ception created by an earlier paper[1] that the virus of infectious papillomatosis was never serially transmissible in domestic rabbits. Experiments attempting to determine accurately the conditions necessary for the regular serial transmission in domestic rabbits are in progress.

7876 C

Attempts to Produce Poliomyelitis in Refractory Laboratory Animals.*

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Since the successful transmission to monkeys of the virus of poliomyelitis by Landsteiner and Popper,[1] many attempts have been made to produce the disease in the other laboratory animals. This work has been adequately reviewed by Shaughnessy *et al.*[2] and Harrington.[3] Experiments concerned with transmission of the virus to any animal other than the monkey have given only negative results. In this work, the passage and multiplication of the virus of poliomyelitis was attempted in mice, rats, guinea pigs and rabbits. It was thought possible to overcome the factors which make these animals naturally resistant to the virus, by using very young animals, by lowering their resistance and by passing the virus serially in the hope of adapting it to the new host.

First, to determine the time of survival of the virus in the brains of these animals, they were injected intracerebrally and after various intervals of time, the site of inoculation was removed, emulsified and injected into monkeys to determine the presence of virus. Rabbits received 0.3 cc., guinea pigs 0.2 cc., rats 0.1 cc., and mice 0.03 cc. of virus suspension. Table I indicates the time of removal of

* This work was aided by grants from the New York and Rockefeller Foundations and a fund from Mr. Jeremiah Milbank.

[1] Landsteiner, K., and Popper, E., *Z. f. Immunitätsforsch. und exp. Therap.*, 1909, **2**, 377.

[2] Shaughnessy, H. J., Harmon, P. H., and Gordon, F. B., *J. Prev. Med.*, 1930, **4**, 59, 89.

[3] Poliomyelitis, International Committee, 1932, p. 101. Williams and Wilkins, Baltimore, 1932.

brain tissue and the subsequent reaction in monkeys inoculated with this tissue. The results are summarized in Table I.

TABLE I.

Animal	Amount of virus suspension injected	Interval between inoculation of virus and removal of brain days	Result of injection of brain tissue into monkeys.
Rat (6 weeks old)	0.1 cc. of 10% suspension, intracerebrally	5	no paralysis
		3	" "
		2	" "
Guinea Pig (2 days old)	0.1 cc. of 20% suspension, intracerebrally, and 0.3 cc. intraperitoneally	4	" "
		3	" "
		2	" "
Guinea Pig (adult)	0.2 cc. of 20% suspension intracerebrally and 0.3 cc. intraperitoneally	3	" "
		2	" "
Rabbit (adult)	0.2 cc. of 20% suspension intracerebrally	5	" "
		3	" "
		2	paralysis 11 days
Mouse (adult)	0.03 cc. of 20% suspension intracerebrally	5	no paralysis
		3	paralysis 7 days

It appears that the mouse is the best of the refractory animals for use in this work, since the virus survived in its brain for from 3 to 5 days. In the rabbit brain the virus is demonstrable 2 to 3 days after injection.

Attempts to Adapt the Virus to Mice and Rats by Serial Passage. White, male rats, six weeks old, received combined intracerebral, intraperitoneal and subcutaneous injections of 0.15 cc., 0.5 cc., and 0.3 cc., respectively, given 2 to 3 times during 24 hours. Six hours after the last injection, the animals were sacrificed, the sites of inoculation including part of the peritoneal epithelium were removed and suspended in distilled water. To this material was added an equal amount of active virus and the mixture was injected into another rat by all 3 routes, 2 to 3 times during 24 hours. Further serial passage was then carried out in this manner for 16 passages, using animals of the same age. The rat on the 16th passage received 1 to 3 injections daily over a period of 13 days, and after a 2-day rest period was again injected on the 16th and 17th days in the hope that the brain trauma increased its susceptibility. At no time did this animal or those of earlier passages show any evidence of poliomyelitis.

With mice, the same technic was used as with rats. In subsequent passages, the amounts of active virus added to the material of the site of injection were decreased, the interval between the last dose and the time of sacrificing the animals was increased and greater amounts of parts of the central nervous system, other than the site of injection, were used. A summary of the passages is as follows:

1. *First 10 passages.* A mixture of equal amounts of a suspension of brain tissue from the site of inoculation and active virus was given to mice twice daily. The animals were sacrificed for passage 1 to 3 hours after the second dose.

2. *Passage 10 to 15.* Serial passages were carried out every 36 hours. The animals were sacrificed 4 hours after the second dose. Mixtures injected consisted of 2 parts of passage material to 1 part of active virus.

3. *Passage 15 to 20.* Mice were sacrificed 8 to 12 hours after the 3rd injection of a combination of 1 part of active virus to 3 parts of transfer material.

4. *Passage 20 to 25.* Material transferred was made up of 3 parts of brain and brain stem and one part of active virus. The animals received 4 injections over a period of 48 hours and were sacrificed at 12 to 18 hours after the last injection.

5. *Passage 26 to 33.* Mice were sacrificed at 12 to 18 hours after the last injection of a series of 4 given over a period of 3 days.

