possessed a negative charge because of its migration toward the anode.

Bedson and Bland (2) made careful studies on the electric charge carried by virus particles. Brown and Broom (4) studied the action of the electric current on bacteria. Hersberg (6) in his experiments with vaccine virus found that the virus in greater concentrations migrated with greater speed. Kligler and Olitsky (8) found that typhus virus migrated to the positive pole. Thompson (13) conducted his studies on normal and chemically treated pneumococci. Olitsky and Boez (12) in working with foot and mouth disease virus, found that under ordinary conditions it carries a positive charge and they also called attention to the fact that it differs from the known cultivable bacteria which are usually electronegative. Douglas and Smith (5) working with vaccine virus found that the virus was rendered inactive at a pH below 5.0 and that it carries a negative electric charge over a range of from pH 5.5 to pH 8.4. Lapine (10) worked on the cataphoresis of fowl pest. Hobbs (7) made detailed studies on the myxoma virus of rabbits. Lewis and Michaelis (11) in their investigations with the Rous sarcoma found that the etiologic agent was active between the pH of 4.0 to 12.0 and carries an electronegative charge.

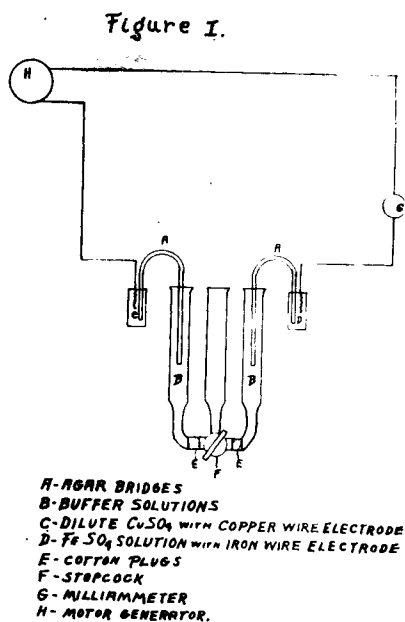
The contributions made by these investigators and many others,

carrying on their studies with viruses which are responsible for many infectious diseases, have given us considerable information concerning the physical and chemical properties of these agents. Although all do not agree in their findings and the interpretations of their experimental results, many of their findings have been confirmed. When one considers the possibility of diverse strains of the viruses responsible for certain specific diseases, the variations in the technique and the irregularities experienced in the reaction of experimental animals to infection, it is not surprising to find disagreement between investigators.

The review of the literature pertaining to the investigations carried out on the virus of hog cholera does not reveal its behavior toward electrophoresis. With this in mind, this series of experiments was conducted to determine the migration of hog cholera virus when subjected to electrophoresis.

TECHNIQUE

The apparatus used is similar to that used by Todd, with some modifications. The two side arms of a large three-way stopcock were turned to a vertical position two inches from the center tap. Pyrex test tubes, 2 x 15 cm., were drawn out from the base and welded to the three arms of the stopcock in a vertical position as shown in Figure 1. The capacity of the entire apparatus was 75 cc. Figure 1 shows the type of apparatus used.



The side chambers of the apparatus were connected by two inverted U tubes, 5 mm. in diameter, with two small glass bottles containing non-polarizable electrodes. These tubes were filled with 1% saline in 2% agar. The incorporation of air bubbles in the agar bridges must be avoided because of their influence on the conductivity of the current through the agar medium. The cathode, made of copper wire, was submerged in dilute copper sulphate solution. The anode, constructed of iron wire, was submerged in a solution of ferric sulphate. In some preliminary experiments dilute copper sulphate solution and a copper electrode were used in place of iron wire electrode and ferric sulphate solution. The copper solution had a tendency to diffuse through the agar bridge during the course of the experiment. Copper sulphate in sufficient concentration is toxic to both plant and animal life. For this reason ferric sulphate was substituted. If sufficient copper sulphate reached the side chamber containing virus suspension the possibility of inactivating the virus would have to be considered as having an influence on the results of these experiments. The ferric sulphate solution did not show any marked tendency to diffuse through the agar bridge. If, however, some of this solution did diffuse through the agar bridge or reach the side chamber because of the contraction of the agar from the sides of the glass tubes, there would be less probability of inactivating the virus, if present, as ferric compounds are less toxic than those of copper.

