THE SURVIVAL OF THE HOG-CHOLERA VIRUS IN LABORATORY ANIMALS, PARTICULARLY THE RAT.

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In beginning an investigation of the filterable virus of hog-cholera one line of work decided upon was to study the effect of the virus on various laboratory animals in the hope that some reaction or local infection might be produced that previously had been overlooked. Although Uhlenhuth and Haendel (1) state that the mouse, rat, guinea pig, rabbit, dog, cat, horse, donkey, sheep, cow, goat, pigeon, chicken, goose, and duck are not susceptible to inoculation with the virus, it was nevertheless hoped that by using some of the less common methods of inoculation and by carefully observing the animals some species other than swine might be made available for work on hog-cholera. The method chosen was to inoculate several animals of a given species in one or more ways, to observe them closely, and after approximately 7 days to determine whether the virus was still present in their bodies by inoculation of susceptible pigs.

HISTORICAL.

Craig (2) has reported work along the same line. He injected large amounts of virus (15 cc.) intravenously into rabbits and 8 days later made a suspension of their organs and tissues, and, after passing it through a Berkefeld filter, injected it into pigs. In several instances the animals came down with hog-cholera, showing that the virus had remained alive for 8 days in the body of the rabbit. Attempts to pass the virus from one rabbit to another were negative.

Sir Stewart Stockman (3) attempted to infect wild rats with hog-cholera by feeding them the intestines of pigs showing lesions of the disease. Two lots of rats were fed daily, one for 12 and the other for 11 days, after which they were killed and as much blood as possible was obtained from the heart. The temperature of the pig inoculated with the blood from the first lot rose to between 104° and 105°F. on the 6th day after the injection and remained there for 3 days, after

which it returned to normal. The animal was killed 16 days after the inoculation and no lesions of hog-cholera were found. The pig inoculated with the blood from the second lot of rats showed no temperature or other signs of illness for 42 days following the injection. The result of the inoculation of the first pig must be looked upon as doubtful, but even though it were negative the conclusion that could be drawn would be that the rats did not have the virus in their blood stream and not "that rats are not, as has been suggested, pathological carriers of swine-fever," for the virus might be carried in some organ in the body and eliminated with the urine or feces and still not be present in the blood.

EXPERIMENTAL.

Experiment 1.—Object: To determine whether after inoculation with hog-cholera virus rabbits will show a febrile or other reaction and to determine whether the virus is destroyed in their bodies in 12 days.

A rabbit weighing 1,038 gm. was inoculated intravenously with 1 cc. of sterile, but unfiltered, serum that was proved, by inoculation into a pig, to contain the virus of hog-cholera. On the same day a rabbit weighing 970 gm. was inoculated intraperitoneally with 1 cc. of the same serum. A third rabbit weighing 1,815 gm. received into the testicle 1.4 cc. of the same virus.

The temperature of these rabbits was normal for the next 10 days and there were no visible signs of illness. The rabbit injected into the testicle showed no swelling or local lesion in this organ.

12 days after inoculation the first two rabbits were killed and their abdominal and thoracic viscera and brains fed to a pig weighing 94 pounds. The temperature of this pig was normal for the next 17 days and later it was shown to be susceptible to hog-cholera virus.

Experiment 2.—Will the virus live in the body of the guinea pig 6 days?

A guinea pig weighing 495 gm. was given an intraperitoneal injection of 5 cc. of virulent virus. The animal immediately showed anaphylactic symptoms and its temperature fell to 35.4°C. Another experiment showed that the serum from a normal pig will produce the same effect in guinea pigs. Several hours later the temperature of this animal was normal and it remained so for the next 6 days. It was then chloroformed and a suspension made of pieces of its liver, kidney, lungs, and heart. The spleen was contaminated and was not included. 5 cc. of this thick suspension were injected intramuscularly into a pig weighing 48 pounds. The animal showed no temperature reaction and gained 12 pounds in the next 14 days. It was then inoculated with a virus of very low virulence and showed no reaction. Later it was proved to be immune by inoculation with an active virus and by exposure. I am inclined to believe that the inoculation with the virus of low virulence gave this pig an immunity, but the fact that it was immune makes the interpretation of the experiment doubtful.

Experiment 3.—Object: To determine whether the virus of hog-cholera can be demonstrated in the bodies of pigeons 7 days after inoculation.

