INFECTION OF GUINEA PIGS BY APPLICATION OF VIRUS OF LYMPHOCYTIC CHORIOMENINGITIS TO THEIR NORMAL SKINS

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The virus of lymphocytic choriomeningitis has been found capable of producing infection in certain laboratory animals when inoculated by a variety of routes. These include the common methods of inoculation, such as the intracerebral (1), subcutaneous (2), intraperitoneal (3), intravenous (3), and intralingual (5). Infection is produced also when virus is applied to the mucous membranes of the nose (3), vagina (4), urethra (4), and rectum (5). Furthermore, it has been shown by Findlay and Stern (6) that, when this virus was rubbed on the lightly scarified skins of mice they did not exhibit apparent infection but the virus could be recovered from their spleens and kidneys. These investigators also showed that, when the virus was rubbed (6) on the lightly scarified skins of two rhesus monkeys, one showed a slight febrile reaction. Shaughnessy and Milzer (7) demonstrated that guinea pigs exhibited the typical picture of the disease when the virus was placed on skin so lightly scarified that blood was not drawn. In the experiments presented here, a preliminary report of which has been published (8), we wish to present more detailed evidence that the virus may penetrate the normal, unscarified skin of guinea pigs.

The possibility that passage of some viruses through normal skin may occur has been tacitly considered by some observers. Thus, it is common practice to administer antirabic vaccine to persons whose hands have been licked or otherwise contaminated with the saliva of rabid dogs. However, we were not aware of any experiments bearing on this question until after our preliminary report had been published. We then learned of the experiments of Bauer and Hudson (9) on the passage of yellow fever virus through the skin. Their methods differed from ours in several important respects. Bauer and Hudson rubbed their virus preparations on the skin while ours were merely deposited on the skin. Furthermore, they made no attempts to exclude the possibility of the monkeys scratching the virus into the skin, or transferring it to mucous membranes. In our experiments the virus was...
placed on an inaccessible region of the body and covered in such a manner as to protect against abrasion of the skin or transfer of the virus by other routes.

**Methods and Materials**

*Virus.*—The W. E. strain of lymphocytic choriomeningitis virus, isolated by Rivers and Scott (2) in 1935, was employed in these studies. In this laboratory the virus was maintained by serial intracerebral passages in guinea pigs. The brains were removed aseptically from the animals immediately following death. The virulence of the virus was such that, when 0.25 cc. of a 1 per cent suspension of an infected guinea pig brain was injected intracerebrally into guinea pigs, they showed signs of the disease (rise in temperature) within 24 to 48 hours, and died 6 to 8 days following inoculation.

In these experiments the virus to which the animals were exposed, consisted of a 10 per cent suspension of a freshly obtained, infected guinea pig brain. The dilutions were made with a heart infusion broth, except in some of the later experiments in which either distilled water or buffered saline solution was used. In each case the suspension was tested for sterility by culturing in heart infusion broth. To establish the virulence of the virus in each experiment, guinea pigs were inoculated intracerebrally with 0.25 cc. of the 10 per cent virus suspension, simultaneously with the experimental animals. The virus was considered of standard virulence if the intracerebrally injected animals showed evidences of the disease within 24 to 48 hours and died in 6 to 8 days.

The first signs of infection shown by the guinea pigs following intracerebral inoculation were a rise in body temperature to about 104°F., ruffled hair, refusal of feed, and the assumption of a humped position. Later they showed labored breathing, excessive salivation, and a marked loss of weight. As a rule, at death each had lost about one-third of its body weight. Some showed a severe seropurulent conjunctivitis. In the guinea pigs that were infected through the skin the disease was similar except that the incubation period was much longer. The first signs usually appeared 5 to 6 days after exposure, and death rarely occurred before the 10th day.

*Animals.*—In the actual studies of infection through the skin, guinea pigs were employed, while albino Swiss mice were used as an aid to the identification of the virus causing death in the experimental guinea pigs.

The guinea pigs each weighed between 275 and 450 gm. and represented a variety of stocks which were purchased from several commercial breeders in the vicinity of Chicago. They did not exhibit any immunity, and those inoculated intracerebrally showed uniform susceptibility, indicating that they were neither infected by, nor carriers of, the virus.

