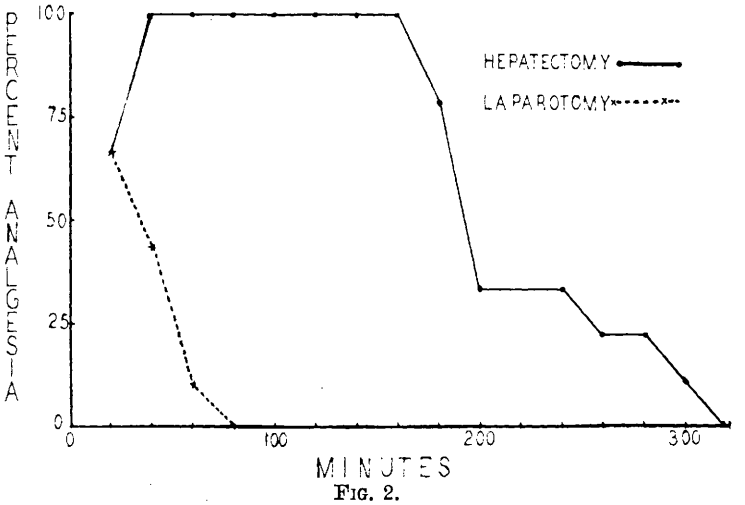

FIG. 2.

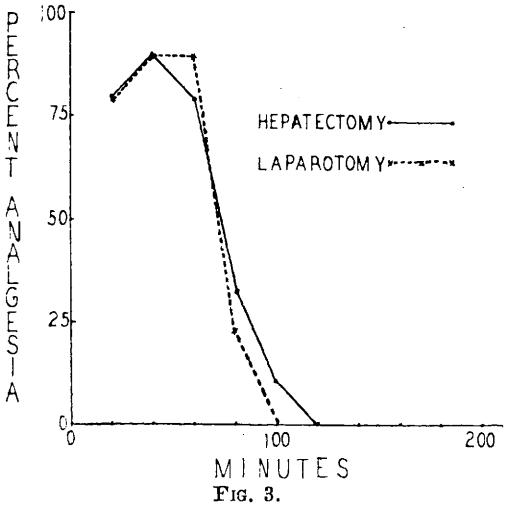

FIG. 3.

the control period, it cannot be taken to indicate that complete regeneration has taken place. In fact it has been stated that this takes 28 to 30 days(3) or possibly longer.

Summary and conclusions. A comparison of the analgetic response of 1.5 mg/kg intraperitoneally of 1 Methadone in normal, sham-operated and partially hepatectomized rats was carried out 5 days postoperatively, and 15 days postoperatively.

These experiments suggest that the liver is the chief site for the destruction of 1 Methadone.

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Persistence of Brunhilde Poliomyelitis Virus in Rodent Brain without Evidence of Adaptation.* (17984)

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Although all evidence indicated that non-Lansing types of human poliomyelitis virus would be difficult to adapt to nonprimate hosts, it seemed worthwhile to reinvestigate the problem using a virus of high titer and a reasonably large number of animals. The method of alternating passage between rhesus monkeys and other animals was employed in addition to passage from rodent to rodent.

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The former method of alternation of the passage of a virus between hosts of known susceptibility and hosts which were less susceptible has apparently achieved success in the adaptation of certain viruses to less susceptible hosts(1-3).

Material and methods. Brunhilde virus pool II(4) with an intracerebral titer of 0.8 ml x $10^{-5.9}$ was used in all experiments. This virus is antigenically distinct from viruses of the Lansing type. Rhesus monkeys of approximately 5-6 lb in weight were inoculated intrathalamically with 0.8-1.0 ml of 20% brain emulsions. The inoculum was divided equally between left and right thalami. Monkeys were observed daily and were sacrificed at the onset of symptoms or after 30 days when no symptoms were observed. Histological confirmation of the diagnosis was made of all paralytic and non-paralytic monkeys. The cotton rats *(Sigmodon hispidus hispidus)* (Say and Ord), golden hamsters *(Cricetus auratus)*, meadow voles *(Microtus pennsylvanicus pennsylvanicus)* (Ord), and deer mice *(Peromyscus leucopus noveboracensis)* (Fischer) were of the stock of Tumblebrook Farm, Brant Lake, New York. Swiss albino mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The cotton rats were approximately 5 weeks old, and the other rodents about 3 weeks old at the time of inoculation. Routinely all rodents were inoculated intracerebrally; the quantity of the inoculum employed for cotton rats and hamsters was 0.05 ml and for voles and deer mice, 0.03 ml. Certain samples of central nervous system tissue when inoculated as a 20% suspension caused the immediate death of up to 30% of rats. This non-specific death rate was sometimes reduced practically to zero by dividing the inoculum between both sides of the brain.

