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ULTRASTRUCTURE OF PERIPHERAL NERVES OF MICE INOCULATED WITH RABIES VIRUS

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Fourty adult female albino mice were inoculated in the right hind leg with rabies viruses of the street type. The mice were sacrificed with an interval of 24 hours each, starting in the next day after inoculation. From the 10th day ownwards the animals started presenting signs of paralysis, first on the leg where the viruses were inoculated and later in the other ones. Twenty-four hours after the initial signs, ultrastructural abnormalities were found in peripheral nerves compatible with axonal degeneration with secondary demyelination but the rabies viruses were not found in the axoplasm, myelin sheet, Schwann cell cytoplasm, endoneural or in the epineural structures.

MATERIAL AND METHODS

The material used in this investigation is derived from fourty adult female albino mice. They were inoculated with 0,5 ml of rabies preparation having a titer of 10^3 LD 50/0,03 ml I.C. The viruses were of the street type and they were inoculated intramuscularly in the right hind leg of all mice. Twenty-four hours later the first animal was sacrificed and both sciatic nerves were taken and divided into three segments (distal, medial and proximal). The same procedure was carried out twenty-four hours later and then successively until the 18th day, when the remainder of the mice succumbed to the viruses. After being removed the specimens were laid immediately on a piece of card and kept slightly stretched by means of pins applied to either end of the specimen. The specimens were then immersed in a cold 4% glutaraldehyde in a cacodilate buffer. After two hours of fixation the specimens held by the pins were released and cut in small pieces of about 1mm

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thick. They were washed in two changes of cacodilate buffer for 15 minutes each. Post fixation was carried out for 30 minutes at room temperature in cold 2% osmium tetroxide in cacodilate buffer. After being washed in distilled water and dehydrated in ascending grades of alcohols, they were embedded in a mixture of Polylite. Thin sections were cut on a Sorvall ultratome and collected on copper grids, stained by uranil acetate and lead citrate (Reynolds, 1963) and examined in longitudinal and cross sections in a "Philips 300".

RESULTS

It was observed that from the 10th day onwards the mice still alive started showing paralysis of the right hind leg; from the 11th day onwards the same was observed with regard to the controlateral leg and after the 14th day the mice showed signs of paralysis in the four limbs. On the 18th day the remaining animals died and by imuno-fluorescent techniques the viruses were detected in their brain.

Electron microscopic evaluation of the peripheral nerves revealed that those originated from mice sacrificed from 24 hours after inoculation to the 8th day did not show any structural abnormalities. But from the 9th onwards, it was observed that the specimens originated from the right ciatic nerves had ultrastructural changes like those seen in axonal degeneration and a slight degree of secondary demyelination.

The same could be noticed in the specimens coming from the left ciatic nerves after the 11th day of the initial date of the experiment.

The axonal degeneration was characterized by alterations of the axoplasm (Fig. 3,5), decrease in volume of the axis cylinder (Fig. 5,8) and myelin break down into large ovoids (Fig. 4). Mastocytes were only occasionaly seen in the endoneuriun (Fig. 2) but phagocytes which usually contained myelin in their cytoplasm occurred frequently between the nerve cells (Fig. 3,7). At times, remyelinated fibers could be seen (Fig. 6). The ultrastructural changes observed were more intense in the mice with the longest period of disease, but no particles resembling rabies viruses were seen in the examined structures at any time.

COMMENTS

It is well known that the rabies virus is a Rhabdovirus and has an external capsule containing RNA inside. The virus has an elongated form ("bullet-shaped") and its size has been estimated to be 100-150mμ in the transverse section and 150-250mμ in the longitudinal. Figure nº 1 shows rabies viruses in cross section found in the brain of the animal sacrificed at the 15th day after inoculation. The manner by which the rabies viruses gain access to the central nervous system in natural infections and experimental modelss is controversial. Various theories of haematogenous and neural pathways have been proposed; those in favor of the neural pathway state that the viral particles travel along or in the nerves from the site of exposure to the CNS. According to Johnson (1965) "the relationship of the early sensory and motor symptoms to the site of exposure in man and dogs is evidence for the invasion of the