The mice were between 6 and 8 weeks of age and were obtained from a healthy stock. Representative animals were tested by the intracerebral inoculation of 0.03 cc. of either 2 per cent starch solution (10), or bouillon broth (3), and were found not to be carriers of the virus. In addition, they did not possess any immunity as shown by their uniform susceptibility. The mice as well as the guinea pigs were examined and found to be free of ectoparasites.

*Methods of Infecting the Animals.*—The guinea pigs were exposed by placing from 0.5 cc. to 1.2 cc. of the virus suspension on the normal skin of the lateral dorsolumbar
The hairs were spread apart by means of the tip of a glass Luer syringe. At the same time the desired amount of the suspension was carefully deposited on the skin from the same syringe without even touching the syringe to the skin.

In order to minimize the opportunities for chance infection by the many possible means resulting from such an exposure, screw-top screen capsules, of the type devised by Jellison and Philip (11), were employed. The hair of the animals was not cut and the capsules were attached to the lateral dorsolumbar position by means of an adhesive tape girdle. The virus was placed on the skin, as described, through the opening of the capsules, the covers of which were then replaced.

It was thought possible that the wearing of the capsules might in some way facilitate infection, such as by irritation of the skin. As a control, guinea pigs were exposed under the same conditions but without the attachment of the screen capsules.

The skin of the animals was examined with a hand lens for abrasions, both before the virus was placed on it and after infection had occurred, but none could be found. The suspension dried, slightly matting the hair, but the skin in every case was free of any scaly, dried material which might abrade the skin and thus provide a portal of entry for the virus.

The mice were given a light ether anesthesia and each was injected intracerebrally, with 0.03 cc. of a 1 per cent dilution of a brain from a test guinea pig.

Methods of Observing and Caring for the Animals.—The guinea pigs were kept in individual cages of metal construction with wire mesh covers. The temperature, weight, and condition of each animal were recorded daily during the 20 days or more that they were kept under observation. To prevent cross infection, the cages were spaced about 10 inches apart on the shelves, and before and after observation of each animal, the operator thoroughly washed his gloved hands in a 20 per cent solution of cresol compound, followed by washing with soap and water. Temperatures of each animal were taken with individual thermometers which were kept between observations in individual bottles containing 10 per cent formalin. Just before using a thermometer, it was held under running tap water to wash off the formalin. The scale pan was covered with heavy wrapping paper which was changed before weighing each guinea pig.

The entire procedure was controlled by placing a cage containing a guinea pig, which was not exposed to the virus by application of the virus to its skin or by any other artificial method, between cages containing the exposed animals. The control animals were subjected to the same methods of observation. In addition, an experiment was designed to rule out cross infection by exposing groups of guinea pigs in rooms other than the one containing the intracerebrally inoculated virus control animal. One group was observed and cared for by a person who had no contact with the intracerebrally injected guinea pig or any other source of the virus. The second group was cared for by another person who also observed the third group, containing the intracerebral control. This individual made an entire change of laboratory clothing between the observation of the two groups. One group of animals was observed in the morning and the other in the afternoon. The procedure was further controlled by spreading the virus suspension on the feed and litter in the cages of selected animals.

The mice were kept in metal cages with perforated covers. Generally, 3 mice were injected with a single specimen and these were kept in one cage. In caring for these animals precautions were taken to avoid any possibility of cross infection.
Throughout the experiment, both the guinea pigs and the mice were maintained on a balanced ration and were kept in rooms which ranged in temperature from about 70-74°F. in winter and from about 75-82°F. in summer.

Cultural Studies.—The brain and heart blood of each animal that died in the course of the experiment were cultured aerobically and in many selected cases anaerobically. When indicated, cultures were made also on other organs. Whenever a bacterial contaminant was encountered, attempts were made to identify the organism and its pathogenicity was studied by animal inoculation.