Two pigeons were each given an intravenous injection of 1 cc. of active virus and two others were each given an intracerebral injection of ½ cc. of the same virus. During the next 7 days their temperatures were slightly more irregular than those of two uninoculated pigeons kept with them. All the pigeons appeared well and the inoculated ones were chloroformed in 7 days and suspensions made from their livers, spleens, kidneys, hearts, lungs, brains, and pieces of their breast muscle. These organs and tissues were removed, using aseptic precautions, and the suspension was not filtered but was injected directly into a pig weighing 33 pounds. The animal showed no signs of illness for the next 25 days when it was inoculated with virulent virus to which it succumbed. The experiment shows that intravenous and intracerebral injections of hog-cholera virus into pigeons failed to produce disease and 7 days after inoculation the virus could not be demonstrated in their organs and tissues.

Experiment 4.—Four half grown white rats were inoculated with active, sterile, but not filtered, serum from a pig with acute hog-cholera. Two of the rats were given intraabdominal injections of 1 cc. and the other two intracerebral injections of 0.5 cc. of the serum. One of the latter rats died soon after the injection so it will not be considered further. The rats showed no effects from the inoculation and were chloroformed at the end of 7 days. At autopsy no abnormalities were noted. The lungs, hearts, spleens, kidneys, brains, and portions of the livers and muscles of the thigh were passed through a tissue grinder and suspended in an equal amount of sterile salt solution and infused for 5 hours. At the end of this time 9 cc. were injected intramuscularly into the thigh of a susceptible pig weighing 25 pounds. The temperature of this animal was normal for 3 days after which it went up to 40.3°C. on the 4th, 40.5°C. on the 5th, and 40.9°C. on the 6th day following the inoculation. The temperature then fell for 2 days after which it went to 41.5°C. During this time the pig was very sick. It was killed on the 10th day following the injection and showed moderately congested lymph nodes, hemorrhages under the capsule of the kidney, and small ulcers around the ileocecal valve. Cultures from the liver, spleen, and kidney showed no growth. The bacteria-free serum when injected into a susceptible pig produced hog-cholera. We may conclude from this experiment that virulent hog-cholera virus remains in the body of the white rat for at least 7 days.

Experiment 5.—Object: To confirm the results of Experiment 4 and to determine whether both types of injection are effective in keeping the virus in the rat's body.

Four rats weighing between 120 and 144 gm. were selected and two of these were each given an intraperitoneal injection of 1 cc. of the serum of the pig used in Experiment 4. The other two were each given an intracerebral injection of 0.5 cc. of the same serum. 7 days after inoculation the two rats that received intraabdominal injections were chloroformed and their livers, spleens, kidneys, testicles, lungs, hearts, and brains passed through a tissue grinder and a 20 per cent suspension was made in salt solution. 10 cc. of this suspension, which represented 2 gm. of tissue, produced typical acute hog-cholera when injected into

a susceptible pig. After sterilizing the instruments and grinder the two rats which had received the intracerebral inoculation were chloroformed, the same organs and tissues as in the other rats were passed through the grinder, and a 20 per cent suspension was made in salt solution. 10 cc. of this suspension also produced typical acute hog-cholera in a susceptible pig. We may conclude from this experiment that the virus of hog-cholera may be demonstrated 7 days after its injection into white rats, whether the injection is intraabdominal or intracerebral.

Experiment 6.—Object: To determine whether the virus is present in the rat 10 days after either intraperitoneal or intracerebral injection.

Two rats were each given an intraperitoneal injection of 1 cc. of the same virus used in Experiment 5, and two other rats were each given an intracerebral injection of 0.5 cc. of the same virus. They were chloroformed after 10 days and a 10 per cent suspension of their organs and tissues was made according to the method used in Experiment 5. The injection of 10 cc. of both of these suspensions into susceptible pigs produced no effect in the month's time that the animals were under observation. The susceptibility of the pigs was not tested but the other animals in the litter were susceptible, so we may conclude that in this experiment the virus of hog-cholera failed to live in the body of the white rat for 10 days after either intraperitoneal or intracerebral injection.

All the above experiments were made with a single strain of virus that had been obtained from Dr. V. A. Moore. It had been passed from pig to pig by inoculation for many generations and it is conceivable that as a result it had been modified so that it was not so susceptible to destructive action of the rat tissues as would be the natural virus.

Experiment 8.—Object: To determine whether another strain of virus recently obtained from the field would live in the rat for 7 days and also to determine in which of the abdominal organs it can be found.

