INFECTIOUS MOTOR PARALYSIS IN YOUNG RABBITS.*

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PLATES 3 AND 4.

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The paralysis described in this article was observed during experiments planned to confirm, if possible, the rat and flea transmission theory of infantile paralysis as outlined by Richardson.[1,2] The investigations began about February 1, 1919, and continued over a 10 month period. In general it was planned to infect young rabbits through intracerebral inoculations with the virus of infantile paralysis and then to determine whether the infection could be transferred from the sick animal to the healthy through the bites of fleas.

For the primary intracerebral inoculations four viruses were secured.[3] The rat flea (*Ceratophyllus fasciatus* Bosc[4]) was used in most of the experiments. In a few cases dog and cat fleas (*Ctenocephalus*

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[1] Richardson, M. W., The rat and infantile paralysis—a theory, *Boston Med. and Surg. J.*, 1916, clxxv, 397.

[2] Richardson, M. W., The rat and infantile paralysis—a theory, *Am. J. Pub. Health*, 1918, viii, 564.

[3] Two straight monkey viruses were kindly furnished by Dr. George W. McCoy of the United States Hygienic Laboratory, Washington, D. C., and two mixed monkey viruses by Dr. Simon Flexner of The Rockefeller Institute for Medical Research.

[4] Craighead, E. M. Observations on certain Siphonaptera, *Entomol. News*, 1921, xxxii, 303.

canis Curtis, and *Ctenocephalus felis* Bouche) were used. In the preliminary intracerebral inoculations from 1 to 0.5 cc. of a suspension of glycerolated spinal cord in $\frac{N}{1}$ salt solution was used.

Fifteen rabbits were inoculated intracerebrally with virus from the Hygienic Laboratory without pathological effect. Eight guinea pigs and three rabbits were inoculated intracerebrally with Rockefeller Institute virus, and all remained well but one rabbit, No. 12. Beginning 12 hours after inoculation fleas were allowed to feed alternately first on inoculated and then on normal animals, and this sequence was continued daily until the fleas died, or until the secondary animal showed paralysis. About 150 fleas were used at each feeding.

March 20, 1919. Rabbit 12 (5 weeks old) was inoculated intracerebrally with 0.1 cc. of a suspension in $\frac{N}{1}$ salt solution of glycerolated spinal cord. March 25. The animal showed some tremor and developed slight paralysis of the fore quarters. April 22. Died. The pathological findings were negative.

Fleas which had bitten Rabbit 12 were transferred daily to Rabbits 12 B and 18 (7 days old), and the latter developed typical paralysis dying 33 and 40 days respectively after their first flea bites. There was never any possible physical contact with Rabbit 12. Under similar conditions nine other rabbits became paralytic and died, the intervals between the first flea bites and death ranging from 9 to 40 days.

It seemed, therefore, highly probable that a paralytic disease having its origin in human infantile paralysis had been induced in young rabbits and transferred from animal to animal by the bites of fleas. The fallacy of this conclusion was quickly demonstrated, however, by the proper control experiment, in which three normal rabbits were simply placed in cages with the sick rabbits, the intermediate biting of fleas being omitted. Two of these control rabbits developed paralysis and one died.

Thus we had to do, presumably, with a paralytic disease in young rabbits spread by simple contact. Further investigation, moreover, showed it to be associated with a large organism which, as will be seen later, can only by the remotest possibility, have anything to do with human infantile paralysis.

Clinically this rabbit disease is characterized by a persistent drowsiness, followed in most cases by tremor shortly before the development of paralysis. The paralysis may be slight or marked, general or localized, and the mortality is high.

During the disease period stained blood smears were examined daily, but nothing suggestive of microorganisms was found. In ten cases cultures were made upon every available medium from brain, cord, kidney, spleen, and liver but no growth occurred. Cultures and smears from the digestive systems and salivary glands of fleas gave negative results. Attempts to transmit the disease through the bites of bedbugs and mites were negative. Intracerebral inoculations of sedimented urine, also of cord emulsions from sick rabbits, reproduced the paralytic disease in other rabbits.

Examination of the infected rabbits showed the following gross and microscopic factors and conditions.

Spinal Cord and Brain.—The cords from nine animals, together with the brains of five of them, were examined. No definite gross lesions were apparent except softening of the cord.

Microscopic sections show small focal lesions in both the gray and white matter, which rarely occupy an area as large as the field of an oil immersion lens (Fig. 1). They consist in infiltration by small inflammatory cells with rounded or irregular shaped nuclei. Polymorphonuclear leucocytes do not take part in the infiltration. The nerve cells in the infiltration areas are diminished in number or absent. Occasionally a degenerated or necrotic nerve cell is present. The foci of infiltration in the white matter are associated with capillary blood vessels.

The lesions are present in one or more sections of the cord, or cord and brain, of each of the nine animals. In a cross-section of a cord one or more lesions (up to about half a dozen) may be present. In a few instances there is mononuclear cell infiltration about the vessels of the pia mater.