Identification of the Virus.—Two methods were employed for the identification of the virus in the experimental animals. The neutralization test with a specific immune serum, as described by Baird and Rivers (12), was used. However, it was not considered necessary to make this test except in a few selected cases. The presence of the virus in most of the animals was established with the aid of the mouse test. This test, while not entirely specific, is believed to be sufficiently accurate in experiments of this kind where a known virus is being used. The test consisted of inoculating from 3 to 6 mice intracerebrally with either the brain or the blood from the infected animal. The brain was emulsified in a 1–10 dilution in a heart infusion broth, and the blood was inoculated undiluted. After inoculation, the mice were observed for characteristic symptoms.

Lymphocytic choriomeningitis, induced in mice by intracerebral inoculation of the virus, causes characteristic symptoms which are considered pathognomonic (3). Briefly the symptoms are as follows: At the onset of the disease the animals become very sluggish and quiet, show ruffled hair, and refuse food. If a mouse is lifted by the tail, tremors are noted in the front and hind legs. These tremors become more pronounced as the disease progresses, and often if the animal is lifted by the tail during the later stages of the disease, it goes into convulsions, and many die. 2 to 3 days after the onset the animals become badly emaciated, very weak, and die. In many cases the mice also develop purulent conjunctivitis. Death is invariably preceded by spastic convulsions which produce a characteristic picture in the animals after death. Their backs are humped and the hind legs and tails are rigidly extended backward during rigor morris. The first symptoms usually appear on the 7th day after infection and death almost always follows 2 to 3 days later.

EXPERIMENTAL

During the course of this study, many factors arose which required investigation. It was necessary to determine whether or not any of the following experimental conditions in any manner facilitated infection: (1) bleeding from the heart; (2) attachment of the capsules; (3) potency of the virus suspension; and (4) the use of heart infusion broth as diluent. Each of these factors was investigated and will be briefly described.

Experiment 1.—Eighteen guinea pigs were employed in this experiment. These animals were used in another study on the virus of lymphocytic choriomeningitis during the course of which it was discovered that they became infected as a result of placing the virus suspension on their skins. Hence they were included in this report. For the other study it was necessary to obtain daily 0.1 cc. of blood by cardiac puncture from each animal.
Eight guinea pigs were exposed by placing 0.5 cc. of a virus suspension on their skins. Capsules were attached to 5 of this number. Each of 5 animals was exposed by spreading 2 cc. of the suspension on the feed and litter in their respective cages. The potency of the virus was established by injecting 2 guinea pigs intracerebrally. The remaining 3 animals were not exposed to the virus and constituted the control on the technique. The virus suspension was cultured and was found not to be contaminated by bacteria.

Four of the guinea pigs to whose backs the capsules were attached, and the 3 without the capsules showed elevation of temperatures (average 104.5°F.) and a marked loss of body weight in 5 to 7 days after the virus was placed on their skins. They continued to show these manifestations of the disease and died in 7 to 13 days. At the time of death, each animal had lost about one-fourth of its weight. The clinical symptoms shown were typical of those observed in experimental infection of guinea pigs with the virus of lymphocytic choriomeningitis.

The cultural examinations showed that the brain and the heart blood in each case were free of bacterial contamination. The presence of the virus in each guinea pig was established by injecting 4 mice intracerebrally: 2 with blood and 2 with brain tissue. In every case the mice died showing typical symptoms of the experimental disease. The heart blood and brain of the mice were studied culturally and with the exception of a few air contaminants, were free from bacteria.

The remaining guinea pig to which a capsule was attached, died 15 days after exposure without showing any elevation of temperature or excessive loss of weight. Upon culturing, the heart blood showed bacterial contamination but the brain did not. The organism was tested and found not to be pathogenic for guinea pigs. The virus could not be isolated from either the blood or the brain. This animal apparently did not become infected with the virus and probably died of some other cause.

The 2 guinea pigs that were injected intracerebrally had fevers of 105.2°F. and 105.3°F. in 24 hours and died in 6 and 7 days, respectively. These animals showed symptoms identical with the ones shown by the animals that were infected by applying the virus to their skins, except that the course of the disease was much more rapid. The virus was found in these guinea pigs, both in the blood and the brain. The 5 animals that were exposed by spreading the virus suspension on the feed and the litter in the cages, and the 2 control animals did not show any evidence of infection, and the virus could not be isolated from their blood.

