Fig. 1-H taken on the 104th day, and Fig. 1-I the eighth biopsy taken on the 227th day.

Summary and Conclusions. 1. The procedure of peritoneoscopy, as described by Ruddock, is a satisfactory means of inspecting the liver and of securing liver biopsy specimens in the dog. The examination can be accomplished without pain to the animal using local anesthesia, after morphine sedation. Repeated examinations with inspection and biopsy of the liver can be made over a period of time in the same dog.

2. A series of 145 peritoneoscopic examinations in a group of 18 dogs with toxic or dietary liver damage was carried out in a 10-month period. Eight dogs in this group were subjected to peritoneoscopy 10 or more times. A second similar group of 9 dogs were subjected to 36 such examinations in a 2-month period. In each instance multiple liver biopsies were taken.

3. No fatalities attributable to this procedure have occurred even when performed on dogs exhibiting severe liver damage.

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The Intracranial Toxicity of Influenza Virus for Mice.*

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A study of the properties of the influenza A virus, designated IC strain, isolated during the 1943 epidemic at Iowa City, involved the injection of the virus into the brains of Swiss mice to determine its neurotropic qualities. Two strains of influenza virus are known to be neurotropic, one reported by Stuart-Harris[1] and the other by Francis and Moore.[2] These strains propagated in the mouse brain and after several passages produced convulsions and death. Henle and Henle[3] working with non-neurotropic strains showed that active virus injected in sufficient concentrations produced convulsions but they did not obtain evidence of virus propagation. Although the investigation herein reported was completed before the publication of Henle and Henle[3] this work will serve as a confirmation of their experiment. Evans and Rickard[4] found that the injection of type A and type B influenza virus in the rabbit's eye produced corneal opacity without multiplication of the virus. Rake and Jones[5] using viruses of lymphogranuloma venereum, mouse pneumonitis, and meningo-pneumonitis have shown that these viruses exert a toxic effect.

All virus and antibody titers given in this report were determined by the Hirst[6] hemagglutination test as modified by Salk.[7]

Preliminary experiments indicated that allantoic fluid containing the IC strain of influenza virus, injected in 0.03 ml doses intracranially in mice, resulted in convulsions in a majority of cases after 48 hours, with death occurring on the second or third day, rarely as late as the fifth. The mortality rate was 50-100% depending upon the concentration of the virus. That the convulsions were not caused by bacterial infection was demonstrated

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1 Stuart-Harris, C. H., *Lancet*, 1939, **1**, 497.

2 Francis, T., Jr., and Moore, A. E., *J. Exp. Med.*, 1940, **72**, 717.

3 Henle, G., and Henle, W., *Science*, 1944, **100**, 410.

4 Evans, C. A., and Rickard, E. R., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 73.

5 Rake, G., and Jones, H. T., *J. Exp. Med.*, 1944, **79**, 463.

6 Hirst, G. K., *J. Exp. Med.*, 1942, **75**, 49.

7 Salk, J. E., *J. Immunol.*, 1944, **49**, 87.

TABLE I.
The Effect of Dilution and Heat Inactivation on the Convulsive Factor.

Material injected	No. of mice injected	No. developing convulsions
Undiluted IC virus in allantoic fluid	10	8
Same, heated at 56°C	10	0
IC virus in allantoic fluid diluted 1:10	10	0
Undiluted normal allantoic fluid	10	0

by failure to isolate bacteria from the brain by both aerobic and anaerobic cultural methods. Although the virus could be recovered from the brain at the time of convulsions, it was present in very small quantities. However, all attempts to passage from brain to brain of mice were uniformly unsuccessful. In no case could the virus be recovered from the second passage by inoculation of brain material into the allantoic sac of chick embryos.

Since there was no evidence of propagation on the part of the IC influenza strain in the mouse brain, it seemed reasonable to think that other non-neurotropic strains would give the same results. Therefore, an experiment using the PR8 influenza virus was tried. The same symptoms and results were produced. Mouse brains removed 48 hours after intracranial inoculation and at the time of convulsions, were injected into the allantoic sac of embryonated eggs. The virus was isolated in some, but not all instances, again giving evidence that little, if any, propagation took place in the mouse brain.