By appropriate staining methods peculiar bodies are demonstrable in most of the lesions. These are considered to be microorganisms and will be referred to as such. They are elongated, sharply and smoothly outlined, with rounded or conical ends, and are slightly variable in shape and dimensions. Their length and thickness are

probably never more than 4 and 1.5 microns respectively. As a rule, they do not stain homogeneously, but show one or two lighter staining areas. In general appearance they closely resemble bacilli. They stain by Gram's method and with methylene blue. Wright's stain does not show any nuclear structure in them and stains them only faintly. They are acid-fast to a certain extent. The best method of demonstrating them consists in first staining the section with carbolfuchsin (diluted 1:4), mordanting and decolorizing with undiluted formaldehyde,[5] and counterstaining with methylene blue. By this method the microorganisms are stained red and the nuclei of the cells blue.

The microorganisms are found in varying numbers in the lesions. They occur scattered among the infiltrating cells and occasionally, in large number, in nerve cells, or in compact aggregations in smoothly outlined rounded or oval spaces in the tissue, of about the size of a nerve cell. The cytoplasm of a nerve cell containing the microorganisms may be vacuolated and the cell body more or less transformed into a shell (Fig. 2). The spaces filled with microorganisms seem to represent destroyed nerve cells. In one instance a nerve cell in a spinal ganglion is thus apparently represented by a compact mass of the microorganisms.

Nerve cells invaded by the microorganisms and the aggregations in spaces are not always closely associated with the focal infiltration by inflammatory cells, and there may be no evidence of inflammatory reaction about them. The appearances seem to indicate that invasion and destruction of nerve cells is the first step in the development of some of the focal lesions.

Kidney.—The kidneys from five rabbits all showed essentially the same lesions, which were either not apparent to the naked eye or appeared as grayish spots and streaks on the cut surface. The lesions are chiefly in the medulla. They consist essentially of multiple small foci of infiltration by small cells with round, indented, or fragmented nuclei, and are marked by degeneration and disappearance of the epithelial cells in the infiltrated areas. Microorganisms like

[5] The use of formaldehyde for this differential staining of the microorganisms was suggested by Dr. William H. Smith, who has long employed it successfully as a mordant in staining certain bacteria.

those in the central nervous system are present. They occur chiefly in closely packed aggregation in epithelial cells and in the lumina of tubules in varying numbers along with free cells and detritus (Fig. 3). In the denser infiltrated areas they are generally few or not demonstrable. The number of epithelial cells harboring the microorganism is relatively small and they are scattered. In sections stained with fuchsin and methylene blue, as above described, there may be seen in tubules, along with the characteristic red-staining microorganisms, blue-staining bodies of varying shape and size which seem to be degenerated forms.

The microorganisms were found in the urine during life. The staining method above described served to differentiate them from the bacilli present, which stained blue (Fig. 4).

Spleen.—Sections of the spleen from four rabbits show the presence in the pulp of large mononuclear phagocytic cells containing what seem to be degenerate forms of the microorganisms. These are variable in size and shape and show an affinity for methylene blue instead of fuchsin.

Liver.—A section from only one animal was examined and this showed two or three small focal lesions.

Myocardium.—Sections from two animals were examined and a few focal lesions found.

SUMMARY.

1. The attempt to infect young rabbits and guinea pigs with material containing in all probability the virus of human infantile paralysis failed.

2. Failure to infect the primary animals almost of necessity brought failure with the secondary flea-bitten animals. It is, however barely conceivable that a non-infectious form of an organism might circulate in the blood of the primary animal and that this form, through development in an intermediate host, the flea, might become virulent for the secondary flea-bitten animal.

3. Incidentally, and presumably accidentally, a paralytic disease was observed in young rabbits associated with the presence of an organism showing certain definite characters. So far as we know this paralysis and the associated organism have not been previously described.

4. This organism is found widely distributed in the organs of the affected animals and can be demonstrated in the urine. The active destruction by the organism of the nerve cells of the spinal cord is particularly striking, and gives complete explanation for the paralysis observed clinically.

5. With the organism present in the urine the spread of the disease by contact can be easily understood.

6. The transfer of the infection from animal to animal by fleabites is possible but not probable.

7. The nature of the observed organisms is in doubt. They represent probably an intermediate stage in the life history of some protozoan parasite.

EXPLANATION OF PLATES.

PLATE 3.