The results in the preceding experiment showed that the virus of lymphocytic choriomeningitis infected guinea pigs when it was placed on their intact skins. However, as was explained, these animals were subjected to daily bleedings from the heart, and it was thought that possibly this operation weakened the animals and rendered them more susceptible to the infection. For this reason, Experiment 2 was undertaken.

Experiment 2.—Six guinea pigs were prepared with capsules and 0.5 cc. of a virus suspension was placed on the skin of each. To determine the virulence of the virus a guinea pig was inoculated intracerebrally. Another animal was not experimentally exposed to the virus and was employed as the control. Cultural studies of the virus suspension were negative. None of the animals in this experiment were bled from the heart.
LYMPHOCYTIC CHORIOMENINGITIS VIRUS APPLIED TO SKIN

Of the 6 animals to which the capsules were attached, only 1 became infected and died. This guinea pig did not show typical symptoms of the disease. It died on the 6th day after exposure without showing any change in temperature and only a slight loss in body weight. The cultural tests showed that the heart blood and the brain were bacteriologically sterile, and the presence of the virus was established in the blood and the brain by mouse inoculation. These results indicate that although the animal did not show the usual symptoms of the disease, it was infected with the virus and possibly died as a result of such an infection. The remaining 5 guinea pigs did not become infected. They were kept under observation for 27 days and throughout this time their temperatures remained normal and each gained weight.

The intracerebrally inoculated guinea pig developed a fever of 104.0°F. 6 days after inoculation and died in 12 days. At the time of death, the animal had lost only 36 gm. of its weight. The heart blood and the brain were free of bacterial contamination, and the virus was present in the brain. The prolonged incubation period and the relatively mild form of the disease shown by this animal, indicated that the potency of the virus employed was below standard. The control guinea pig did not become infected.

32 days following the exposure, 3 guinea pigs that did not become infected as a result of placing the virus on their skins, were inoculated intracerebrally with 0.25 cc. of 0.1 per cent suspension of a virus of standard potency. These animals developed typical lymphocytic choriomeningitis, and died within 8 days. These results showed that the animals were susceptible to the virus and that they did not become immunized as a result of placing the virus suspension on their skins.

In the above experiment two factors were involved which were not present in Experiment 1. The guinea pigs were not bled from the heart and the potency of the virus suspension was below standard. For this reason the succeeding experiment was designed to determine which of these factors was the controlling one.

**Experiment 3.**—In this experiment 18 guinea pigs were used. Capsules were attached to 4 and 3 were used without capsules. Three of the animals with the capsules were bled daily from the heart as in Experiment 1. The remaining guinea pig with the capsule and the ones without the capsules were not bled. They were employed to control the effects of the bleedings, and the attachment of the capsules. Each guinea pig in this group was exposed by placing 1 cc. of a virus suspension on its skin.

Eight guinea pigs were brought in contact with the virus by spreading 2 cc. of the suspension on the feed and the litter in the cage of each. The virulence of the virus was established by inoculating a guinea pig intracerebrally. The technique of the experiment was controlled by incorporating in it 3 animals that were not exposed to the virus. The virus suspension was cultured and found to be negative for bacteria.

Two of the guinea pigs with capsules, that were bled, developed a typical form of the disease and died. The remaining animal in this group did not become infected. All the others to whose skins the virus was applied, became infected and died. The guinea pigs to whose feed and bedding the virus was added, as well as the unexposed control animals, did not show any clinical signs of infection and gained in weight. The animal that was injected intracerebrally showed a fever of 104.4°F. 30 hours after injection, and died in 4
days. In the case of each guinea pig that died, the cultural studies of the heart blood and the brain were bacteriologically negative, and the presence of the virus was established by mouse inoculation.

21 days after the guinea pigs were placed in contact with the virus, 5 that did not become infected were tested for immunity by intracerebral inoculation with a virus of standard potency. Of these 4 were taken from the group to whose feed and bedding the virus suspension was added and the other was taken from the group that was bled and had capsules attached. All these animals developed the usual symptoms of the disease and died, showing that they did not become immune to the virus as a result of the above methods of exposure.

The next experiment was designed to secure additional proof that the animals did not become infected as a result of cross contamination.