The glass apparatus was washed, boiled in water and sterilized in a hot air oven. Two small loose cotton plugs boiled in physiologic

saline solution were introduced through the side chambers into the horizontal portions of the tube about one inch from the stopcock. The stopcock was lubricated sparingly with sterile stopcock grease to insure proper operation. In preliminary tests with dyes there was no indication of diffusion beyond these cotton plugs for twenty-four hours which was a far greater period of time than was required to run an experiment.

In a series of preliminary experiments the electric current was obtained from a series of storage batteries. This necessitated the use of variable resistances in the circuit for the purpose of controlling the amounts of electricity passing through the apparatus. This source of electricity was not entirely satisfactory as the current varied considerably making it impossible to maintain a uniform current. A motor generator equipped with a volt meter and a field rheostat proved to be quite satisfactory and was therefore used. A milliammeter was placed in the circuit to indicate strength of current passing through the apparatus. It was also noted that as the experiments proceeded the temperature of the liquids in the apparatus increased, thus increasing the conductivity of the media in the apparatus. Since high temperatures would prove detrimental to the potency of the virus, it was thought advisable to conduct all experiments at a uniform temperature, preferably at 37° C. The apparatus was submerged up to within one inch of its top in a water bath maintained at a temperature of 37° C. throughout the period of the experiment.

Another difficulty experienced by many investigators was the comparatively high resistance of buffers such as acetic acid and sodium

acetate which are poorly ionized in solution. Todd suggested the addition of physiologic saline solution with the pH 7.0. He found that in every case where the pH of the buffer was measured after the addition of saline that there was no appreciable change in the hydrogen ion concentration. The pH of the buffers was also measured after the experiment and in no case was it found to be appreciably altered. The buffers commonly used in this work are sodium hydroxide, hydrochloric acid, glycocoll, disodium hydrogen phosphate (secondary), potassium hydrogen phosphate (primary), sodium citrate and sodium baborate. In these experiments sodium hydroxide and hydrochloric acid in physiologic saline were used. These solutions after being adjusted to the proper hydrogen ion concentration were sterilized, their pH was determined again after which they were stored in the refrigerator. The serum virus adjusted to the desired pH value was filtered through a Berkfeld "W" filter to remove possible bacterial contamination, placed in a sterile test tube which was tightly stoppered and poured into the center chamber of the apparatus.

The hydrogen ion concentration was determined by the early investigators by the colorimetric method. Douglas and Smith objected to this method because of the interference of small quantities of serum found with the tissue proteins in the colorimetric changes. In these experiments the hydrogen ion concentration was determined by means of the glass electrode. The apparatus employed was the same used in determining the hydrogen ion concentrations in the experiments described in Part I. Care had to be taken to see that no air locks formed in the apparatus when the stopcock was opened. These may cause increased

resistance or stop the flow of the electric current entirely. The cotton plugs were boiled not only to sterilize them, but also to remove all air bubbles incorporated in them. The most convenient means of removing air bubbles from the apparatus was to fill the side chambers with the sterile buffer solution and to open the stopcock a little allowing the liquid to force the air from the tubes and passages of the stopcock. The liquid which was admitted into the center chamber was removed with a sterile pipette and the serum virus was then placed in the apparatus. The height of the liquids in the three chambers of the apparatus after the agar bridges were in position was adjusted to about the same level, with that of the center chamber slightly lower so that, when the stopcock was opened, the movement caused by the equalization of the liquid levels of the three chambers would be toward the center chamber containing the serum virus. This precaution seemed necessary to prevent possible migration of the serum virus into the side chambers due to the hydrostatic pressure.

