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Attempted Adaptation of the Virus of Poliomyelitis to Wild Rodents.

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Since the publication of the report by Armstrong[1] that the Lansing strain of poliomyelitis could be transmitted to the eastern cotton rat, *Sigmodon hispidus hispidus*, studies were undertaken to repeat his findings using other strains of poliomyelitic virus, both recently isolated and those with long passage through monkeys. Toomey and Takacs[2] had obtained negative results using 9 strains of virus, while Kessel and Stimpert[3] reported unfavorably on the use of several wild species of mice and rats. Jungeblut and Sanders,[4] however, obtained what they called a murine strain of virus after passage of the S.K. poliomyelitic virus to cotton rats. Hammon[5] reported on negative findings using the Duran-Reynals factor to accelerate the action of the virus in rats. But Toomey and Takacs[6] have recently been able to obtain passage of the MV strain of poliomyelitis to the cotton rat after addition of their colon-typhoid-paratyphoid broth filtrate. Typical poliomyelitis could be produced in monkeys after the 6th and 8th generations of the virus in rats.

Several species of wild rodents, *Sigmodon hispidus eremicus, S. h. texianus, Microtus californicus* and *M. montanus* were obtained from the field and later raised by Dr. F. Evans of the Hooper Foundation. All but one species, *S. texianus*, were indigenous to California.

Large animals of unknown age brought in from the field were used for the first passage experiments. They were given 0.03 to 0.05 cc intracerebrally, 0.5 cc intraperitoneally and about 0.2 cc intranasally of a 10% suspension of 6 strains of poliomyelitis; Jackson, isolated in California in 1934; Reichle from Switzerland in 1934; Geise and Dyer in 1939 and Caldwell in 1940 from California. The doses mentioned were employed throughout all the succeeding inoculations unless otherwise recorded. The 13 *Microtus californicus*

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1 Armstrong, C., *U. S. Pub. Health Rep.*, 1939, **54**, 1719.

2 Toomey, J., and Takacs, W. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 536.

3 Kessel, J., and Stimpert, F. D., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 665.

4 Jungeblut, C., and Sanders, M., *J. Exp. Med.*, 1940, **72**, 407.

5 Hammon, W. McD., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 124.

6 Toomey, J., and Takacs, W., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 364.

and the 13 *Sigmodon h. eremicus* inoculated failed to show any symptoms of disease after 4 weeks' observation. Brain material from 3 other cases suspected of having poliomyelitis was also used but both the monkeys and the rodents proved negative.

Younger animals varying in age from 2 to 3 weeks were then employed. Eight *Microtus montanus,* 3 *M. californicus,* 52 *Sigmodon h. eremicus* and 7 *S. h. texianus* were inoculated, not only with the poliomyelitis strains just enumerated but with the MV strain and fresh brain material from 4 new cases, including 2 in California and 2 in Tacoma, Washington. No symptoms of poliomyelitis were noticed in any of the animals after 4 to 6 weeks' observation.

A group of wild mice of the 2 species were inoculated with the Jackson strain of poliomyelitis in the amounts previously mentioned and from 2 to 3 of these were killed at intervals of 1, 2, 3, and 4 days, and 1, 2, and 3 weeks, respectively. Each lot was then passed into 2 to 3 other normal wild mice and also to a monkey. The second generation mice coming from the original groups killed on the first, fourth, and seventh days were killed and passed to a third generation. A few of the mice died of an intercurrent bacterial infection in 2 to 3 days after inoculation, but otherwise all the others survived without any typical symptoms suggestive of poliomyelitis. On the other hand, the monkeys given the mouse brains removed on the third and fourth days after injection, respectively, both developed temperatures and typical symptoms of poliomyelitis. The wild mice inoculated with the same material, however, failed to carry on the virus.

A similar experiment was undertaken by inoculating 15 Sigmodons, 13 to 23 days old, with the Jackson strain of virus. Three to 4 animals were killed in 3 days, in 2, 3, and 4 weeks, respectively after the inoculation and their brains were passed on into 4 more cotton rats. Brain suspensions from all the rats, except those killed on the 4th week were also inoculated into monkeys but without positive results.

The Sigmodons killed on the fourth day were passed to 3 others, one of which developed a paralysis of the hindlegs on the third day. It was killed and its brain transferred to 4 other rats and a monkey. They all remained well. In 2 weeks the rats were killed and passed to a fourth generation of animals. In 2 weeks 3 of these developed a very spastic type of convulsion, characterized by a weakness and slight transient paralysis of the hindlegs, combined with rough hair, general nervousness and hyperexcitability and inclination to show a humped back. The animals usually recovered in a few minutes but were weaker for a short period. One rat died. The 3 showing

spasms were killed and passed into a fifth generation of rats. After a month one showed the same symptoms and was killed. The others remained well, as did the rats of the 6th generation given the brain from the sick animal.

Two other series of experiments were undertaken using both species of Sigmodons, 12 to 17 days of age. One group was given the Jackson strain of virus and the other the Reichle. Part of each group was killed in 4 days after the inoculation and part in 7 days and passed on both to monkeys and to other cotton rats. The former all remained well. The Sigmodons killed on the fourth day were passed through 5 generations for the Jackson strain and 4 for the Reichle, each lot of rats being killed every 4 days and then passed to the next. The latter were killed in 4 days and so on. Monkeys were also inoculated intracerebrally at the same time after each passage but none developed poliomyelitis. On the fourth passage with the rats given the Jackson strain and on the third with those given the Reichle, animals began to show the same type of transient, spastic semi-paralysis as previously described. These animals were killed and their brains transferred to 4 or 5 rats. One of 7 developed slight spasticity in the Jackson group and one of 5 in the other.

