# Minnesota Branch.

*University of Minnesota, December 15, 1926.*

3338

## Sodium Ricinoleate and Active Immunity Against Experimental Monkey Poliomyelitis.

J. C. McKINLEY AND W. P. LARSON.

*From the Division of Nervous and Mental Diseases and the Department of Bacteriology, University of Minnesota Medical School.*

The experiences of one of us (Larson[1]) with the detoxifying action of sodium ricinoleate in the case of several bacteria and several bacterial toxins, suggested the possibility of actively immunizing monkeys against experimental poliomyelitis through the medium of this soap. Consequently we obtained a sample of the "M. A. strain" of poliomyelitis virus from the Rockefeller Institute of New York, through the courtesy of Dr. Simon Flexner, and established the disease in our own animals.

In general, we are proceeding according to the following plan. A virulent sample of poliomyelitis is obtained by producing the disease in an animal by the intracerebral injection of stored glycerinated virus. The animal is then killed by chloroform during the height of the disease, and immediately about a gram of the brain is removed sterilly and ground up in 5 to 10 cc. of physiologic saline solution. This emulsion is used to continue the disease in another animal and to prove the potency of the soap-virus emulsion injected into the animals being immunized. At the same time another gram of the brain is ground up and emulsified with 5 to 10 cc. of 1 per cent aqueous sodium ricinoleate. This soap-virus emulsion is injected by different routes in normal animals to establish the assumed immunity. Some time later these animals are injected intracerebrally with a virulent saline emulsion of poliomyelitis brain to test their resistance.

Approximately 60 injections of the soap-virus emulsion have been administered in the animals by the cerebral, subcutaneous or intraperitoneal route. None of these animals have developed poliomyelitis within the incubation period of the disease following such an injection, though 2 of them showed complete hemiplegia immediately after frontoparietal cerebral injection, and died within 2 or 3 days. This result is readily correlated with the destruction of brain tissue from the action of the soap itself. Injection of 0.5 cc. of soap alone into a rabbit's brain is regularly fatal, and 0.5 cc. or more of the soap is commonly fatal to the monkey by this route, but 10 cc. are easily borne when injected subcutaneously or intraperitoneally. Microscopic examination of the animals dying shortly after cerebral injection of the soap-virus mixture showed absence of the lesions of poliomyelitis in the central nervous system. It appears from these limited observations that sodium ricinoleate attenuates or detoxifies the poliomyelitis virus so that the disease is not produced in animals by injecting soap-virus mixture containing a potent virus.

Animals No. 3, 14, 15 and 17 were treated for the production of immunity and 3 normal animals (10, 19 and 21) were used as controls as follows:

Animal No. 3. Male. Javanese macaque. November 14, 1925, this animal was injected cerebrally on the left side with 0.15 cc. of filtered 5 per cent emulsion of 1 per cent castor oil soap and glycerinated brain tissue from Rockefeller Institute monkey No. 1933. The animal showed a paralysis of his right side on recovery from the anesthesia, no doubt due to the action of the soap on the brain. The control animal inoculated with the same virus in saline solution did not develop poliomyelitis, so we considered it likely that the virus was not potent in this experiment. At that time we did not realize that glycerinated virus is an attenuated virus and often does not infect the animal injected. April 17, 1926, the animal was injected in the left parietal region of the brain with 0.7 cc. of fresh soap-virus emulsion from a paralyzed monkey which was killed the same day at the height of an attack of the disease. The control animal, injected with this material at the same time, developed poliomyelitis in 5 days, and was killed on the 6th day. Microscopic examination confirmed the presence of the disease. We considered that immunity had possibly been induced at this time in animal No. 3.

Animal No. 14. Female. *Macacus rhesus.* May 25, 1926, fresh soap-virus emulsion, prepared from a poliomyelitis animal which

had been killed immediately previously, was injected into the brain of this animal. The monkey showed a hemiplegia on recovery from the anesthetic, due to the destructive action of the soap on the brain tissue, but otherwise remained well. The control animal developed poliomyelitis June 4, as proved by the microscopic findings.