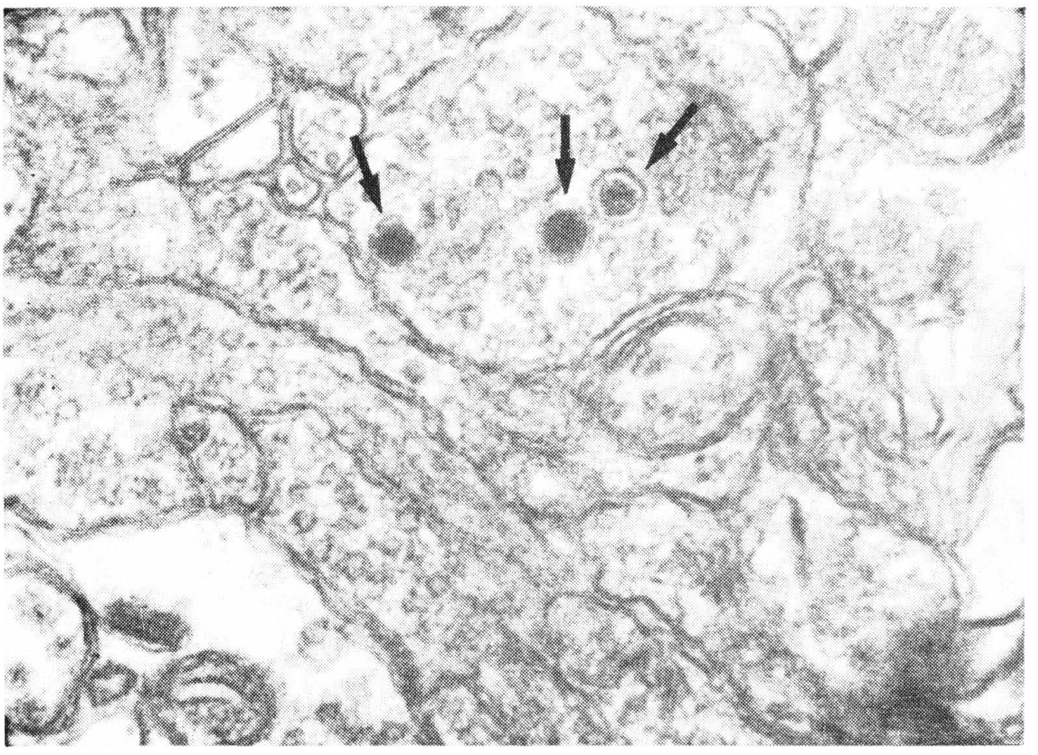

Fig. 1 — A nerve cell of a mouse brain sacrificed at the 15th day after inoculation shows three rabies viruses (arrows). X 77,000