Because this peculiar type of spasticity greatly resembled the symptoms noticed for animals injected with the virus of lymphocytic choriomeningitis, it was thought that this strain might have been activated among the wild rodents. A similar type of experiment was then undertaken by passing on the brains of normal rats *(Sigmodon h. eremicus)* into others, killing them every 4 days as in the previous groups. Animals, raised in the laboratory, from 17 to 20 days old, were used. Four or 5 were inoculated each time with the same doses as for the other experiments and their brains passed to 4 other normal animals, which were killed in 4 days and so on through 7 generations. The rats all remained quite normal through the sixth passage.

Of the 5 inoculated on the seventh generation, 3 remained well, but 2 developed the same spasms as before, on the seventh day after inoculation. These were killed and passed to 3 others, one of which remained normal but 2 developed spasms on the 25th day, the eighth generation. Upon the ninth passage only one out of 7 rats showed the symptoms. No further spasticity was noticed after the eleventh generation.

Those animals that had remained well and had not been killed out of the different groups were then tested for immunity to the virus of lymphocytic choriomeningitis (l.c.m.) by intracerebral

inoculation. All of those tested became ill with typical symptoms for this virus or else died, discouraging the idea that this virus had been present among these animals. Sections of the brains from a number of the rats that had shown atypical spasms were examined by Dr. W. P. Covell of the Hooper Foundation but they failed to reveal any lesions significantly suggestive of the l.c.m. virus.

In examining the data on these transfers with the cotton rat brains, it seemed that while animals developed neurological symptoms suggestive of a virus disease, yet there was no definite evidence for the presence of a virus among the rats. With the exception of the 2 animals given the Microtus material, no monkeys and no white mice tested with any of the brains from the rats having spasms ever developed symptoms indicative of the presence of a neurotropic disease. It was, therefore, decided that the spasms were probably of a dietary nature.

It was then found that the animals that were inoculated and kept in the laboratory under observation for a long period had only been receiving sunflower seeds. They had formerly been given a more adequate diet but during the summer because of an unusual amount of work the caretakers had put in the easiest food at hand. After addition of carrots and alfalfa it was noticed that the spasms ceased among the rats. None had been recorded for the wild mice, but they had always been given oats and bread. However, the surviving rats had grown older and the convulsions had been mainly among younger animals. This would conform to a recent report by Wolbach and Bessey that a vitamin A deficiency in young, one- to two-months-old white rats produces "an overgrowth of the central nervous system in relation to its boney enclosure, resulting in mechanical damage to brain, spinal cord and nerve roots."[7] Neurological spasms might result. Such symptoms were not noticed as much in the older animals on the inadequate diet. Addition of carrots prevented the spasms.

In the present study, since the cotton rats showing these symptoms were mainly young animals and since the return to a carrot diet stopped the convulsions, it would appear that the latter were probably due to these dietary factors rather than to a virus disease. Further evidence was obtained by keeping one group of normal rats on the inadequate diet for 6 weeks, in which time spasms were developed, and then returning them to the carrot ration, when none appeared. Another group on carrots alone for the same period never developed any spasms.

[7] Wolbach, S. B., and Bessey, O., *Science*, 1940, **92**, 483.

Another observation brought out in this study referred to the susceptibility of these wild rodents to the virus of lymphocytic choriomeningitis. Because it was thought that the convulsions might be due to this virus, a large number of animals were tested for susceptibility. 0.03 cc of a 10% suspension of the W.E. strain, kindly sent by the laboratory of Dr. T. Rivers of the Rockefeller Institute, was inoculated intracerebrally into old adults (over 6 months of age), immature animals (2-6 months), and very young animals (1-2 months) of both species of Microtus and Sigmodons. It was interesting that all of the 23 *Microtus californicus* in the 3 age groups survived the inoculations, while of the 50 *M. montanus,* all survived except 54.8% of the immature group. All of the 9 adults and the 10 young remained well.

There was less resistance among the Sigmodons. 91.1% of the 35 young *S. h. eremicus* died, as did 68.4% of the 38 immature and 50% of the adults. Among the *S. h. texianus* group the resistance was higher, as 91.7% of the young and 46.7% of the immature animals survived.

It may be seen, therefore, that the *Microtus californicus* were very highly resistant to this virus, the *M. montanus* less so, while among the rats, the *Sigmodon h. texianus* were more resistant than the other species which showed a highly susceptible younger group.

Summary. Attempts to transmit the virus of poliomyelitis, both old and newly isolated strains, to the cotton rats, *Sigmodon hispidus eremicus* and *S. h. texianus* and the mice, *Microtus californicus* and *M. montanus,* were unsuccessful by the methods employed. The virus, however, could survive in the brain of the Microtus for 3 to 4 days and be transferred to monkeys but not mice. It was found that the convulsive spasms that developed among both inoculated and the normal rats were probably due to a deficient diet and not to a virus disease. *Microtus californicus* is highly resistant to intracerebral inoculation of the virus of lymphocytic choriomeningitis, *M. montanus* less so, while the Sigmodon species are more susceptible.