Animal No. 15. Female. Javanese macaque. One attempt was made to immunize this animal with glycerinated virus on December 4, 1925, but was considered unsuccessful as the control animal did not develop the disease. The injection was performed subcutaneously in the back. April 17, 1926, the animal was injected along with No. 3 with 5.0 cc. of the same soap-virus mixture, but the injection was performed intraperitoneally rather than cerebrally. As indicated under the description for No. 3, the control developed the disease on the 5th day and was killed the 6th day.

Animal No. 17. Male. *Macacus rhesus.* May 25, 1926, was injected intraperitoneally with 4.0 cc. of fresh soap-virus mixture at the same time that animal No. 14 was treated. As described under animal No. 14 the control developed the disease June 4.

June 6, 1926, in order to test the possible immunity of the 4 animals, they were injected cerebrally with 1 cc. of fresh virus in physiologic saline along with 3 normal control monkeys; No. 10, male, Javanese macaque; No. 19, male, *Macacus rhesus,* and No. 21, female, *Macacus rhesus.* Control No. 10 was found dead July 12, 1926. Control No. 19 showed the first symptoms of poliomyelitis July 14 and was chloroformed July 16. Control No. 21 showed the first symptoms July 13 and was chloroformed July 16. The microscopic examination of the central nervous systems of the control animals showed the typical lesions of poliomyelitis in each case. The experimental animals No. 3, 14 and 15 all remained perfectly well. No. 3 died September 2 from an extensive pulmonary and visceral tuberculosis, as proved by autopsy. Microscopic examination of the brain and spinal cord revealed no evidence of poliomyelitis. Animals No. 14 and 15 are living and well at the present time. Animal No. 17 developed mild signs of poliomyelitis 13 days after injection, lived 15 days with the disease, and was chloroformed at this time to obtain autopsy material in which the acute lesions of poliomyelitis were found.

Since 3 of the 4 treated animals remained well, and only one developed a delayed and slow case of poliomyelitis, while the controls all died during an attack of poliomyelitis, the deduction is permissible that complete immunity was induced in 3 of the animals and partial immunity in the 4th. But the interpretation of this experiment must be conservative because certain facts vitiate its sig-

nificance. Aside from the small number of animals experimented on, the most important of these is that we have been unable to produce the disease with more than 70 per cent to 75 per cent of the inoculations. On the other hand, in favor of the hypothesis that these animals were actually immunized, it is our general experience that if one animal of a number inoculated at a given time becomes infected, the rest of the controls usually succumb also, as in the final stage of the experiment just cited.

While this experiment is obviously inconclusive, it is just as obviously suggestive that sodium ricinoleate may be a medium for actively immunizing against poliomyelitis, and we intend to test this idea until we are satisfied that it is either true or false.

[1] Larson, W. P., and Nelson, E., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 278. Larson, W. P., Evans, R. D., and Nelson, E., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 194. Larson, W. P., Halvorson, H. O., Evans, R. D., and Green, R. G., *Colloid Symposium Monograph*, 1925, iii, 152. Larson, W. P., and Eder, H., *J. Am. Med. Assn.*, 1926, lxxxvi, 998. Larson, W. P., Huenekens, E. J., and Colby, W., *J. Am. Med. Assn.*, 1926, lxxxvi, 1000. Larson, W. P., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 497.

3339

## The Growth of the Spinal Axis of the Human Body in Prenatal Life.

LEROY A. CALKINS AND RICHARD E. SCAMMON.

*From the Department of Anatomy, University of Minnesota.*

It has been previously pointed out[1, 2, 3] that the growth of the external dimensions of the human body in the fetal period is directly proportional to the growth in length of the body as a whole. The same law holds true for many of the lineal dimensions of the internal organs and parts, and is particularly well illustrated by the growth of the spinal column in prenatal life.

We have studied these relationships by measurements of the total length of the spine and its parts in 148 specimens ranging from 2.5 to 55.0 cm. in total or crown-heel length, and have placed the results in the form of empirical formulae. These formulae were computed from the mean spine lengths for 5 cm. intervals of crown-heel length by the method of averages, weighting by the square root of the number of cases in each interval.

In all instances we have found that the relationship of the spine and its parts with the total body-length is a rectilinear one which may be represented by the expression: