most likely hypothesis is that the serum exerted beneficial clinical effect because of an antitoxic effect. It has been shown that a "toxic factor" is associated with the epidemic and murine strains of typhus rickettsiae cultivated in yolk sac cultures and that this "toxic factor" can be neutralized specifically by convalescent serum.[3-6] This may explain why such relatively small amounts of serum as were employed in Cases 11, 13, 20 and 24 exerted a beneficial clinical effect. The hyperimmune rabbit serum used in these cases was prepared with infected yolk sac cultures.

Conclusion. 1. In 11 cases of epidemic typhus treated with hyperimmune rabbit serum, strains were isolated immediately before and again at variable periods after the administration of serum. 2. The only effect observed was that in the postserum isolations the period of incubation in the inoculated guinea pigs was usually, but not always, prolonged. 3. While the effect of the serum upon the agent may be rickettsiostatic, it is believed that a more likely explanation of the favorable clinical effect was due to its antitoxic effect.

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Propagation of Hog Cholera Virus in Rabbits.

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The literature in regard to negative results in attempts to transmit hog cholera virus to hosts other than swine, is too voluminous to be cited. The only evidence of actual transmission of the virus in other hosts has been furnished by Zichis[1] who was able to propagate the virus for 10 passages in domestic sheep. Cultivation of the virus in hog tissue outside the body of the animal has been reported by Hecke[2] and TenBroeck.[3]

Even though all evidence indicated that hog cholera would be a difficult virus to adapt to other hosts, it seemed that in view of the knowledge gained with other viruses—such as: poliomyelitis,[4] dengue fever,[5] Colorado tick fever[6,7] and rinderpest,[8-11] the problem should be reinvestigated.

For some time attempts have been made in this laboratory to adapt hog cholera virus to some host other than swine. In accordance with the experience of most previous investigators most of our attempts ended in failure. Recently, however, by using an alternating passage method similar to that reported by Baker[10] in his work with rinderpest virus in rabbits, we have obtained results indicating that hog cholera virus may be successfully passed for a number of generations in rabbits.

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TABLE I.
Summary of Hog Cholera Virus Passages in Rabbit Spleen.

Rabbits				Swine	
Rabbit passage*	No. inoculated	No. with febrile reaction	Highest temp, °F	No. injected	Results
1	7	4	104.8		
3	2	2	104.7		
5	4	3	104.6		
6	3	3	105.2	2	1 sacrificed when sick on 6th day. 1 survived with development of immunity (see text).
8	4	3	105.1	2	2† died on 18th day with typical hog cholera lesions.
9	4	3	104.8	1	1† died on 18th day with typical hog cholera lesions.
11	4	2	105.6	2	2 survived. 1 showed delayed febrile reaction.

* Refers to number of passages counting from the 6th alternating pig-to-rabbit to pig-to-rabbit passage.

† Normal swine placed in the same pen with these animals contracted hog cholera and died from the disease.

The presentation of experimental data is the subject of this report.

Material and Methods. The hog cholera strain was the stock virus used by the Lederle Laboratories in St. Joseph, Missouri* for the production of hog cholera virus vaccine and immune serum. Pigs were bought from local farmers whose premises were known to be free from hog cholera and who employed no hog cholera vaccines. The pigs were obtained immediately after weaning and care was taken to ascertain that none of the animals were exposed to hog cholera or were sick prior to purchase. Upon arrival in this laboratory, the pigs were kept in strict quarantine for

* We are indebted to Mr. Frank Cooper, of the St. Joseph plant, for his cooperation in the initial stages of this work.