In all experiments a sufficient number of groups of rats and hamsters was inoculated so that the effects of each suspension could be observed in 25-30 animals for a period of at least 6 weeks. Excluding animals dying immediately or on the first day after inoculation, a total of 732 cotton rats and 97 hamsters was kept under observation. Voles were inoculated in groups of 12 to 18, and deer mice in groups of 18 to 24. A total of 256 of the former, 52 of the latter were inoculated.

Experimental. (a) *Alternation of passages*: The alternation of passages between cotton rats and rhesus monkeys was initiated by the intracerebral inoculation of cotton rats with 20% Brunhilde virus. On the 1st, 3rd, and 5th days after inoculation, 6 rats were sacrificed, and their brains pooled and tested for virus by the inoculation of 2 rhesus monkeys with each pool. Virus was found to be present in each emulsion of rat brains as indicated by the onset of paralysis and subsequent demonstration of typical histological lesions in the inoculated monkeys.

A 20% emulsion was made of the pooled lumbar and cervical enlargements of the monkeys inoculated with the pool of rat brains which had been harvested on the 5th day. This monkey cord emulsion was inoculated into other groups of rats. Six rats of the latter groups were killed and their brains harvested and pooled on the 5th and 10th days after inoculation. Virus was demonstrated to be present in both pools by the inoculation of rhesus monkeys. The remaining rats in this group were kept under observation for 6 weeks. This alternation of passage of virus from the brains of rats sacrificed on the 5th and 10th day back to monkeys and into rats again was continued for 5 cycles. It was clear (Fig. 1) that virus could be recovered from the brains of rats up to the 10th day after inoculation. The rats from which virus was recovered were, however, symptomless. Brains of 9 rats were examined histologically 10 days after inoculation with 20% Brunhilde virus. No lesions, other than could be accounted for by the inoculations, were found. During the 6 week observation period, any rat show-

1. Baker, J. A., *Am. J. Vet. Research,* 1946, v7, 179.

2. Koprowski, H., James, T. R., and Cox, H. R., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 178.

3. Baker, J. A., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 183.

4. Bodian, D., Morgan, I. M., and Howe, H. A., *Am. J. Hyg.,* 1949, v49, 234.

FIGURE SHOWING PASSAGES OF BRUNHILDE VIRUS IN RHESUS MONKEYS AND COTTON RATS

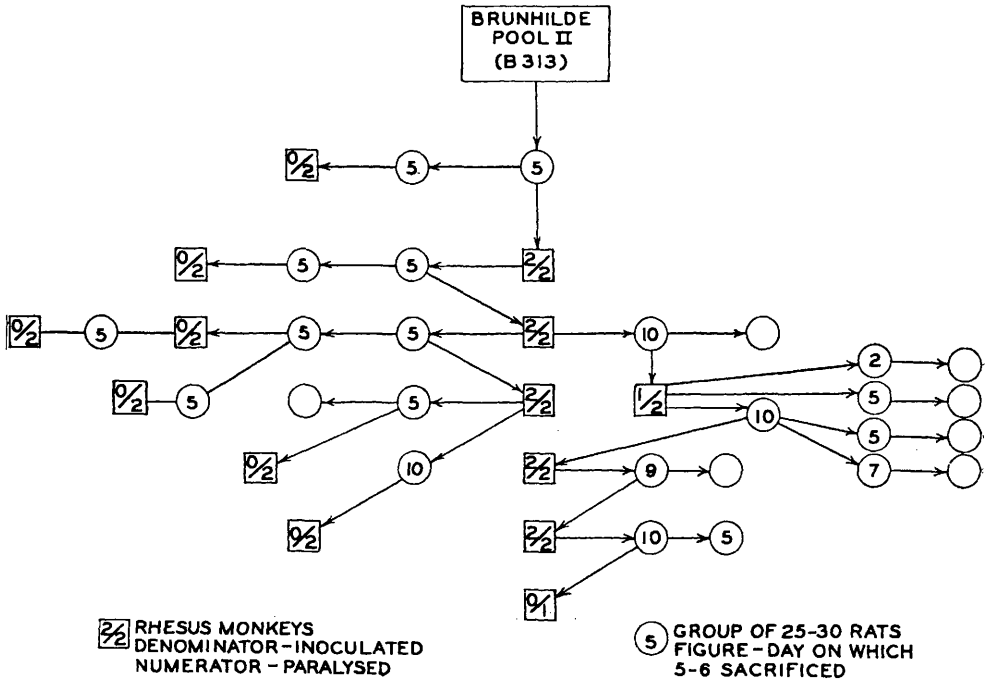

FIG. 1.

ing the least abnormality was sacrificed. In 18 of these sacrificed rats, the brain was passed to other rats, and the cord from 5 of them was examined histologically. In 4 other sacrificed rats, histological examination of the cord was made. In none of these animals was there any visible evidence of virus proliferation.