The Lee strain of influenza B virus was tested in the same manner and no difficulty was encountered in duplicating the above results. No attempt was made to recover this virus from the brain at the time of convulsions.

Since there was no evidence of propagation of virus in the brain it was decided to determine if an inactive virus would give these neurological symptoms. Also included in this experiment was the effect of dilution on the convulsive factor. A control of normal allantoic fluid was added. The IC strain was grown in the allantoic sac of embryonated eggs. The fluid was removed and pooled from several embryos after 48 hours incubation at 37°C and was found to have a hemagglutination titer of 1:3,200. Intracranial injections of 0.03 ml of undiluted infected allantoic fluid were made into the brains of month-old Swiss mice and a similar group was inoculated with an equal volume of 1:10 dilution of the virus-containing material. Another group of mice received this volume of undiluted virus-containing fluid which had been heated at 56°C for 45 minutes. The control mice were given undiluted normal allantoic fluid in the same manner. The results are shown in Table I.

Table I shows that the undiluted, unheated material produced convulsions while the heated and the diluted allantoic fluid failed to elicit such reactions. The normal fluid had no effect.

Because propagation of the influenza virus in the brains of mice seemed unnecessary for the production of convulsions, it was thought that these symptoms might be caused by the toxicity of the virus particle itself, or by some soluble substance that was not absorbed and eluted from the chicken red blood cells as was the virus. With this in mind, an experiment was planned to test these possibilities, and also to determine whether the convulsive factor passed an unglazed porcelain filter. Included in the test was the effect of inactivation by heat on the concentrated virus.

The PR8 strain of influenza virus was used after growth in the allantoic sacs of embryonated eggs. After 48 hours incubation at 37°C the pooled fluid having a hemagglutination titer of 1:12,800 was tested for sterility and during the sterility test the allantoic fluid containing virus was stored on dry ice. After proving that the fluid was free from bacteria, an equal volume of 3% sterile suspension of chicken red blood cells was added to a portion. This mixture was incubated for 2 hours at 4°C with occasional gentle shaking, centrifuged in the cold, the supernatant fluid re-

TABLE II.
The Association of the Convulsive Factor with the Virus Particle.

Material injected	Amount intracranially	No. of mice injected	No. developing convulsions
Unfiltered concentrated virus; titer 1:102,400	0.03 ml	10	10
Conc. virus; titer after filtration 1:51,200	0.03 ml	10	10
Supernatant fluid after 1 absorption with equal volume 3% sterile red blood cells	0.03 ml	10	0
Conc. virus; titer 1:102,400; heat inactivated at 56°C for 45 min	0.03 ml	10	0

moved, and .9% sterile saline added to the sedimented red blood cells in such amount that the virus was concentrated ten times after elution. The virus was eluted by a 2-hour incubation at 37°C and after removal of the red blood cells the titer was found to be 1:102,400. Eight ml of the concentrated virus suspension was filtered through a Coor's porcelain filter of 3 porosity. The hemagglutination titer of the filtrate was 1:51,200. Thus a substantial amount of the virus was removed by filtration.

Swiss mice, 5 weeks old, were injected intracranially with 0.03 ml each of the following materials: Concentrated virus with a titer of 1:102,400; filtrate of concentrated virus with a titer of 1:51,200; the supernatant fluid after one absorption with an equal volume of 3% sterile red blood cells; and concentrated virus inactivated at 56°C for 45 minutes. The results of this experiment are given in Table II.

It is obvious that the convulsive factor was absorbed on and eluted from the red blood cells. It did not remain in the supernatant fluid after one absorption in sufficient concentration to produce symptoms. The convulsive factor was not removed by filtration through unglazed porcelain, and inactivation at 56°C wholly destroyed the ability of the concentrated virus to produce convulsions.

Another experiment was devised to determine whether or not the convulsive factor was specifically neutralized by immune serum. For this test sterile undiluted allantoic fluid containing PR8 influenza virus, having a hemagglutination titer of 1:6,400, was mixed with sterile anti-influenza A and anti-influenza B immune mouse serum. The anti-hemagglutination titer of the anti-A serum was 1:5,120 and that of the anti-B 1:81,920. The following mixtures were made:

TABLE III.
Specific Neutralization of the Convulsive Factor by Antiserum.