THE VIRUS

The serum virus used in these experiments originated from field cases of hog cholera. It was filtered and passed through susceptible swine to eliminate the possibility of bacterial contamination. The dilution of serum virus was the same as that used in the filtration series of experiments, i. e., 25 cc. serum virus and 75 cc. sterile physiologic saline solution. The pH range used in these studies was 5.0 to 9.0. The potency of the virus within this range was not materially affected. The virus and the experimental animals used are recorded in summaries of this series of experiments.

EXPERIMENTAL

A current of 120-125 volts from a direct current motor generator was found necessary to maintain a flow of 20 milliamperes. In preliminary experiments this strength of current was found suitable and so far as could be determined did not materially affect the potency of the virus. For the sake of uniformity all experiments were conducted in the same manner and under as nearly the same conditions as were possible to maintain. All experiments were conducted for a period of three hours, with no interruptions to the current at any time. There was no interference with the apparatus in any way during the course of the experiments so that diffusion of the serum virus with resulting errors were not possible. At the end of the period in which the serum virus was subjected to the action of the electric current, the stopcock was closed, and the electric circuit was completely broken so that no possible movement of the liquids could occur when the agar bridges were removed. The liquids were withdrawn from the chambers with sterile pipettes and placed in sterile test tubes which were tightly closed with cotton stoppers. In drawing these liquids from the chambers it was impossible to disturb the cotton plugs in the apparatus because the pipettes could not be lowered into the tubes of smaller diameter in which the cotton plugs were placed.

The experimental animals were placed in the isolated units previously described and a series of preinoculation temperatures was

taken. Two cc. of liquid from each of the chambers were injected into susceptible pigs. The pH was checked on the remaining portions of the liquids. The animals injected with the liquid from the center chamber of the apparatus acted as a check on the potency of the virus.

Temperatures of the experimental animals were recorded and symptoms observed. The diagnoses were made from the post mortem examinations. Cultures were made from the heart blood, liver and spleen in order to detect any possible bacterial infection. These cultures proved negative. Control animals were kept for the duration of the experiments and were subsequently used in other experiments to test their susceptibility to hog cholera infection. The experimental animals which did not contract the disease as a result of the intramuscular injections of these liquids were used in other tests by which their susceptibility was ascertained.

EXPERIMENT I

Serum virus 4711

Voltage - 125

Milliamperes - 20

pH - 5.0

Time - 3 hours

Temperature - 37°C.

Animal Inoculation

Inoculum	Protein test	Test pigs	Results	Diagnosis
Positive pole	+	4712	+	H. C.
Center chamber	+++	4714	+	H. C.
Negative pole	-	4715	-	- -

Pig 4715 susceptible as proven by subsequent test.

H. C. = positive diagnosis of hog cholera.

EXPERIMENT II

Serum virus F.6(field virus)

Voltage - 120

Milliamperes - 20

pH - 6.0

Time - 3 hours

Temperature - 37° C.

Animal Inoculation

Inoculum	Protein test	Test pigs	Results	Diagnosis
Positive pole	+	4740	+	H. C.
Center chamber	+++	4760	-	- -
Negative pole	-	4749	-	- -

Pig 4749 susceptible as proven by subsequent test.

Pig 4760 immune.

H. C. = positive diagnosis of hog cholera.

EXPERIMENT III

Serum virus 4745

Voltage - 122

Milliamperes - 30

pH - 7.0

Time - 3 hours

Temperature - 37° C.

Animal Inoculation

Inoculum	Protein test	Test pigs	Results	Diagnosis
Positive pole	+	4753	+	H. C.
Center chamber	+++	4750	+	H. C.
Negative pole	-	4759	-	- -

Pig 4759 susceptible as proven by subsequent test.

H. C. = positive diagnosis of hog cholera.

EXPERIMENT IV

Serum virus 4745

Voltage - 120

Milliamperes - 20

pH - 8.0

Time - 3 hours

Temperature - 37° C.

Animal Inoculation

Inoculum	Protein test	Test pigs	Results	Diagnosis
Positive pole	+	4751	+	H. C.
Center chamber	+++	4752	+	H. C.
Negative pole	-	4755	-	- -

Pig 4755 susceptible as proven by subsequent test

H. C. = positive diagnosis of hog cholera.

EXPERIMENT V

Serum virus 4751

Voltage - 120

Milliamperes - 20

pH - 9.0

Time - 3 hours

Temperature - 37° C.