Two white rats were each given an intraperitoneal injection of 1 cc. of a virus two generations removed from the natural strain; that is, the virus had been passed through two of our swine. The rats were chloroformed 7 days after the injection and with separate sterile forceps the spleens, kidneys, and portions of the livers were removed and transferred to weighed Petri dishes. The weights of the organs having been determined, they were ground with sterile sand and 5 per cent suspensions made in salt solution. After standing at room temperature for 3 hours they were injected into pigs. The data for this part of the experiment and the results are given in Table I.

The experiment indicates that 7 days after an injection into the peritoneal cavity of the white rat the virus of hog-cholera is present

in the spleen, it is not present in the liver, and the results of the experiment in as far as the kidneys are concerned are uncertain. It is probable that the pig injected with the kidney suspension suffered from a mild hog-cholera which made it immune. The experiment also shows that this nearly natural strain of hog-cholera virus will live in the rat for at least 7 days.

The above experiments clearly demonstrate that the virus of hog-cholera will live in the body of the white rat for at least 7 days. Rats are constantly associated with swine and it is possible that they

TABLE I.

Pig.		Intramuscular injection of 5 cc. of 5 per cent suspension of rat.	Result.
No.	Weight.		
	lbs.		
A	28½	Spleen.	Temperature up on 4th day. Killed 16 days after inoculation. Kidney hemorrhagic. Ulcers in colon. Filtered urine and serum produced hog-cholera in other swine.
B	28	Liver.	No effects from inoculation. Exposed to Swine A 9 days after inoculation, as a result contracting acute hog-cholera.
C	31	Kidneys.	Temperature slightly above normal on the 5th day after the inoculation but the animal did not appear sick. Subsequent inoculation with filtered urine from typical case of hog-cholera and exposure to pigs with hog-cholera were negative. Killed. No evidences of an old hog-cholera infection found.

may act as intermediate hosts, so the following experiments were made to determine whether or not they could be infected in a more natural way than by injection.

Experiment 9.—Four white rats were fed muscle from a pig that was killed 10 days after it had been injected with virus and while it was moribund. 8 days after the feeding of this infected material two of the rats were chloroformed and a suspension of their spleens, kidneys, hearts, lungs, and portions of their livers was injected into a pig weighing 30 pounds. The animal was under observation 35 days during which time its temperature and general appearance were normal. At the end of this period it received a test inoculation with hog-cholera virus and after 10 days it died, showing on autopsy typical hog-cholera lesions.

The other two rats were allowed to live a month, during which time they appeared normal and when chloroformed and autopsied no abnormalities were found.

In this experiment we were unable to demonstrate the virus of hog-cholera in the bodies of white rats 8 days after they had been fed virus in the shape of meat from a diseased pig.

Experiment 10.—Object: To repeat the attempt to infect rats by feeding material containing hog-cholera virus and in addition to determine whether the virus could be demonstrated in their urine.

Two rats, one weighing 108 and the other 145 gm., were allowed to fast for several hours and then fed pieces of spleen and kidney from a pig dying of hog-cholera. 2 days later they were transferred to an improvised metabolism cage arranged in such a way that their feces were caught in a funnel and their urine was filtered through into a sterile test-tube. Urine was collected on the morning of the 3rd, 5th, and 6th days after the inoculation and placed at once in a refrigerator at approximately 7+° C. The total amount of urine collected from the two rats was about 7 cc. and as this was contaminated with feces it contained many bacteria, but the amount was so small that it did not seem wise to attempt to filter it. The next day after the major portion of urine was collected the whole amount was injected subcutaneously into a pig weighing 51 pounds. The only result of the inoculation was a large abscess at the site of the inoculation. 13 days after the urine was injected the pig was given an intramuscular injection of hog-cholera virus which produced a typical attack with lesions characteristic of hog-cholera.

The rats fed with the virus were killed after 7 days and a 5 per cent suspension was made of their spleens of which 5 cc. were injected intramuscularly into a pig weighing 36 pounds. The animal showed no effects from the inoculation and 15 days later it was given an intramuscular injection of hog-cholera virus to which it succumbed.

In this experiment the attempt to infect white rats by feeding material containing the hog-cholera virus was unsuccessful, as was the attempt to demonstrate the virus in the urine and feces of these same rats.

Experiment 11.—Object: To determine whether a combined infection of hog-cholera virus and hog-cholera bacilli produces in the rat a condition that differs from that caused by the injection of either of these viruses alone.