6. *Passage 33 to 45.* Serial passages were carried out every 4 days, during which time the animals received 4 doses. The animals were sacrificed 24 hours after the fourth dose. The passage material was a mixture of 1 part of active virus and 4 parts of a suspension of mouse brain and brain stem. The material from the 24th and 45th passages injected into a series of mice in multiple inoculations, produced no effect in the mice nor did the virus in either of these passages survive for a longer time than in the preliminary experiment, when it was demonstrated in the mouse brain, 3 but not 5 days after intracerebral injection.

Inasmuch as Nungester[4] reported a paralysis in mice using poliomyelitis virus and mucin, attempts were made to produce the disease by the addition of mucin to the active virus and the material of the 24th passage. Results of this experiment were negative.

In the case of several virus diseases, young animals have been reported to be more susceptible than fully matured animals, so a series of five, 3 weeks old mice and 20 guinea pigs, 14 hours to

[4] Nungester, W. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1128.

2 days old were injected intracerebrally and intraperitoneally, but with negative results.

Attempts to Reduce the Resistance of Animals so as to Render Them More Susceptible. The first method used was to blockade the reticuloendothelial system by the intravenous inoculation of colloid particles. The colloid used was thorium dioxide (thorocotrast), which was kindly supplied by Mr. F. A. Degener of the Heyden Chemical Corporation, New York City. From the literature and from experiments I have carried out with staphylococcal infection in rabbits it appears that the resistance to bacterial diseases can be reduced by doses of colloid particles, large enough to paralyze or partially paralyze the reticuloendothelial system. Using rabbits, 8 cc. per kilogram was injected. However, no attempt was made to determine whether or not the blockade was complete by bilirubin determinations. The dose used gave the usual marked leucocytic blood changes, consisting of a rapid drop followed by a rapid rise in the number of white cells.[5, 6] For mice, almost double the rabbit dose per kilogram of body weight was used, which was very close to the lethal dose. Soon after the intravenous injection of the thorocotrast, the animals received a dose of 20% suspension of active virus intracerebrally and intraperitoneally. In the case of the mice, the 40th serial passage material was also used.

Although little evidence exists for the belief that resistance to poliomyelitis is connected with the glands of internal secretion, an attempt was made to produce the disease in animals in which the hypophysis had been removed, inasmuch as its removal produces atrophy and probably loss of function of all the internal secretory glands. The removal was carried out by Dr. Hans Selye of the Department of Biochemistry, McGill University, Montreal. Single inoculations and a series of 16 passages in animals with the glands removed, were done. Serial transfers were carried out as described above and all with negative results.

Summary. From these experiments, it appears that of all the ordinary laboratory animals, the mouse should be the best in attempting to produce poliomyelitis, for the virus survives in its brain for a longer time than in that of the guinea pig, rabbit or rat. Serial passage of the virus of poliomyelitis, for 45 generations in the mouse and 16 in the rat, failed to adopt the virus to either host. Likewise, the use of very young mice or guinea pigs proved ineffective. Blockade of the reticuloendothelial system with thorium

[5] Elvidge, A. R., *J. Path. and Bact.*, 1928, **31**, 33.

[6] Gottlieb, R., *Can. Med. Assn. J.*, 1933, **28**, 496.

dioxide failed to reduce the resistance to the disease in mice or rabbits, nor did the addition of mucin to the virus aid in using mice, or hypophysectomy in using rats.

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Cataract Formation in Rats Fed on a Diet Containing Galactose.*

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Mitchell and Dodge[1] report the occurrence of cataracts in rats fed on a high lactose diet. A similar investigation conducted in our laboratory corroborated their ocular findings. It was therefore considered of interest to determine whether cataracts could be provoked with galactose.

Eight female albino rats, 21 days old and weighing approximately 40 gm. were fed a diet consisting of 50% galactose, 20% cornstarch, 15% caseine, 9% crisco, 4% (Osborn and Mendel) salt mixture, and 2% cod liver oil. In addition, 5 drops of cod liver oil and 0.5 gm. dried yeast powder were fed separately each day.

The rats on this diet appeared well nourished and grew as well as the animals on the standard laboratory ration described by Bing and Smith.[2] In the course of 12 to 14 days, the young animals developed changes in the lens of the eye. The manifestation was bilateral. It was apparent from daily examination of the eyes that the lenticular changes developed in the nucleus of the lens.

Six animals were sacrificed for histological study and 2 were allowed to stay on the same diet. At autopsy no gross pathological changes were noted. The liver, kidney, adrenal, thyroid, and parathyroid glands were fixed for future histological study.

At the end of 7 weeks the remaining 2 animals have matured cataracts in both eyes and are still growing well. They show none of the deficiency manifestations noted in albino rats on diets lacking vitamin A or G. At no time during the experiment was a gastrointestinal disturbance noted.

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1 Mitchell, H. S., and Dodge, W. M., Jr., *J. Nutrition*, 1935, **9**, 37.

2 Smith, A. H., and Bing, F. C., *J. Nutrition*, 1928, **1**, 129.