Animal Inoculation

Inoculum	Protein test	Test pigs	Results	Diagnosis
Positive pole	+	4756	+	H. C.
Center chamber	+++	4757	+	H. C.
Negative pole	-	4758	-	- -

Pig 4758 susceptible as proven by subsequent test.

H. C. = positive diagnosis of hog cholera.

SUMMARY AND DISCUSSION

This series of experiments on the serum virus of hog cholera shows clearly that the migration takes place toward the positive pole within the specified hydrogen ion range. In Experiment II pig 4760 which was inoculated with liquid from the center chamber of the apparatus did not develop hog cholera. Later tests with virus of known potency demonstrated beyond doubt that the pig was not susceptible to hog cholera. It was not considered necessary to repeat this experiment because the experimental animal inoculated with liquid from the center chamber acted only as a check on the potency of the virus at that particular hydrogen ion concentration at the termination of the experiment. This necessary check was furnished by pig 4740 which was inoculated with liquid from the positive pole and developed a typical case of hog cholera. Pig 4760 which was inoculated with liquid from the negative pole and did not develop hog cholera proved to be susceptible as the result of a subsequent inoculation. The control pigs used in this series of experiments did not develop hog cholera.

CONCLUSIONS

1. The serum virus of hog cholera migrates toward the positive pole at pH values from 5.0 to 9.0.
2. The virus of hog cholera either carries a negative electric charge or is carried toward the positive pole by the associated proteins.
3. It is not possible within the pH range studied to separate hog cholera virus from the associated proteins by the electrophoretic method employed.

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PART III. EXPERIMENTS ON THE ATTENUATION OF VIRUS AND THE
PRODUCTION OF IMMUNITY TO HOG CHOLERA

INTRODUCTION

The protection of swine against hog cholera by the use of anti-hog-cholera serum was fully established in 1908 as the result of the investigations of Dorset, McBryde and Niles (5). Since that time either the serum treatment or the simultaneous treatment has been successfully used in almost all localities in which a considerable number of swine are raised. The efficiency of this method was greatly increased by the improved methods of manufacture resulting in the production of immunizing agents of known potency and free from bacterial contamination. No doubt the government supervision of biologic production exerted a great influence. Other methods of producing immunizing agents for hog cholera have been published but extensive studies along these lines have not been carried on.

In studying the possibilities of developing a protective agent for the prevention or treatment of an infectious disease, it is natural to inquire into the characteristics of the etiologic factor. Dinwiddie (4) as the result of his studies concluded that the virus of hog cholera was contained principally within the blood cells, but that it also occurred in the serum and plasma. Duval (6) stated in his work that the virus of hog cholera at no period during the infection is intracellular

in so far as the blood cells are concerned. Rederick and Schalk (13) found as the result of their studies that erythrocytes of cholera infected swine carried virus even after nine washings in physiologic saline solution. Ruess and Murray (14) clearly demonstrated that erythrocytes were highly infective after being thoroughly washed in physiologic saline solution. This establishes the fact that hog cholera virus is intracellular and that the virus found in the tissues of the various organs of an infected pig are incorporated in the tissue cells as well as the body fluids which are incorporated in them. This fact in itself would make possible the development of tissue vaccines provided the active agent could be attenuated or rendered capable of producing immune bodies in susceptible swine without experiencing high mortality in the process.