FIG. 1
TEMPERATURE CURVE OF RABBIT
INJECTED WITH THE EIGHTH RABBIT SPLEEN-PASSAGE VIRUS

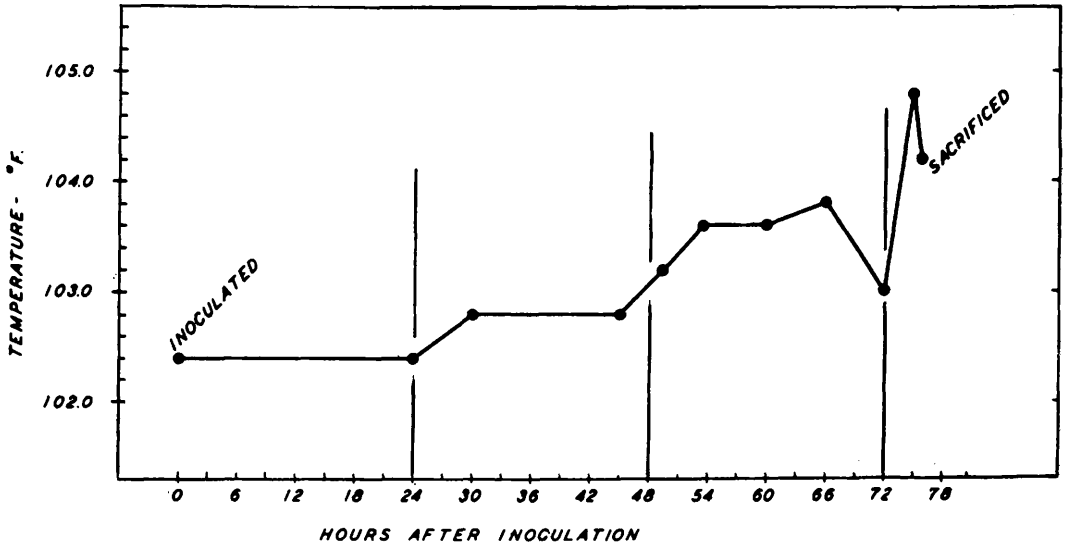
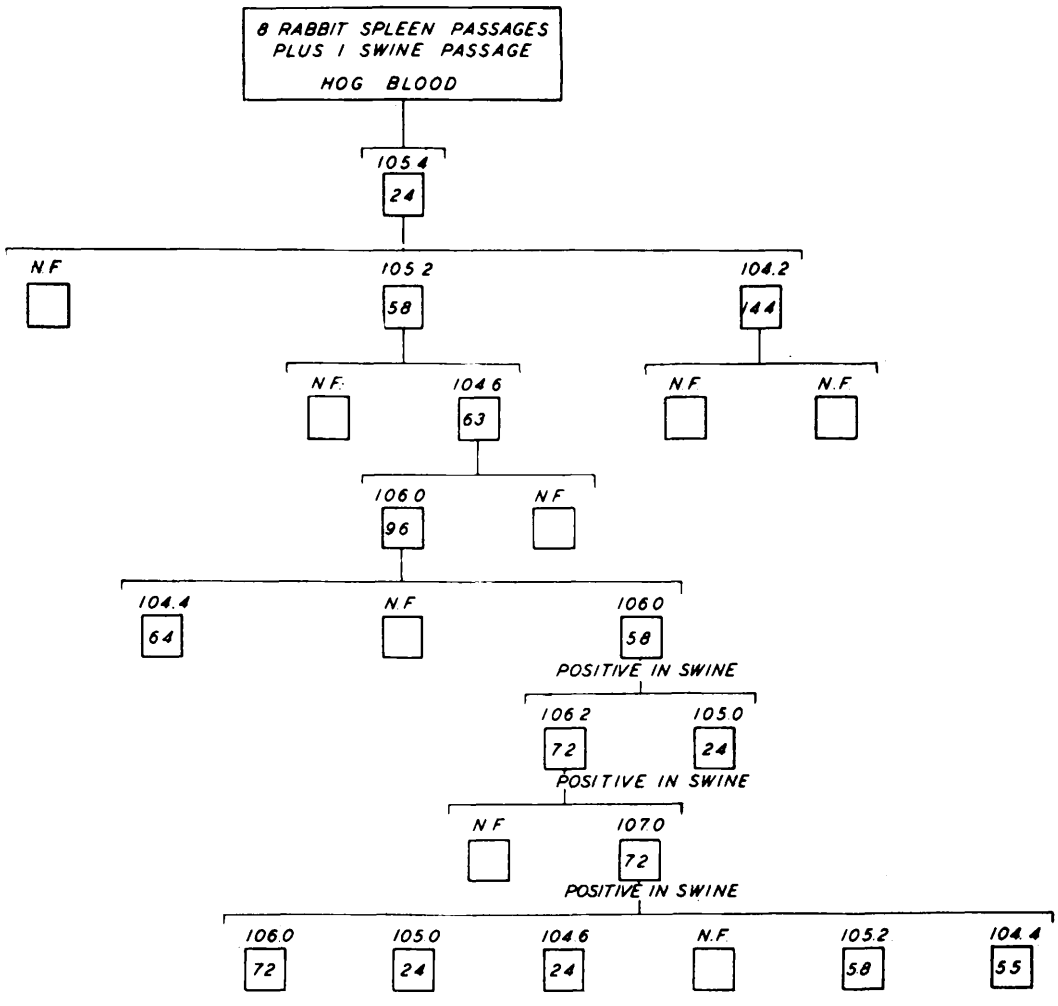