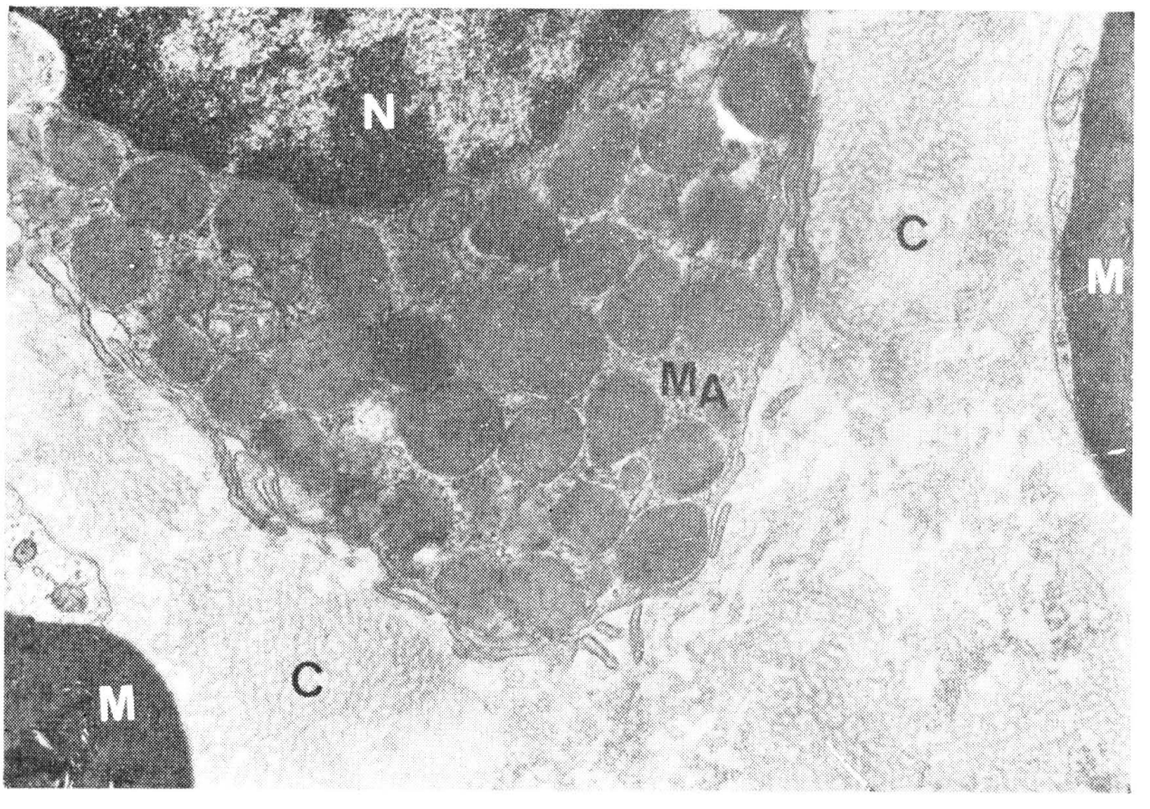

Fig. 2 — Electron micrograph of part of a mastocyte between nerve cells. Collagen fibrils are abundant between the structures. X 25,000. 12h day. C = collagen, Ma = mastocyte, M = myelin, N = nucleus.

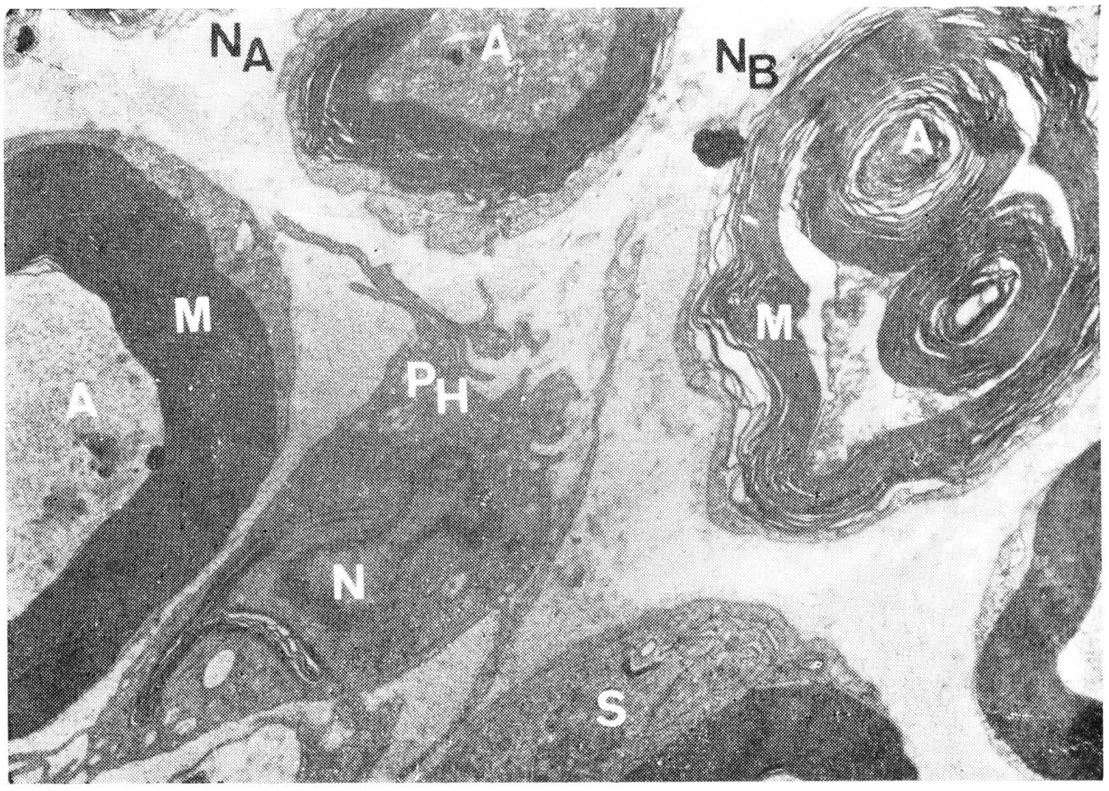

Fig. 3 — A phagocyte with myelin debris in its cytoplasm is seen among nerve cells, two of which (NA and NB) are undergoing axonal degeneration. That of the right upper corner shows almost no axon and the myelin sheath is in dissolution. X 16,000. 14th day. A = axon, M = myelin, N = nucleus, Ph = phagocyte, S = Schwann cell cytoplasm.