Among the investigators who have attempted to prepare vaccines as mentioned by Hutyra and Marek (9) were Marxer, Uhlenhuth, King, Pfeiler and Standfuss, Reynolds and Kraft. These workers employed blood, serum and preparations made from various tissues treated to attenuate the virus and produce immunity in susceptible swine. Their attempts were unsuccessful. Graham and Bruckner (7) failed to produce an immunizing agent by attempted attenuation using various degrees of heat. Healy and Gott (8) using a mixture of hog cholera virus and hyperimmune blood incubated at 37° C. obtained variable results. More recently Boynton (2,3) carried on a series of experiments in an attempt to produce an effective tissue vaccine. He tried a vaccine similar to that he used in his work with rinderpest, a modification developed by Kelser (10) and a method used by Kakizaki (11) without obtaining dependable results. His

experiences with formalin as used by Laidlaw and Dunkin (12) proved to be no more successful. Benner (1) was also unsuccessful with formalin preparations. Boynton (3) however claims to have produced a vaccine efficient as a prophylactic against hog cholera but of no therapeutic value. He used eucalyptus oil as an attenuating agent and tissue which included red bone marrow and lymphatics taken from infected swine. Boynton is the only investigator who seems to have had any degree of success in producing a prophylactic agent for protection against hog cholera.

The use of anti-hog-cholera serum and virus in the production of an active immunity against hog cholera has proven effective for a good many years. The cost of this operation is considerable even though these products are made on a large scale and produced in an efficient manner. The danger of spreading infection by injudicious use of virus is also a factor to be considered. For these reasons it would be desirable to produce a vaccine which would afford suitable protection for susceptible swine and which would not itself be infectious. Further studies along these lines are indicated until an efficient vaccine can be produced.

TECHNIQUE

The blood serum used for desiccation of serum virus was obtained from pig 3869 which was destroyed several days after showing symptoms of hog cholera. The blood was collected in a flask under aseptic conditions and allowed to clot. The serum was drawn off with a sterile pipette and 20 cc. were placed in each sterile petri dish. These petri dishes were placed in a sterile desiccator after which the petri dish covers were removed and the desiccator was sealed. The rubber stopper in the top of the desiccator was fitted with glass tubes, one of which extended to the base of the desiccator forming the inlet for the circulating air, and the other tube extending only one inch below the rubber stopper which served as the outlet. The air passed through a large glass tube filled with cotton which was previously sterilized serving as an air filter. The air then passed through a series of four wash bottles containing concentrated sulphuric acid which was used as a desiccating agent to remove the water from the air. The dry air circulated through the desiccator removing the water content of the serum virus. An additional wash bottle containing concentrated sulphuric acid was placed in the series between the desiccator and the vacuum pump to absorb the moisture from the air as it passed from the desiccator. This prevented the possibility of moisture condensing on the walls of the glass and rubber tubing. A water vacuum pump was used to maintain the air circulation. The serum was desiccated after two days time, forming a

hard amber colored sheet on the bottom surface of the petri dishes. The outlets to the desiccator were closed and disconnected from the wash bottles. A small amount of phosphorus pentoxide was placed in the desiccator and allowed to remain for seven days to insure complete desiccation. The desiccated serum was removed from the desiccator and petri dishes, sealed in a dark brown sterile bottle and placed in a refrigerator maintained at a temperature of 36-38° F. Two cc. of 50% suspension in physiologic saline solution were tested on susceptible pigs to determine the virulence. When animal inoculation demonstrated that the preparation was no longer virulent, two injections of 10 cc. of 50% suspension were made intramuscularly at ten day intervals. Ten or more days following the last inoculation, the immunity test was carried out by injecting 2 cc. of potent hog cholera virus. Temperatures were recorded, symptoms observed and the diagnosis was determined from post mortem examination.

When the desiccated brain and spinal cord were used the tissues were removed as carefully as possible from the infected animal. The outer surface of the tissue was seared with a heated spatula to destroy any bacterial contamination thereon. Sterile instruments were used to dissect out thin pieces of tissue from the central portion of the brain and cord. This material was placed on sterile gauze supported by wire and placed in the desiccator. The same methods of desiccation as previously described were employed. Essentially the same technique as described was used in testing this preparation.

