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Further Experiments with Poliomyelitis Virus in Cotton Rats.*

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It has been shown that Flexner's M.V. poliomyelitis strain carried in monkeys can be acclimated to rats[1] and subsequently retransferred from rats to monkeys, in which latter typical disease was produced.[2] The first 2 experiments of this report were performed in order to determine whether the virus could be retransferred from the collateral monkey generation back to rats.

Experiment I. Monkey No. 2379 was 4 generations removed from the original rat transfer. Generation I monkey had received intracerebrally 1 cc of a 10% lineal generation VI cotton rat cord virus. Generations II, III, and IV monkeys were given 1 cc of a 10% cord virus obtained from the generations I, II, and III monkeys, respectively.

Generation I Rat Retransfer. On 11/11/40, 5 cotton rats were injected with 0.06 cc IN, 0.06 cc IC, 0.5 cc SQ, and 1 cc IP of a 10% cord virus suspension of generation IV, Monkey No. 2379. (The doses mentioned here are the same in all the subsequent experiments of this type.)

Two animals died—1 on 11/27, the sixth day, and 1 on 11/28, the seventh day; both were weak, but neither had paralysis.

Generation II Rat Retransfer. On 11/29/40, the cords of the 2 animals dying in rat generation I were injected into 3 cotton rats. One rat died on 12/1 with no evidence of paralysis; the other 2 animals had paralyses and died on 12/5.

Generation III Rat Retransfer. On 12/6/40, a cord suspension made from the 2 animals that died on 12/5 in rat generation II was injected into 3 animals, all of which developed paralysis.

Thus, one can transfer Flexner's M.V. strain from the monkey to the cotton rat, back to the monkey and then to the rat again. It will be noted that the animals dying in generation I rat retransfer did not have typical paralysis. Typical paralysis never has been seen by us

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

2 Toomey, John A., and Takacs, William S., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 22.

in a generation I rat transfer. In these experiments, "filtrate" was not necessary to initiate the transfer.

Experiment II. It was thought that a transfer from a rat cord to a generation I monkey might leave enough of "rat specificity" in the acclimated animal to produce immediate paralysis if this monkey cord, only one generation removed from the rat, were transferred back to the rat. Monkey No. 2387 was a first generation take from generation XI rat cord virus. This animal, injected 11/8/40, had quadriplegia on 11/16.

The monkey cord virus was injected into 2 cotton rats on 11/19, 1 of which had weakness and died 11/27. Three other animals were injected on 11/25, 1 of which had weakness and died on 12/3. Three animals lived.

Generation II. A suspension of the cord and brain of the first animal dying in generation I was injected into 3 cotton rats on 11/29/40. All had weakness; 1 rat died on 12/10, 1 on 12/16 and 1 on 12/20.

Generation III. A combination suspension of the cord and brain of the animals in generation II that died on 12/10 and 12/16 was injected into 5 animals on 1/2/41. One animal died accidentally after the injection. The 4 remaining animals had paralysis; 1 on 1/14, 2 on 1/16, and 1 on 1/18.

Thus, it was again possible to transfer Flexner's M.V. strain from monkeys to rats, to monkeys, and back to rats. It would seem, however, that heterologous monkey virus, even though but one generation removed from the parent rat stock, must again become acclimated to the rat and that 2 or 3 transfer generations are necessary before paralysis can be produced.

Experiment III. The third experiment was done in order to determine whether or not we were dealing with a true poliomyelitis virus in these experiments. A 4% suspension of Flexner's M.V. strain of acclimated generation XV rat cord virus was made. To different lots of this suspension were added equal quantities of: (a) horse serum obtained from an animal which lacked any virucidal antibodies as proven by many previous neutralization experiments in monkeys;[3] (b) horse serum obtained from the same animal after it had undergone a long series of injections with Flexner's M.V. virus, fortified with enteric organisms and toxins;[3] a serum which was found highly virucidal in previous neutralization experiments in monkeys; (c) pooled convalescent poliomyelitis human serum; (d) saline. One group of rats received convalescent serum only and,

[3] Toomey, John A., *Am. J. Dis. Child.*, 1937, **53**, 1492.

another, horse serum only (plus saline in each instance to make up a comparable dosage). Serums, saline, etc., were added to the virus mixture as indicated, the combination shaken, incubated for 1 hour and placed in the icebox for 24 hours, warmed and injected as follows: 0.06 cc IC, 0.06 cc IN and 0.5 cc SQ. The final injection mixture was a 2% suspension of virus.

Protection may be said to have been demonstrated if the animals which received the neutralized virus lived at least 21 days without having paralysis after the test was done.

Every one of the positive controls developed paralysis and died within 10 days after the injection. All of the animals, injected with the horse serum lacking any neutralizing antibody titer, also died. None of the animals injected with either the normal pooled convalescent serum or specific horse serum died or developed disease. In the pooled human convalescent serum protected group, 4 of the 20 animals died, a 20% mortality rate. Of the 20 animals injected with the specific neutralizing horse serum and virus, 1 was killed by other rats the first day after the injection; of the 19 that remained, 1 died without signs of paralysis on the thirteenth day. Only one of the 19 died, a 5+% mortality rate as compared with that of 100% following the injection of serum obtained from the same horse prior to immunization.

These specific neutralization tests demonstrate that the virus material transferred to the cotton rat was truly a poliomyelitis virus strain. Since the original acclimations to the cotton rat were made with Flexner's M.V. strain, it can be assumed that the poliomyelitis strain which had been carried in the rat was the Flexner's M.V. strain.

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Salt After Adrenalectomy. IV. Urinary Nitrogen and Survival Time of the Fasted Adrenalectomized Salt-treated Rat.*

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Adrenalectomized rats kept under optimal living conditions and allowed to drink 1% NaCl solution store fed glucose as liver glycogen

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