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Poliomyelitis Virus Acclimated to Small Laboratory Animals.*

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Flexner's M.V. strain of poliomyelitis can produce paralysis in Eastern cotton rats.[1] Another experiment was started on 8/13/40 to see if our previous results could be duplicated.[1] The usual injections of cord brain suspension in these experiments were 0.06 cc intracerebrally (IC), 0.06 cc intranasally (IN) and 0.5 cc subcutaneously (SQ) of a 10% suspension in "enteric organism filtrate." The starting injection in the first generation was made with monkey cord. Each subsequent generation got the rat cord and brain of the preceding generation. Ten animals in Generation (Gen.) I were injected. All died between the 16th and 30th day; none had paralysis. Three animals in Gen. II were injected on 8/29 with Gen. I rat which died on the 16th day. These animals died between 42 and 46 days. Three animals in Gen. III were injected on 10/16 with a combination of all 3 animals from Gen. II. This time an additional injection of 1.0 cc was made IP. Two animals died on the 3rd and 1 on the 5th day; all were weak; none had paralysis. Three animals in Gen. IV were injected on 10/21 with a combination of all 3 rats of Gen. III. Twenty per cent cord suspensions were used in this experiment. One animal died on the 2nd, 1 on the 7th and 1 on the 8th day; the latter 2 were weak. Gen. V: On 10/30, 2 animals were injected with a 10% saline suspension combination from Gen. IV—the second 2 rats above. One animal was weak on the 5th and 1 had definite paralysis on the 6th day. Gen. VI.: The 2 animals injected on 11/7 with Gen. V had paralysis on the 7th day. Gen. VII: The 2 animals injected on 11/18 with Gen. VI had paralysis on the 7th day. Thus, this method of isolation was successful a second time.

An attempt was made to adapt Flexner's M.V. strain to cotton rats by a modified procedure. The idea was to condition the rats by injecting them with "enteric organism filtrate." *B. coli* isolated from one of the rats was used to make up the enteric organism filtrate.[1] On 8/13, 8/19, 8/24, 8/27, 8/31, 9/3 and 9/6, 3 rats

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1 Toomey, John A., and Takacs, William S., PROC. SOC. EXP. BIOL. AND MED., 1940, 45, 364.

were injected SQ with 0.5 cc filtrate. A second group of 5 animals was injected similarly on 8/31, 9/3, 9/6, 9/10, 9/13, 9/17 and 9/30. Monkey cord virus was injected in the usual way—0.06 cc IC, 0.06 cc IN, 0.5 cc SQ and 1.0 cc IP. Five rats died, 1 each on the 5th, 6th, 7th, 14th and 31st days following the cord injections. The other animals were weak. A 20% saline suspension from these Gen. I rats dying the 5th, 6th and 7th days was injected into 3 animals. Two animals remained well. One died. A 10% suspension of cord of the latter was injected into 5 other animals, without any "takes". The modified method did not help us to get successful "takes", and the experiment must be regarded as negative.

The acclimated rat cord virus was not lymphocytic choriomeningitis, for 1 cc of a 10% suspension of rat cord and brain of the 11th lineal generation transfer injected SQ into 20 guinea pigs failed to produce a disease condition in any of the animals. A neutralization test was done with lymphocytic choriomeningitis antiserum in mice, using a serum obtained from Dr. Thomas M. Rivers of the Rockefeller Institute capable of neutralizing 10,000 units or more of WE virus strain per cc. Twice the volume of the serum was combined with one volume of 30% ground mouse cord (1 cc of serum plus 0.5 cc 30% mouse brain virus); the whole was shaken in a mixing machine for 1 hour in the icebox and incubated for 1 hour at 37°C. The control injection suspension was made up with twice the volume of normal saline. The dose was 0.03 cc injected IC. A negative control experiment was done with 30 other animals wherein 0.03 cc of saline only was injected. There was a 10% accident rate following the latter injections. The results were clear-cut. Lymphocytic choriomeningitis antiserum instead of neutralizing seemed to boost the pathogenicity of the mouse cord suspensions, for by the 5th day 100% of the animals injected with this combination had died. One hundred per cent of the controls died by 11/26, 10 days after the experiment was begun. Thus, this acclimated virus was not a strain of lymphocytic choriomeningitis.

Could this acclimated virus be transferred from cotton rats to monkeys? There were 3 monkeys in Gen. I. On 8/16, 1 was injected IC with 1 cc of a 10% suspension of the cords and brains of cotton rats from the 6th lineal generation transfer. On 8/27, it developed quadriplegia. A second animal was injected on 9/9 with the same amount of virus in "filtrate" from the 7th lineal gen. It developed quadriplegia on 9/16. A third animal was injected IC with 1 cc of a 20% saline suspension of rat cord and brain from the 7th lineal gen. and developed quadriplegia on 9/17. Three monkeys