The fresh tissues used in connection with chemical inactivation of hog cholera virus included sections taken from the liver, spleen and brain. The various organs were carefully removed from the infected animal which had previously been carotid bled. All blood and fragments of connective tissue were removed from the outer surfaces. The surfaces were cleaned with cotton saturated with 70% alcohol and seared with a heated spatula. Sections were taken from the interior of the organs, placed in a sterile mortar and triturated to a very fine pulp. This was forced with the pestle through a fine sterile sieve to separate the connective tissue from the parenchymatous tissue. Fifty grams of this finely macerated tissue were weighed out in sterile, covered crucibles. To this was added an equal amount of the diluting agent composed of equal parts of glycerin and physiologic saline solution. The chemicals used were added in the amounts indicated. The preparations were thoroughly mixed, placed in bottles which had been previously sterilized and sealed. These preparations were placed in a refrigerator maintained at a temperature of 36-38° F. and agitated once daily.

A preliminary series of tests was run to determine the effect of the saturation of blood of infected animals with various gases. As there seems to be no record in the literature of the effect of gases on viruses it was thought possible to attenuate or inactivate virus in this manner with the possible production of a substance possessing sufficient antigenic properties to stimulate immunity to hog cholera in susceptible swine. One hundred cc. of distilled water were placed in 500 cc. flasks

fitted with rubber stoppers through which two glass tubes were placed; one served as inlet for the gases extending to the base of the flask and the other tube extending about one inch below the base of the rubber stopper served as an outlet. The infected animal was carotid bled under aseptic conditions and 100 cc. of whole blood were added to each flask and gently agitated. Distilled water was used to dilute the blood rather than physiologic saline solution so that the erythrocytes would become hemolyzed. These flasks were taken to the laboratory and connected with the cylinders containing the various gases. The inlet was connected to the cylinder by means of small rubber tubing. The stopcocks on the inlet and outlet of the flask were opened and the gas was allowed to flow slowly through the diluted blood. It was necessary to agitate the contents of the flasks gently and to admit only a very small amount of gas at a time, as gas passing through diluted blood causes the formation of gas bubbles which are rather persistent due to the viscosity. The associated proteins contained in this diluted blood were of sufficient concentration to cause considerable viscosity. After 20 minutes the outlet was closed allowing considerable gas to accumulate within the flask and then the intake was closed. This left the 300 cc. of the flask not occupied by the diluted blood filled with gas. After this series of preparations had been treated with the various gases they were placed in the refrigerator for 24 hours. The contents of these flasks were agitated for several minutes every hour for six consecutive hours. This insured thorough saturation of the flask contents with their respective gases. Two cc. of these preparations were injected into experimental

animals to test the virulence of the preparations. The animals which did not show any reaction were inoculated with 10 cc. followed by a similar treatment ten days later. Two of the preparations which congealed during the gas treatment were further diluted to 50% strength with sterile distilled water. The experimental animals were later injected with 2 cc. of potent hog cholera virus to determine whether these preparations had any value as immunizing agents.

The experimental animals used were susceptible pigs raised on the farm. They were placed in the isolated units previously described which had been thoroughly cleaned and disinfected. Preinoculation temperatures were taken to avoid the possible use of diseased animals. The temperatures were recorded, symptoms observed and the diagnoses were based on post mortem examinations.

EXPERIMENTAL

The results of the experiments conducted to determine the immunizing properties of these preparations are summarized as follows:

Desiccated Tissues

EXPERIMENT I

Material - Brain and spinal cord of pig 3738

Desiccated - 1/31/31

Test of Virulence - Two cc. of 50% suspension of pulverized desiccated tissue in physiologic saline solution were injected into test animals as follows:

Date	Test Pig	Symptoms	Diagnosis
6/3/31	3764	4th day	Hog Cholera
10/2/31	3869	5th day	Hog Cholera
12/9/31	3887		Normal

Treatment - Ten cc. of 50% desiccated tissue suspension in physiologic saline solution were injected into pig 3887 on 12/23/31 and 1/2/32. The immunity test was made 21 days later.

Immunity Test - Two cc. of virus 3884 were injected into pig 3887 intramuscularly. The animal showed symptoms of hog cholera on the 7th day. Post mortem examination showed distinct lesions of hog cholera. Cultures made from the heart blood, liver and spleen were negative.