An experiment similar to the above was set up in which alternation of passage of virus between rhesus monkeys and hamsters was carried out. Virus was recovered in monkeys from the brains of hamsters up to the 5th day after the inoculation of virus. No later tests were made. This experiment was abandoned in the 2nd cycle because of difficulties in obtaining sterile brain emulsions from hamsters. No evidence of virus activity was observed in any of the inoculated hamsters.

(b) *Rodent to rodent passages.* After it had been established that Brunhilde virus was present in cotton rat brains up to the 10th day after inoculation, 20% emulsions of brains of rats killed on the 5th, 9th, or 10th days were inoculated into other rats as shown in Fig. 1. Virus was not recovered in rhesus monkeys after more than one cotton rat passage, nor was there any evidence of disease in the rats themselves.

In addition to these direct passages of 20% brain suspensions, the following passages were made. A pool was made of the cervical and lumbar enlargements from monkeys which had become paralysed after inoculation with cotton rat brains containing virus. The supernate of this pool was concentrated ten times by ultracentrifugation,‡ and 12 rats were inoculated with the concentrate. Five groups, each of 6 rats, were given 3 to 5 inoculations intracerebrally and intraperitoneally daily or on alternate days with suspensions

‡ This preparation was made by Dr. C. E. Schwerdt.

of monkey cord or rat brain containing Brunhilde virus. Brunhilde virus was diluted in 10% normal rat brain supernate in another experiment, and inoculated intracerebrally into 24 rats. By none of these methods was any adaptation accomplished.

Brains of hamsters sacrificed on the 5th day after inoculation with Brunhilde virus were passed to other hamsters. Although Brunhilde virus was recovered in rhesus monkeys after a 5 days sojourn in the brains of symptomless hamsters, there was no evidence of transmission of the virus to other groups of hamsters.

Voles were inoculated with 20% Brunhilde virus. Unfortunately, voles appear to have a high non-specific death rate under laboratory conditions. The medulla and cord from 19 voles which were sick or died after inoculation of Brunhilde virus were examined histologically. In none of these animals was there any evidence of virus proliferation. Passages were made to groups of 10-20 voles with the brains of 5 of the dead or sick voles, with evidence of bacterial infection in 2 cases.

The susceptibility of the vole to Lansing virus does not appear to exceed that of the cotton rat. In 2 titrations of Lansing virus derived from monkey cord and employing 10-fold dilutions with 5-7 voles per dilution, there were no paralyses above 10^{-3}. This material (Pools IVa and IVa-1) had a median titer of $10^{-3.5}$(5), in cotton rats. A Lansing pool of mouse origin, J. Bell(5) with a mean titer of $10^{-4.6}$ in cotton rats was 3.7 in a single titration in voles using 12 animals to a dilution. The titration, however, was very irregular since even at the lower dilutions, 100% paralysis was not obtained. The test was further complicated by a number of deaths not preceded by observable paralysis. In view of the high non-specific death rate among voles, these deaths were excluded from the titration which ran as follows: $10^{-1.5}$ 8/10; $10^{-2.5}$ 4/7; $10^{-3.5}$ 6/10; $10^{-4.5}$ 5/8. It seems likely, however, that although the end point was not definitely reached, it probably would not have exceeded that in cotton rats.

A similar experiment to the above was made with deer mice. There was no evidence of virus adaptation to these animals.

In view of the persistence of virus in the brains of hamsters and cotton rats up to the 5th and 10th day respectively after inoculation, an experiment was done to see if the virus could be maintained in mouse brains. Thirty-two Swiss albino mice were inoculated intracerebrally with 0.02 ml of 20% Brunhilde virus. This quantity of inoculum was chosen so that the dilution factor in the mouse brain would be of the same order as that of virus inoculated into cotton rat brains. Two groups of symptomless mice were sacrificed on the 5th and 10th days after inoculation. The brains from each group were pooled separately and each of the four were inoculated into 2 rhesus monkeys. All the inoculated rhesus monkeys except one developed symptoms of poliomyelitis indicating that Brunhilde virus had remained present in mouse brains up to 10 days after inoculation.

Summary and conclusions. Adaptation of Brunhilde virus to cotton rats or hamsters was not achieved by the method of alternation of passage between these animals and rhesus monkeys.

Brunhilde virus was found present in the brains of symptomless hamsters up to 5 days after intracerebral inoculation and up to 10 days after intracerebral inoculation of cotton rats and Swiss albino mice (no tests at longer intervals were made). There was no evidence of the virus persisting after more than one cotton rat or hamster passage, and there was no histological evidence of virus proliferation. This is highly suggestive that no multiplication had occurred in the brains of these animals. This persistence of virus in symptomless animals is similar to that observed by Smith(6) in attempts to establish St. Louis encephalitis in rats and guinea pigs.

5. Bodian, D., Morgan, I. M., and Schwerdt, C. E., *Am. J. Hyg.*, 1950, v51, 126.

6. Smith, M. G., *J. Inf. Dis.*, 1939, v64, 307.

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