Experiment 4.—The animals were divided into three groups of 4. Each group was placed in a separate room and was observed and cared for as explained. In each group, capsules were attached to 3 and not to the other. 0.5 cc. of a virus suspension was placed on the skins of 2 animals with capsules, and on the skin of the one without the capsule. The remaining animal with the capsule, in each group, was not exposed to the virus and served as the control on the technique. The potency of the virus was established by inoculating a guinea pig intracerebrally. This animal was kept with group 3. The virus suspension was cultured and was found to be free of bacterial contamination.

Of the guinea pigs with the capsules, 2 in group 1 and 1 in each of groups 2 and 3 became infected and died. In each case, the heart blood and the brain were free of bacterial contamination, and the virus was shown to be present in the brain by means of the mouse test. The guinea pig that was injected intracerebrally developed the disease after a prolonged incubation period. 5 days after inoculation it had a fever of 105.0°F. and died on the 9th day. The cultural studies did not reveal any bacterial contamination and the virus was found in the brain by the mouse test.

In each group the guinea pigs without the capsules and the ones in groups 2 and 3 with the capsules on whose skins the virus suspension was placed, did not show any signs of infection. The unexposed controls also remained in good health throughout the experiment as evidenced by definite gains in body weight.

As shown by the above results, only guinea pigs to which the capsules were attached, became infected with the virus. Although the potency of the virus in this experiment was below standard, the results seemed to indicate that the attachment of the capsules, possibly through irritation of the skin, was responsible for the infections. Experiment 5 was undertaken to determine if the infection of the animals was brought about by the attached capsules.

Experiment 5.—This experiment was divided into two parts.

1.—1.2 cc. of a virus suspension was placed on the skins of each of 7 guinea pigs. Of this number, 3 were prepared with capsules. One was inoculated intracerebrally and another was not exposed to the virus and constituted the control.
The 3 guinea pigs with the capsules and 3 without the capsules became infected, exhibited the characteristic symptoms of the disease, and died. In the case of each animal, the cultural studies were negative and the presence of the virus was established in the brain by mouse inoculation. In addition, the virus from the brain of a guinea pig in each group was identified by neutralization test with a specific immune serum. The remaining animal in the group without the capsules did not become infected.

The intracerebrally inoculated guinea pig showed a fever of 104.6°F. 24 hours after inoculation and died in 7 days. At death it had lost almost one-half of its body weight. The control animal and the remaining one without the screen did not become infected.

II.—Eight guinea pigs were exposed by placing, on the skin of each, 1.2 cc. of a virus suspension. Capsules were attached to 4 of them. To establish the potency of the suspension, a guinea pig was injected intracerebrally. One animal was used as the unexposed control. The inoculum was bacteriologically negative.

Four of the guinea pigs with the capsules, and 3 without the capsules became infected and died. Each of these developed typical symptoms of the disease as characterized by high temperature and loss of weight. The presence of the virus was demonstrated in the brain of each by the inoculation of mice. The remaining animal without the capsule did not show any evidence of infection.

The guinea pig that was injected intracerebrally developed a temperature of 103.8°F. 24 hours following injection. It ran the typical course of the disease and died in 6 days. The heart blood and the brain were culturally negative for bacteria and the mouse test for the virus was positive. The control guinea pig did not become infected.

It has been shown repeatedly that a virus will pass through clay filters when suspended in a meat infusion broth, whereas it will not when suspended in saline. It is also well known that histamine-like substances alter the permeability of the skin. In the foregoing experiments, heart infusion broth was used as the diluent. In view of these facts, Experiment 6 was designed to investigate whether or not the broth diluent altered the characteristics of the virus or of the skin in such a manner as to enable infection to take place through the intact skin. This study was carried out by determining the infectivity of the virus suspended in heart infusion broth, buffered normal saline, and distilled water, respectively.

Experiment 6.—A virus-infected guinea pig brain was thoroughly ground and portions of this were used to prepare each suspension. With each diluent a 10 per cent suspension by weight was made. Each suspension was tested and found to be free of bacterial contamination.