Desiccated Serum Virus

EXPERIMENT II

Material - Serum virus 3869

Desiccated - 10/21/31

Test of Virulence - Two cc. of 50% suspension of pulverized desiccated virus in physiologic saline solution were injected into the test animals as follows:

Date	Test Pig	Symptoms	Diagnosis
5/11/32	3771	5th day	Hog Cholera
6/3/32	3765	4th day	Hog Cholera
11/12/32	3883		Normal

Treatment - Ten cc. of 50% serum suspension in physiologic saline solution were injected into pig 3883 on 11/24/32 and 12/4/32.

Immunity Test - Two cc. of virus 3869 were injected into pig 3883 on 12/14/32. The animal showed symptoms of hog cholera on the 5th day. Diagnosis was hog cholera determined by post mortem examination.

Formalized Tissue

EXPERIMENT III

Materials - Liver, spleen and brain from pig 3770

Prepared 4/6/31; kept at 36-38°F.

Test of Virulence - Ten cc. of 50% suspension of macerated tissue pulp diluted with a diluting agent consisting of equal parts glycerin and physiologic saline solution with 1% formalin added were injected into test animals.

Treatment

Dates of Injection	Pig	Preparation	Volume	Reaction
5/2/31 and 5/12/31	3785	Liver	10 cc.	None
5/7/31 and 5/17/31	3782	Spleen	10 cc.	None
5/7/31 and 5/17/31	3783	Brain	10 cc.	None

Immunity Test

Date	Test Pig	Virus	Symptoms	Diagnosis
5/22/31	3785	3771	4th day	Hog Cholera
5/27/31	3782	3771	5th day	Hog Cholera
5/27/31	3783	3771	5th day	Hog Cholera

The diagnoses on these pigs were based on post mortem findings. All animals showed well defined lesions of hog cholera.

Phenolized Tissue

EXPERIMENT IV

Materials - Liver, spleen and brain from pig 3883

Prepared 12/24/31; kept at 36-38°F.

Preparation made the same as in Experiment III with the addition of 2% phenol as the virus inactivating agent.

Test of Virulence - Ten cc. of these preparations were injected into the experimental animals.

Treatment

Dates of Injection	Pig	Preparation	Volume	Reaction
1/15/32 and 1/25/32	3888	Liver	10 cc.	None
1/15/32 and 1/25/32	3889	Spleen	10 cc.	None
1/15/32 and 1/25/32	3892	Brain	10 cc.	None

Immunity Test

Date	Test Pig	Virus	Symptoms	Diagnosis
2/5/32	3888	3884	5th day	Hog Cholera
2/5/32	3889	3884	4th day	Hog Cholera
2/5/32	3892	3884	5th day	Hog Cholera

The diagnoses of these animals were based on post mortem examinations.

All animals showed distinct lesions of hog cholera.

Saturation of Virus with Gases

EXPERIMENT V

Material - Virus blood 4758

Dilution - 50% with sterile distilled water

Gases used - Oxygen, carbon dioxide, sulphur dioxide, nitrogen
chlorine and hydrogen.

Time - Saturated for 24 hours and kept in refrigerator at 36-38° F.

Test of Virulence

Date	Virus	Gas	Test Pig	Volume	Symptoms	Diagnosis
1/5/34	4758	Nitrogen	4766	2 cc.		Normal
1/5/34	4758	Chlorine	4767	2 cc.		Normal
1/5/34	4758	Sulphur dioxide	4768	2 cc.		Normal
1/5/34	4758	Oxygen	4769	2 cc.		Normal
1/5/34	4758	Carbon dioxide	4759	2 cc.	5th day	Hog Cholera
1/5/34	4758	Hydrogen	4765	2 cc.		Normal

Treatment

Dates of Injection	Pig	Preparation	Volume	Reaction
1/20/34 and 1/30/34	4766	Nitrogen	10 cc.	None
1/20/34 and 1/30/34	*4767	Chlorine	10 cc.	None
1/20/34 and 1/30/34	*4768	Sulphur dioxide	10 cc.	None
1/20/34 and 1/30/34	4769	Oxygen	10 cc.	None
1/20/34 and 1/30/34	4765	Hydrogen	10 cc.	None

*Preparations congealed upon saturation with the chlorine and sulphur dioxide.