FIG 2

SUMMARY OF HOG CHOLERA VIRUS PASSAGES IN RABBIT BLOOD



- LEGEND -

54 - - FIGURES SHOW HOURS AFTER INOCULATION · BEFORE RABBITS WERE BLED

104.0 - - FIGURES SHOW TEMPERATURES AT THE TIME THE RABBITS WERE BLED

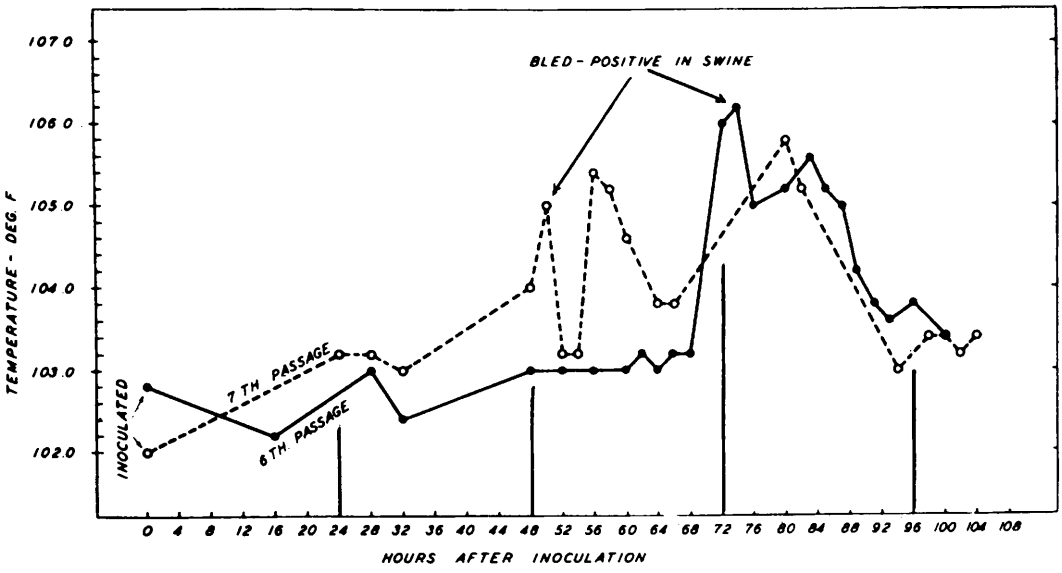
N.F. - - NO FEBRILE RESPONSE OBSERVED

2 weeks and released for experimental use only if signs of disease were absent in the entire lot. Inoculated pigs were housed in individual pens under strict isolation so that any possibility of cross-infection was ruled out. White rabbits of the "New Zealand" strain were used throughout the work. The animals were 5-8 lb. in weight and were kept individually in cages. The pigs were inoculated intramuscularly in the groin and the rabbits were inoculated in the marginal ear vein. Rectal temperatures were taken on the animals twice daily, at 8:30 a.m. and 4:30 p.m., unless otherwise stated.