Four guinea pigs were exposed to each suspension, and of these 2 were prepared with capsules. The dose to which they were exposed in every case was 1.2 cc. The potency of each suspension was established by inoculating a guinea pig intracerebrally. Four animals were not exposed experimentally to the virus and were employed as controls.

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1 Supplied through the courtesy of Dr. T. M. Rivers of The Rockefeller Institute for Medical Research.
Of the 12 guinea pigs on whose skins the virus was placed, 7 became infected and died. The number that died as the result of exposure to each suspension was as follows: 4 in the broth series; 1 in the saline, without capsule; and 2 in the distilled water, 1 with and 1 without the capsule. The remaining 5 animals did not develop any signs of the disease. Those intracerebrally inoculated developed the disease and died. None of the controls became infected.

The cultural studies, with the exception of a specimen from 1 animal, which showed a non-pathogenic contaminant, were negative. The presence of the virus in the brain of each animal was established by mouse inoculation. In addition, the virus from the brains of guinea pigs 2, 8, and 13, which received virus on their skins in the form of suspensions made in broth, buffered saline, and distilled water, respectively, and from the brain of guinea pig 16, intracerebral control on the broth suspension, was identified by neutralization tests with specific antisera.2

DISCUSSION OF RESULTS

In the course of this study several factors were encountered which required investigation. At the conclusion of Experiment 1, the results indicated that the virus infected guinea pigs when it was placed on their normal skins. However, these animals were bled from the heart daily and it was thought that possibly this operation impaired their resistance and rendered them more susceptible to the infection. When Experiment 2 was completed, in which only 1 animal out of 6 became infected, the suspicion was increased that the bleeding was responsible for the infection. But in this instance the potency of the virus was also below standard. For this reason it was necessary to reinvestigate both of these factors.

In Experiment 3, guinea pigs were bled from the heart as in Experiment 1, and others were exposed with and without capsules. These results showed that all the animals were equally susceptible, indicating that the bleeding was not a factor which facilitated infection. The virus suspension in this case was of standard potency.

Only the animals to whose bodies the capsules were attached became infected in Experiment 4. Such results lead one to think that possibly during the course of attaching and wearing the capsule a certain amount of irritation might have been encountered which provided a portal of entry for the virus. If this was so, the irritation was not definite enough to be visible by the aid of a hand lens. However, here again the potency of the virus was below par.

In Experiment 5 it was demonstrated that the attachment of the capsule did not play a part in infecting the animals through their skins. As shown

2 Supplied through the kindness of Dr. T. M. Rivers, The Rockefeller Institute for Medical Research, and Mr. A. Milzer, Michael Reese Hospital, Chicago.
by the results in this experiment, animals with and without capsules were infected to the same degree. The virus used in this experiment was of a standard potency.

The results of these experiments indicate that neither the bleeding from the heart, nor the attachment of the capsules played a determining part in rendering the guinea pigs more susceptible to the virus infections. In each of the experiments where the infectivity was not consistent, the potency of the virus was below standard. On the other hand, in experiments in which the virus was of standard potency, guinea pigs exposed with and without the capsules were infected to a similar degree. In view of these findings it would appear that the potency of the virus suspension was the controlling factor in the infection of the animals through the intact skin.

In Experiment 6, heart infusion broth, buffered saline solution, and distilled water were employed as diluents in which to suspend the virus. All the guinea pigs exposed to the broth suspension, 1 of 4 exposed to the saline, and 2 to the water, became infected and died. Although a small number of animals was used in this experiment, the results suggested that the virus suspended in broth did possess a higher infectivity. The lower infectivity of the virus suspensions in water and saline may have been due to the lessened viability of the virus in these menstrua. The fact that some of the animals receiving water and saline suspensions of the virus on their skins became infected indicates that broth was not the controlling factor in causing infection by rendering the skin more permeable or by causing greater infectivity of the virus.