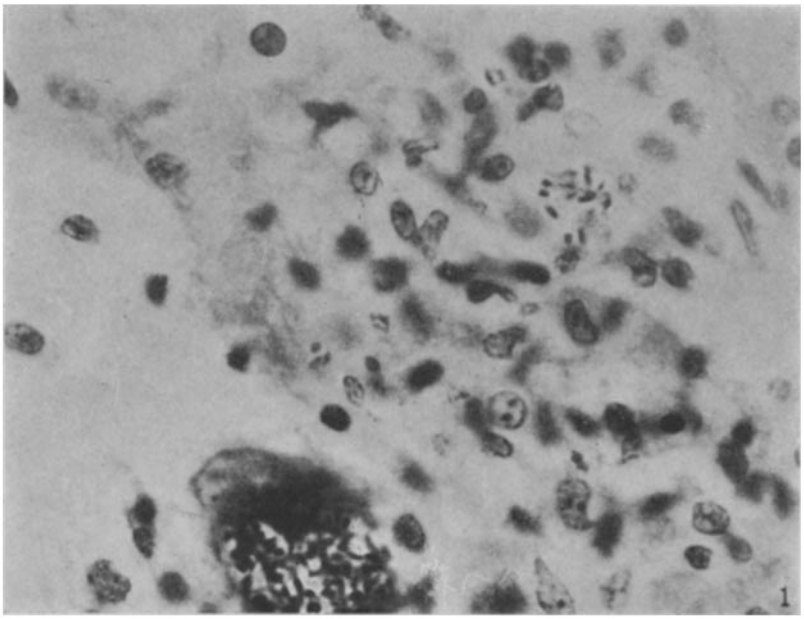
FIG. 1. A focal lesion in the spinal cord showing the infiltration by cells, and the microorganisms, many of which are in a degenerated nerve cell at the lower margin. × 1,000.

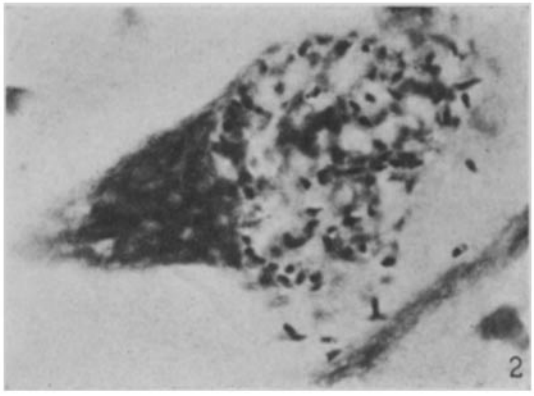
FIG. 2. Nerve cell of the spinal cord, degenerated and invaded by many of the microorganisms. A few microorganisms lie outside of the cell. × 1,000.

PLATE 4.

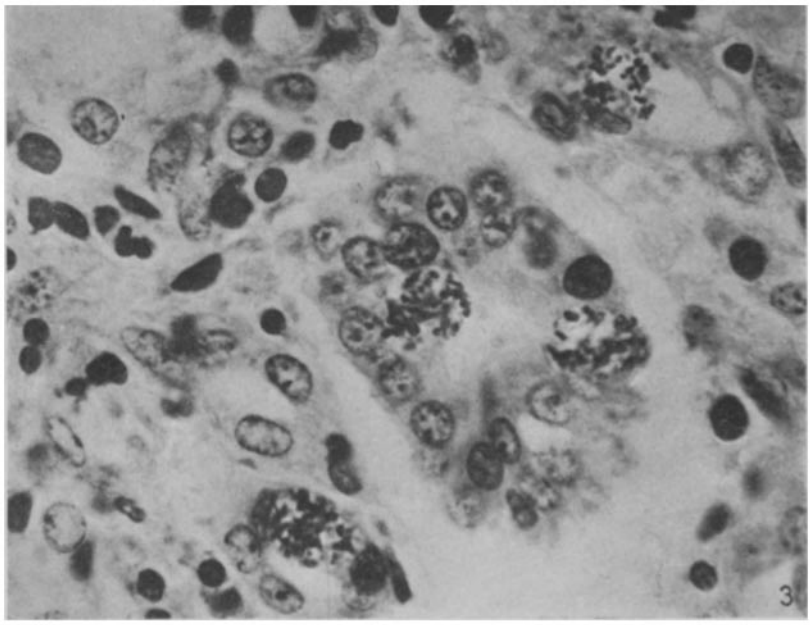
FIG. 3. Groups of microorganisms in four epithelial cells of the kidney. A sparse inflammatory cell infiltration is also shown. × 1,000.

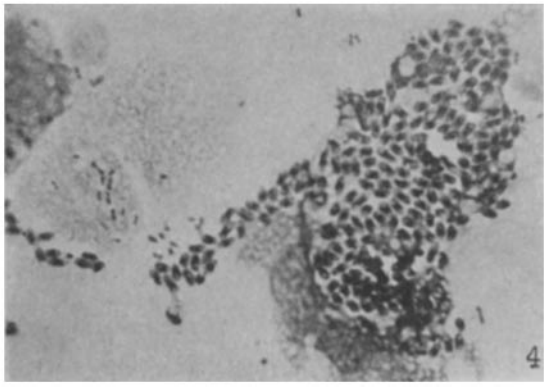
FIG. 4. A mass of microorganisms in a smear preparation from a urinary sediment. Some bacilli are also present. × 1,000.





(Wright and Craighead: Infectious motor paralysis.)





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