Experimental. Infected hog spleen, derived from an animal which was sacrificed on the

FIG 3

TEMPERATURE CURVES OF RABBITS INJECTED WITH THE SIXTH AND SEVENTH RABBIT BLOOD-PASSAGE VIRUS



6th day after inoculation with hog cholera virus, was made into a 10% suspension in saline in a TenBroeck grinder. The suspension was centrifuged for 5 minutes at 1000 r.p.m. and 2 ml of the resulting supernatant were inoculated intravenously into a rabbit. The rabbit showed no elevation of body temperature but was sacrificed on the 3rd day after inoculation and a 10% suspension of its spleen inoculated into a pig. The latter showed fever up to 105-106°F on the 2nd, 3rd and 4th days after inoculation and was sacrificed on the 5th day. A suspension of its spleen, in turn, was injected into a rabbit. The virus was transferred by means of this technic of alternation between the hog and one or 2 rabbit passages until it had been carried through 6 intermittent rabbit passages. Out of 7 rabbits injected with the 6th passage virus, 3 showed slight elevation of temperature on the 2nd day after inoculation, and were sacrificed at that time. Their spleens were pooled and a suspension of them was injected intravenously into another group of rabbits. These animals also reacted with a slight fever and were sacrificed in turn, and the virus was thus transferred for 2 more rabbit passages. At this stage, none of the rabbits showed increased temperatures on routine checking and no virus was detected in their spleens by subinoculation of swine. It thus became obvious that the virus was either merely transferred from one rabbit to another without proliferation or that the technic of taking temperatures only twice during the daytime, following the technic used by Baker[8] in his work with rinderpest virus, was inadequate. In order to test the latter possibility, a rabbit spleen suspension, representing the 3rd rabbit passage, was injected into 3 rabbits, temperatures of which were taken every 2-3 hours throughout the 24-hour period. All 3 rabbits reacted with a febrile response on the 2nd night after inoculation. Curiously enough the elevated temperature lasted only 2-3 hours after which it started to drop. The animals were sacrificed during this febrile period and a pool of their spleens when injected into swine proved to contain hog cholera virus. By adopting the above technic of taking temperatures every 2 or 3 hours, day and night, the virus was carried for 12 continuous rabbit passages by spleen transfer. Febrile response was the only sign of infection in the rabbits. The data are summarized in Table I. A

typical temperature curve of a rabbit injected with the 8th rabbit passage spleen-virus is shown in Fig. 1.

Proof for Identity of the Virus. A pig injected with the 6th rabbit passage virus reacted with a 6-day febrile period but survived, and was subsequently placed in contact with 2 pigs which sickened, following injection with the original hog strain of Lederle blood virus. These animals were kept in an uncleaned pen with 2 normal contact control pigs. The 2 animals injected with the hog strain of the virus died and the 2 control swine showed fever after 5 and 6 days of contact respectively, and subsequently died on the 12th and 19th days. On autopsy, typical lesions of hog cholera were observed in the dead swine whereas the pig, injected with the 6th rabbit passage virus, remained asymptomatic during the entire 30-day observation period. On the 34th day this pig was injected with 2 ml of a 1:100 dilution of swine blood infected with hog cholera virus (Lederle stock strain). Normal swine injected simultaneously came down with typical symptoms of hog cholera whereas the pig which was previously injected with the 6th rabbit passage virus remained afebrile and symptom-free during the whole observation period.

Swine injected with the 8th and 9th rabbit passage spleen-virus (Table I), were autopsied after death and typical lesions of hog cholera were observed.

Propagation of the Virus in Rabbits by Injections of Infectious Blood. A pig injected with a spleen suspension of the 8th rabbit passage virus was bled when febrile on the 5th day after inoculation and 2 ml of its defibrinated blood were injected into the ear vein of a rabbit. The rabbit became febrile (105.4°F) 20 hours after inoculation at which time 20 ml of blood were taken by heart puncture. The blood was defibrinated and 2 ml were injected intravenously into each of 3 rabbits. Two of the animals became febrile and their blood was passed to another group of rabbits. By means of this technic, the virus has been propagated for 8 passages in rabbits. The data are summarized in Fig. 2. It may be observed that not all rabbits inoculated with the infected blood reacted with fever. However, in the later passages more animals showed a febrile reaction, and the temperature peaks reached higher levels and persisted for longer periods than in those rabbits inoculated with the spleen-passage virus, although in the latter case it was difficult to ascertain how long the febrile period lasted because the animals were sacrificed immediately after the supposed peaks of fever were reached.