We have found that at least one of our cultures of the hog-cholera bacillus will produce in the rat a febrile disease either by feeding or by subcutaneous injection. The bacillus can be found in the spleens of these animals at least 21 days after inoculation.

Six rats were weighed, marked, and allowed to fast for 6 hours and then fed meat on which was sprinkled a 24 hour bouillon culture of Hog-cholera Bacillus XII. The next day the rats were divided into lots of three each and the individuals of one lot were each given an intraabdominal injection of 1 cc. of hog-cholera virus that later was proved to be virulent. In addition three uninoculated rats were each given an intraperitoneal injection of the same virus.

The average weights of the three groups of rats show that the gain in weight of the rats that were fed the hog-cholera bacilli was not so great as that in the rats injected with the virus alone, but no visible illness was caused by the combined infection of virus and bacilli.

7 days after the injection of the virus the rats were killed and their abdominal and thoracic viscera fed to pigs weighing about 40 pounds. The animal given the viscera of the rats fed hog-cholera bacilli and injected with hog-cholera virus showed a slight rise in temperature, beginning the 2nd day after the feeding and continuing for 4 days. During this time it was eating and acting normally and for the next 7 days it seemed to be normal in every way. It was then injected with hog-cholera virus to test its susceptibility and died on the 10th day following the inoculation. On autopsy typical lesions of hog-cholera were found and the hog-cholera bacillus was isolated in pure culture from the liver and spleen.

The pig fed the viscera of the rats injected with the virus alone showed no temperature or other evidences of illness during the next 10 days and during this time it gained 15 pounds. It was inoculated with hog-cholera virus to test its susceptibility and 8 days later was chloroformed when it was very sick. Autopsy showed typical lesions of hog-cholera. Cultures were negative.

The experiment shows that a combined infection of hog-cholera bacilli and virus in the white rat caused no more evidences of disease than did the bacilli alone. The attempts to infect pigs by feeding them the viscera of rats that had been injected with hog-cholera virus were negative.

Experiment 12.—Object: To determine whether the virus could be passed from one rat to another.

Three rats weighing between 102 and 113 gm. were each given an intraperitoneal injection of 1 cc. of a serum that later was proved to be active. 7 days after the inoculation the rats were chloroformed and a 10 per cent suspension was made of their ground livers, spleens, and kidneys. 1 cc. of this suspension was injected intraperitoneally into each of three rats weighing between 95 and 98 gm. Several days later these rats were chloroformed and a 10 per cent suspension was made of their livers, spleens, and kidneys. 5 cc. of this suspension were injected intramuscularly into a pig weighing 31 pounds. This pig was apparently normal for the next 24 days. It was then inoculated with virulent virus to which it promptly succumbed, showing that the virus was not present in the second series of rats.

Effect of the Virus of Hog-Cholera on the Rat.

During these experiments the rats inoculated with the virus have been observed carefully and frequently compared with uninoculated animals kept with them. The inoculated rats do not look sick and they gain in weight the same as the controls. Their temperatures show marked fluctuations but this is true of the normal animals and no differences between the two have been detected. With sera and spleen suspensions from inoculated rats and albumin-free but virus-containing urine from hog-cholera pigs as antigen, precipitin and complement fixation reactions have been negative. In other words, the virus apparently produces no disease in the rats.

DISCUSSION AND CONCLUSIONS.

The attempts to demonstrate the virus of hog-cholera in rabbits 12 days after intravenous and intraabdominal inoculations were unsuccessful. Likewise the attempts to show that the virus might be found in the guinea pig 6 and in the pigeon 7 days after inoculation were negative. It was shown, however, that the virus can be found in the bodies of white rats for at least 7 days after either intraabdominal or intracerebral inoculations. One attempt to demonstrate it after 10 days was negative. From the fact that the rats show no evidence of illness such as loss in weight, pyrexia, or visible pathological changes, and that after either intraabdominal or intracerebral inoculation the virus is only found in the abdominal organs and possibly only in the spleen, it seems likely that it does not multiply but that in the rat tissue, particularly in the spleen, it is not destroyed so rapidly as in the organs of other animals. Careful study of the records fails to show that passing one strain of virus alternately through pigs and rats for three transfers in each species changes the virulence for swine or causes the virus to become virulent for rats.

Attempts to introduce the virus into the body of the rat by feeding virulent material and an attempt to pass the virus from one lot of rats to another were unsuccessful, so that we have evidence from the experiment that the rat does not play a part in the transmission of hog-cholera.

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