Diluted to 50% concentration with sterile distilled water.

Immunity Test

Date	Test Pig	Virus	Symptoms	Diagnosis
2/9/34	4766	A.V. ₂	5th day	Hog Cholera
2/9/34	4767	A.V. ₂	4th day	Hog Cholera
2/9/34	4768	A.V. ₂	5th day	Hog Cholera
2/9/34	4769	A.V. ₂		Normal
2/9/34	4765	A.V. ₂		Normal

SUMMARY AND CONCLUSIONS

Many complex problems confront the investigator of such a disease as hog cholera. The only experimental animals which can be used are swine, because this disease is limited to that particular species. The expense involved in this type of work is necessarily great and considerable care has to be taken to conserve experimental animals and to develop technique which will prevent their accidental infection in isolated units. Furthermore for each experiment there must be one susceptible experimental animal for control. The results of these experiments indicate potential possibilities for the production of an immunizing agent against hog cholera. Since there are at the present time no means of detecting the presence of potent hog cholera virus other than animal inoculation, a great number of experimental animals must be used. It is also difficult to determine whether or not virus has been attenuated by certain processes because of the variability in the natural resistance of the experimental animals and the variation in the potency of the infective agent. This makes it difficult to evaluate experimental results conducted for the purpose of securing immunizing agents. This series of experiments indicates many negative results with several potential possibilities. Considerable investigation will be necessary to determine the value of these possibilities. With these considerations in mind certain reservations have to be made in the interpretations of the experimental data presented.

To determine the value of desiccation as a possible attenuating agent for infective material, a series of tests was necessary to determine the virulence of the products used. Because of the limited number of experimental animals available at certain times and the limited number of isolation units provided, these tests were carried on whenever possible. If these products failed to produce symptoms in the experimental animals, two 10 cc. volumes were injected into the test pigs at intervals of ten days. At least ten day were allowed to pass before the animals were tested for immunity to hog cholera. The desiccated preparations used in this series of experiments did not show any promise of being of value for immunizing susceptible swine against hog cholera.

The fresh tissue preparations, using phenol and formalin as inactivating agents, when used in a similar manner, failed to produce immunity against hog cholera.

The treatment of the diluted blood of the cholera infected swine with gases in an effort to attenuate so as to produce a safe immunizing agent in susceptible swine holds some promise. Treatment with carbon dioxide for 24 hours did not however seem to reduce the virulence of the preparation. The other gases used apparently destroyed the virulence of the preparation or reduced it to a point where it no longer produced noticeable symptoms. In the virulence test pigs 4769 and 4765 showed slight elevations in temperature, the former on the 6th, 7th and 8th days and the latter from the 3rd to the 9th days. These temperatures returned to normal again and on subsequent treatment with

these preparations showed no reaction. These two pigs were the only ones in this experimental group to survive the immunity test. The 2 cc. of virus of known potency which produced decided symptoms on the 4th and 5th days in the other animals of this group, with distinct lesions of hog cholera on post mortem examination failed to have any noticeable effect on these two animals. Since there is no way of measuring the natural resistance an animal possesses to infection it is hard to interpret these results. However, they indicate that it may be possible to produce an immunising product of value by this method. There are many more gases that may be tried for this purpose, employing various concentrations or saturation for various periods of time. It seems rather singular that hydrogen and oxygen should be the two gases in this series of tests which should appear to be the most effective. Much more experimental work will have to be carried out on this phase of the investigations before the value of these products as practical immunizing agents can be determined.

CONCLUSIONS

1. The preparations made from infective tissues inactivated by desiccation apparently have no value in producing immunity against hog cholera.
2. The preparations made from fresh tissues inactivated by phenol and formalin in the concentrations used did not seem to be effective in producing any degree of immunity.
3. The saturation of the blood of infected swine with certain gases inactivates the virus.
4. The preparations attenuated or inactivated by oxygen and hydrogen apparently have some value in producing immunity against hog cholera.

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