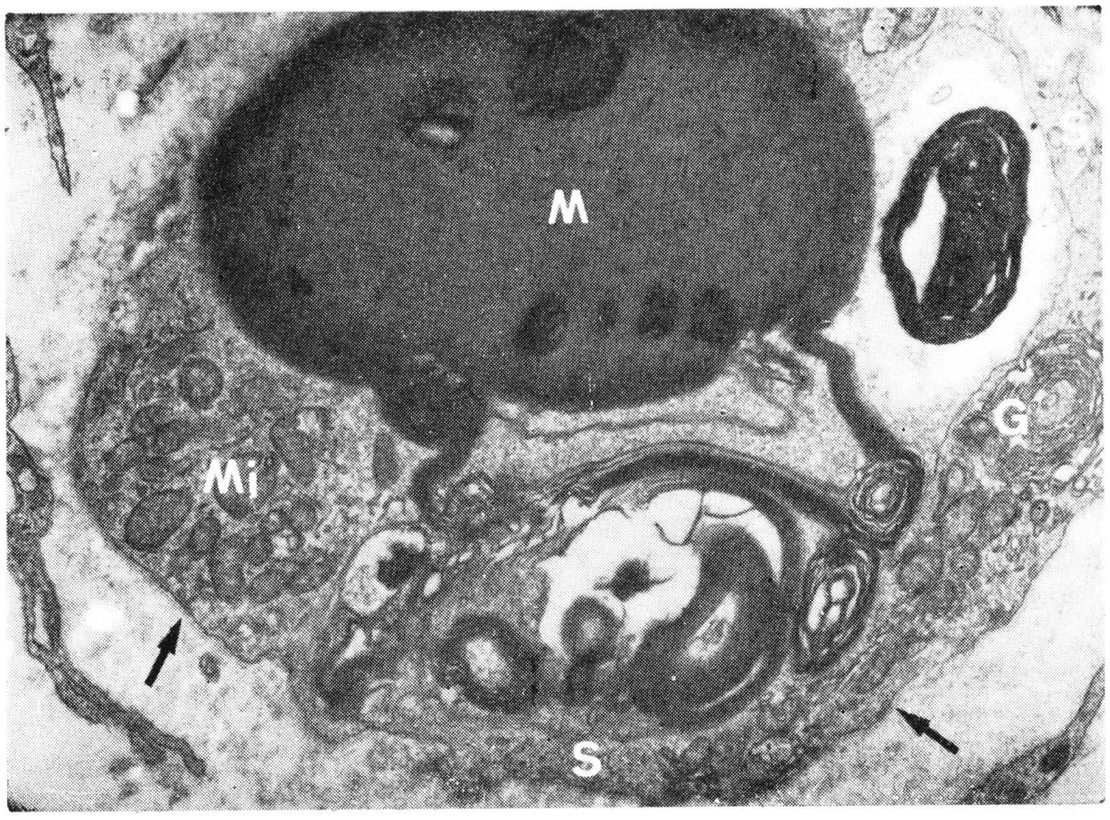

Fig. 4 — A large mass of myelin ovoid and its degradation occur within a Schwann cell cytoplasm which exibits also numerous mitochondria. A layer of basement membrane (arrows) occurs around the cell. X 20,500. 15h day. G = Golgi apparatus, Mi = mitochondria, M = myelin, S = Schwann cell cytoplasm.