were injected in Gen. II—the first on 9/12 with 1 cc of a 20% monkey cord suspension IC from Gen. I and 9/24 with the same amount IC, IP and SQ. On 9/30 and 10/3, it was again injected with 1 cc of a 10% suspension IP and SQ. On 10/22, it died from miliary tuberculosis. Some weakness present might have been due to poliomyelitis, but it was not definite. A second animal was injected on 9/24 with 1 cc of a 10% saline suspension of cord from Gen. I monkey. On 9/24 and 10/2, it received 1 cc of a 10% saline suspension IC. On 10/6, it developed paralysis, chiefly of the upper extremity and neck muscles. A third animal received 1 cc of a 10% suspension in "filtrate" from Gen. I on 9/21 and 10/1. It died from miliary tuberculosis on 10/3. Gen. III: On 10/6, a monkey was injected IC with 1 cc of a 10% saline monkey cord suspension of the second animal in Gen. II and reinjected IC, SQ and IP with the same amount on 10/16. On 10/23, it developed paralysis. Gen. IV: A monkey was injected IC and IP with 1 cc of a 20% saline suspension of the lumbar cord obtained from the animal that died in Gen. III and with 2 cc SQ on 10/23. It received 2 cc IP on 10/31 and 11/4. It developed quadriplegia on 11/7. Gen. V: On 11/11, another monkey was injected IC with 1 cc of a 20% cord suspension of the animal dying in Gen. IV. It developed quadriplegia on 11/22.

Although clinical poliomyelitis was produced, the animals, excepting the monkey in Gen. V, were usually injected several times with a brain cord antigen. Hurst[2] has shown that repeated injections of normal rabbit brain tissue might cause paralysis in the homologous animal. Rivers, Sprunt and Berry[3] repeatedly injected 8 monkeys with emulsions and alcoholic suspensions of rabbit brain, and in 2 animals paralysis was produced. The experimental animal does not develop paralysis after a primary injection. Although the animal in Gen. V developed paralysis after one injection and thus met possible objections because of nonspecific factors, another experiment was performed. On 11/9, a monkey was injected IC with 1 cc of a 10% suspension and IP with 2 cc of cotton rat brain and cord, not from the 6th but from the 11th lineal gen. On 11/16 it developed quadriplegia.

Thus, it has been shown (1) that the original experiments with Flexner's M.V. strain could be repeated in the cotton rat; (2) that rat cord virus obtained from paralyzed animals was not lymphocytic

[2] Hurst, E. Weston, *J. Hyg.*, 1932, **32**, 33.

[3] Rivers, Thomas M., Sprunt, D. H., and Berry, G. P., *J. Exp. Med.*, 1933, **58**, 39.

choriomeningitis; (3) that this acclimated rat cord virus could be transferred to monkeys.

In collateral experiments, we attempted to acclimate Flexner's Philadelphia virus strain to the cotton rat.

Gen. I: On 8/31, 5 cotton rats were injected with a 10% suspension of monkey cord (Philadelphia poliomyelitis strain) in "enteric organism filtrate". The original strain was obtained from Dr. Irving J. Wolman, The Children's Hospital, Philadelphia. The doses in this and in the following animals were: 0.06 cc IC, 0.06 cc IN and 0.5 cc SQ. Four of the 5 animals died—1 the 6th, 1 the 14th and 2 on the 29th day following. No animal was paralyzed. Gen. II: (a) The cords from the first 2 animals that died in Gen. I were ground in "filtrate" and injected in 3 other rats on 9/20. One animal died on the 29th day. (b) On 10/3, 3 other animals were injected with a combination of cords and brains of animals from Gen. I in filtrate. There was an added injection of 1.0 cc IP. Three rats died —1 in 24 hours and 2 within 5 days after the injections. Gen. III: A 10% saline suspension of the cords and brains of the animals in Gen. II that died on the 5th day was injected into 5 animals on 10/10. One animal was found dead on the 2nd, 1 the 5th and 1 the 3rd day; the latter had had paralysis of the front paw. Two died on the 4th day and had weakness. Gen. IV: A 20% saline suspension of the cords and brains of those rats of Gen. III that died on the 3rd, 4th and 5th days was injected into 4 animals on 10/18. The same percentage suspension used in this experiment was used in the next 4 generations. All the animals died—1 the 6th, 1 the 7th and 1 on the 9th day following the injection. All had weakness. Gen. V: On 10/23, 2 rats received material from the first animal that died on the 5th day in Gen. IV. Both died—1 the 5th and 1 on the 11th day. Both were weak. Gen. VI: On 10/28, 2 rats received material from the animal that day on the 5th day in Gen. V—1 died on the 3rd and the other on the 7th day with paralysis. Gen. VII: On 11/4, 2 rats received material from the animal of Gen. VI that died on the 7th day. They died on the 5th day with paralysis. Gen. VIII: In this experiment, it was thought best not to inject the animals IP. This added dose might have been successful in breaking down the general resistance, but when the virus has become acclimated, the additional IP injection is apt so to accelerate the disease that an animal would sometimes die before paralysis would be appreciated. On 11/19, 3 animals were injected with material from the 7th gen. All developed paralysis and died on the 5th, 6th and 7th days after injection.

Thus, Flexner's Philadelphia, as well as his M.V. strain, can be made to produce paralysis in the cotton rat.