Material injected	No. of mice	No. developing convulsions
A antiserum + A virus	10	0
B antiserum + A virus	10	6
Saline + A virus	10	8

0.4 ml allantoic fluid containing influenza A + 0.06 ml anti-A mouse serum.

0.4 ml allantoic fluid containing influenza A + 0.06 ml anti-B mouse serum.

0.4 ml allantoic fluid containing influenza A + 0.06 ml saline (control).

Swiss mice, 21 days old, were injected intracranially with 0.03 ml each of the above mixtures and the results are shown in Table III.

It is evident that influenza A virus neutralized with specific serum was unable to cause convulsions but that the anti-B serum gave no protection against the convulsive factor.

The results of these experiments demonstrated that the convulsive factor is closely associated with the virus particle and that it is absorbed and eluted by red blood cells as is the case of the virus. The convulsive factor is heat labile. No evidence was found that the virus propagated in the mouse brain, therefore the most logical conclusion would be that the virus protoplasm was the toxic substance in a manner similar to the endotoxins of bacteria. However, until some form of inactive virus is found that will produce neurological symptoms, one should have some hesitation in concluding that the convulsions were caused by a toxicity in the manner of bacterial endotoxins.

Summary. Although incapable of propagating in the mouse brain, the influenza virus strains used in these experiments produced

convulsions in this species when injected intracranially. The convulsive factor was absorbed on and eluted from chicken red blood cells as was the virus. The convulsive factor was inactivated by heating at 56°C for 30 minutes. It was specifically neutralized by antiserum.

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Simultaneous Infection in Laboratory Animals with Murine and Classic Typhus. II. Recovery of Strains.*

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In a previous paper[1] we reported on the production of typhus pneumonia in rats and mice with a mixture of murine and classic rickettsiæ. The assumption that both viruses were present was supported by serological and immunological studies. The experiments here reported demonstrate that the mixed infection was still detectable after 10 generations in the lungs of mice and that then both strains were recovered by inoculation in guinea pigs. The means by which we could separate the murine from the classic strain were based on the observation of the behavior of male and female guinea pigs towards the 2 types of typhus infection. Male guinea pigs, when inoculated with murine typhus, develop, as is well known, a characteristic swelling of the scrotum accompanied by a rise of temperature which lasts several days. The same virus inoculated into female guinea pigs produces a considerably milder disease apparent only in a smaller rise of temperature for a shorter period than in male animals. Often the disease is inapparent. The transfer of the strain from female to female is difficult and the strain is often lost after 2 or 3 generations. On the other hand, females react readily to classic typhus infection and the strain can be preserved indefinitely.

Material and Methods. The murine typhus strain used was isolated from a case of human typhus in 1936 in Mexico City. It has been kept by a considerable number of transfers in guinea pigs and constantly produces scrotal swelling in males. In females inoculated with tunica material the infection is restricted to less than 4 days of moderate febrile reaction. The classic virus is the "Breinl" strain which produces a febrile reaction lasting from 6 to 10 days in male and female guinea pigs when inoculated with 0.1 g of brain of an animal killed on the 5th day of fever.

The material for the inoculation of mice by the intranasal route was prepared according to methods described elsewhere.[2] Once the virus was adapted to the mouse lung, a mixture of equal amounts of murine and classic material was inoculated into mice by the intranasal route. At 48-hour intervals transfers were made using sulfathiazole[3] as a preservative to prevent contamination of the lungs by secondary invaders.

The separation of the 2 typhus rickettsiæ was made from the 10th transfer of the mixed virus from lung to lung. Guinea pigs, male and female, were inoculated by intraperitoneal route with an emulsion of an infected mouse lung. The material was diluted conveniently to reduce the chances of peritonitis by secondary invaders. Subsequent transfers from male to male guinea pigs were made using as inoculum the tunica vaginalis, while in female guinea pigs the inoculum was brain emulsion. Transfers from tunica material to female

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