Rigid procedures were instituted to safeguard against cross infection through contact. The inclusion of animals that were not exposed artificially to the virus in each experiment, and their subjection to the same methods of observation, were considered to be effective means of controlling this factor. In addition, Experiment 4 was designed to establish whether or not the guinea pigs were contracting the disease as a result of cross infection. The animals in this experiment were divided into three groups, each of which was kept in a separate room. The intracerebrally injected animal was kept with group 3. As explained, each group was observed and cared for in such a manner as to make cross infection from the intracerebrally inoculated animal or from any other source of the virus quite unlikely. The results of this experiment showed that cross infection was not a factor because guinea pigs in each of the groups contracted the disease. The unexposed control animals supplied further evidence that cross infection was not involved, as none of them showed any signs of infection, nor was it possible to demonstrate the presence of the virus in their blood.
These combined results strongly indicate that the guinea pigs did not become infected as a result of cross contamination.

Attempts to infect guinea pigs by spreading a potent virus suspension on their feed and litter in the cages resulted in a failure. As shown in Experiments 1 and 3 none of these animals became infected and it was not possible either to isolate the virus from their blood or to demonstrate any immunity. It is difficult to explain why these animals did not become infected; however, it is thought that possibly this was due to an inability of the virus to infect through the gastro-intestinal tract, to a less intimate contact of the virus with the skin, and to attenuation of the virus through exposure to drying and other physical factors. Whatever the explanation, the results are in general agreement with those reported by Armstrong and Lillie (1) and Traub (13).

The guinea pigs that became infected as a result of placing the virus on their skins showed an average incubation period of 5 days and died between 8 and 12 days. In contrast, the incubation period in those inoculated intracerebrally was from 24 to 48 hours and death resulted in 5 to 7 days. The mice became sick in about 5 days and died between 6 and 10 days. The mortality rate of the guinea pigs and the mice that developed clinical signs of the infection was 100 per cent. These results are not in agreement with those reported by other investigators (3, 6) who described the recovery of guinea pigs that showed clinical signs of infection. However, we believe

### TABLE I

**Summary of Results**

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<th>Experiment No.</th>
<th>Exposed with screen</th>
<th>Exposed without screen</th>
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<th>Unexposed controls</th>
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No. dead from virus infection............ 22 16 None 10 None
Total No. of guinea pigs used............ 34 23 13 10 17
Total mortality............ 64.7 per cent 69.5 per cent 0 per cent 100 per cent 0 per cent

*Numerator = number of guinea pigs dead.
Denominator = number of guinea pigs used.
that these apparent discrepancies can be reconciled. In some of our earlier, unpublished work we used a strain of virus supplied by Dr. Armstrong of the National Institute of Health. This strain in our hands produced a mild infection in guinea pigs with a long incubation period and recovery from infection in some of the animals. On the other hand the W. E. strain has been consistently virulent, showing relatively short incubation periods and practically 100 per cent fatality. Since this strain of virus has been propagated in guinea pigs in our laboratory for many generations, its virulence for these animals has probably been enhanced. At any rate, it should be emphasized that the high mortality in these experiments probably was due to the fact that they were conducted with a highly virulent strain of virus.

That we were dealing with the specific virus of lymphocytic choriomeningitis was demonstrated by the characteristic symptoms produced in both guinea pigs and mice and by the neutralization of the virus with specific immune sera.

SUMMARY

As shown in Table I, 97 guinea pigs were used in this study. Fifty-seven were exposed by placing a virus suspension on their normal skins. Of this number, 34 had screw-top capsules attached to them. Thirteen were exposed by spreading the virus suspension on their feed and cage litter. Ten were inoculated intracerebrally to establish the potency of the virus. The remaining 17 were not exposed artificially to the virus and were employed as controls to detect cross infection.

Twenty-two guinea pigs, to which capsules were attached, died as a result of virus infection; 1 died of unknown causes and 11 survived without showing any clinical signs of the infection. Sixteen of the animals without the capsules died of virus infection and 7 did not become infected. The 10 guinea pigs that were inoculated intracerebrally became infected and died. None of the animals that were exposed by spreading the virus on the feed and the litter in the cages, or those used as unexposed controls, developed any clinical signs of infection with lymphocytic choriomeningitis.

It is realized that minute abrasions, not visible with a hand lens, may have been present in the skins of these guinea pigs. However, any condition of this nature would be a factor encountered in any normal skin.

In view of these facts, it is believed that these results indicate that the virus of lymphocytic choriomeningitis may infect guinea pigs through the normal, apparently intact, skin.
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