In Fig. 3 are shown temperature curves of rabbits inoculated respectively with the 6th and 7th rabbit blood-passage virus. It may be observed that the temperature of the rabbit inoculated with the 6th passage virus started to rise between the 68th and 70th hour after inoculation, reached its peak on the 74th hour when the animal was bled and remained above normal for 16 hours. Blood of this rabbit produced a typical clinical picture of hog cholera in an inoculated pig which died on the 13th day after inoculation. On the other hand, the temperature curve of the rabbit injected with the 7th rabbit blood passage virus followed a slightly different course. A temperature of 105.0° was reached on the 50th hour after inoculation when the animal was bled. Then the temperature curve dropped rather suddenly and remained at a 103.2° level during 2 hours, returning back to a 105.4° level at the 56th hour after inoculation. During the next 26 hours the temperature was either at a subfebrile (103.8°) level or at a febrile level (105.8°), returning back to normal at the 94th hour after inoculation and remaining normal until the end of the observation period. Blood of this rabbit injected into a pig again produced a clinical picture of hog cholera. Up to now, no symptoms other than a febrile response were observed in rabbits injected with the rabbit blood-passage virus.

Discussion. The data presented above indicate that hog cholera virus has been successfully passed through several generations in rabbits by the use of an alternating passage method. However, it was apparently also essential to take temperatures of the rabbits frequently day and night during the entire observation period—and to make pas-

sage transfers promptly at the time the febrile response occurred.

It may be of interest to mention here that other investigators[10,12-16] have used the concept of alternating passages to maintain, modify, reactivate or adapt viral or rickettsial agents to other hosts or tissues.

It is conceivable that it may not be essential to use the alternating passage method to pass hog cholera virus in rabbits since in work with rinderpest virus, Nakamura and colleagues[8] were able to adapt the virus to rabbits and maintain it for 166 consecutive serial passages without going back to the original host. Edwards[17] also reported success in maintaining rinderpest virus in rabbits for a period of at least 14 months by going directly from the calf to the rabbit and by making successive intravenous transfers in rabbits every 2-7 days. On the other hand Baker[1] found it necessary to use the alternating passage method to achieve the same object. The difference in results obtained by various investigators could be due to the fact that different breeds of test animals or different strains of virus may have been employed.

Summary. Hog cholera virus has been carried for 12 consecutive passages in rabbits by using infected rabbit spleen as transfer material. Starting from the 8th rabbit spleen passage, the virus was passed back to a pig for one passage and was then carried for 8 further passages in rabbits by using infected rabbit blood as transfer material. Aside from a febrile response, no other symptoms were observed in inoculated rabbits.

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Serial Passage of Hog Cholera Virus in Rabbits.

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Successful adaptation of rinderpest virus of cattle to the rabbit[1] naturally led to similar experiments with hog cholera virus since both of these agents are quite specific for their natural hosts. In the original work on rinderpest alternate transfer of virus from its natural host to the rabbit and then back to the calf was the procedure that made for success. With this alternating technic virulence of the rinderpest virus for the rabbit increased progressively and, following this increase, evidence of disease was found in the rabbit. The results of the experiments on hog cholera virus follow.

Virus Strains Used. Strain A was obtained through the courtesy of Dr. TenBroeck[2] and, as reported by him, it was one used for vaccination in the central portion of the United States. In his work this strain was grown in the presence of fresh minced swine testicle on the chorioallantoic membrane of embryonated eggs for 13 transfers, followed by an equal number of transfers on agar, making 26 transfers in all. From the last transfer lyophilized material was kept from April 5, 1941 until March 24, 1945 when it was injected into a pig. For further preservation spleen from this animal was kept frozen with

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