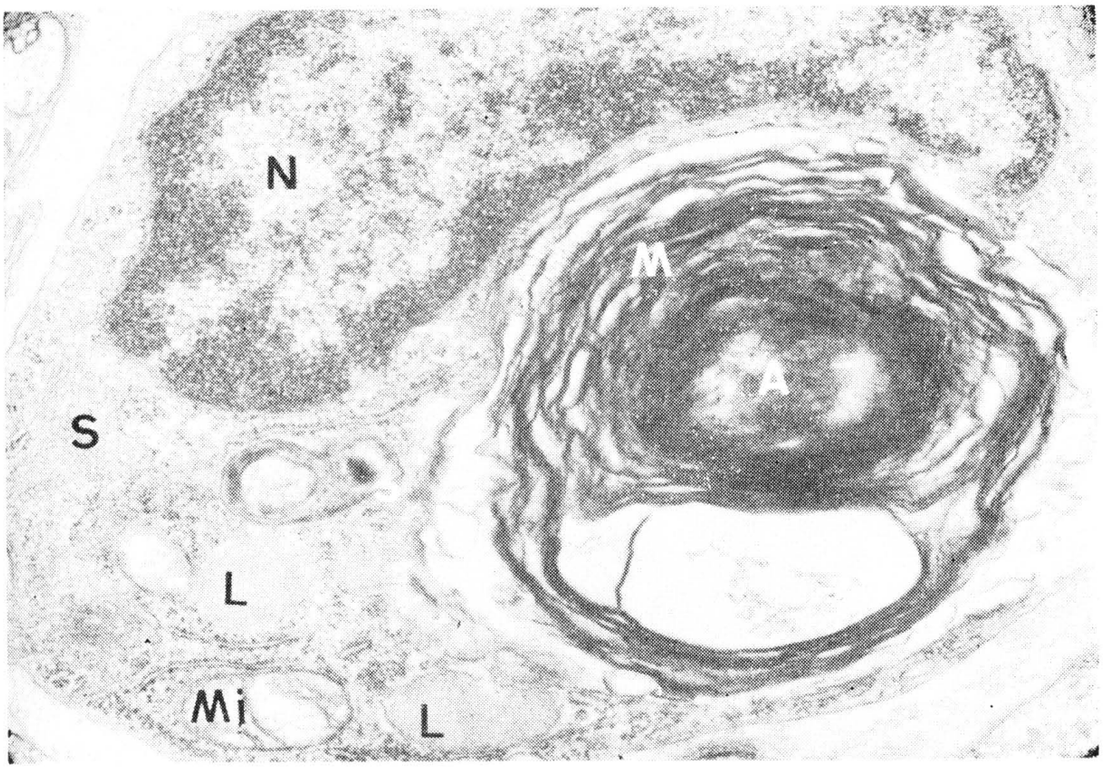

Fig. 5 — This electron micrograph shows a degenerating axon in cross section surrounded by myelin lamellae in dissolution. Note the desproportion between the axon and other parts of the Schwann cell. X 31,000. 12th day. A = axon, L = lipid droplet, N = nucleus, Mi = mitochondria, M = myelin, S = Schwann cell cytoplasm.

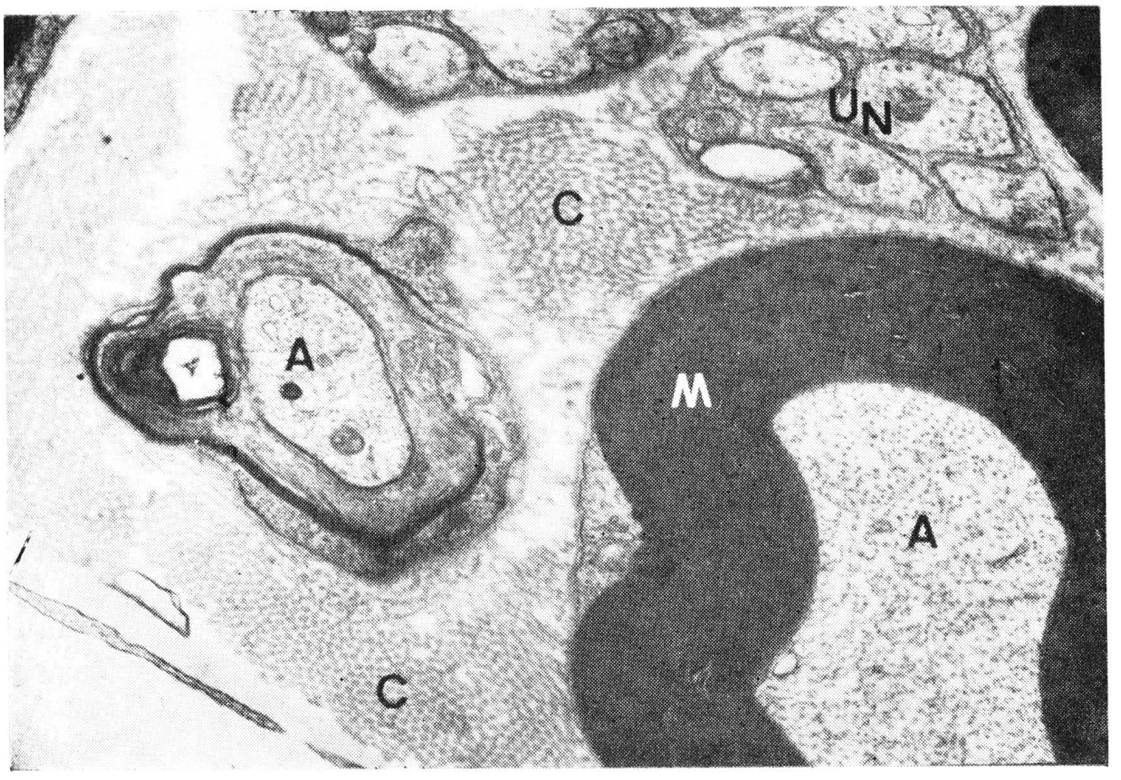

Fig. 6 — Left is a remelinated fiber in transverse section. Note the characteristic thin myelin sheath and lack of compaction of myelin lamellae. Right is a normal myelinated fiber. X 25,000. 13th day. A = axon, C = collagen, M = myelin, Un = unmyelinated nerve fibers.

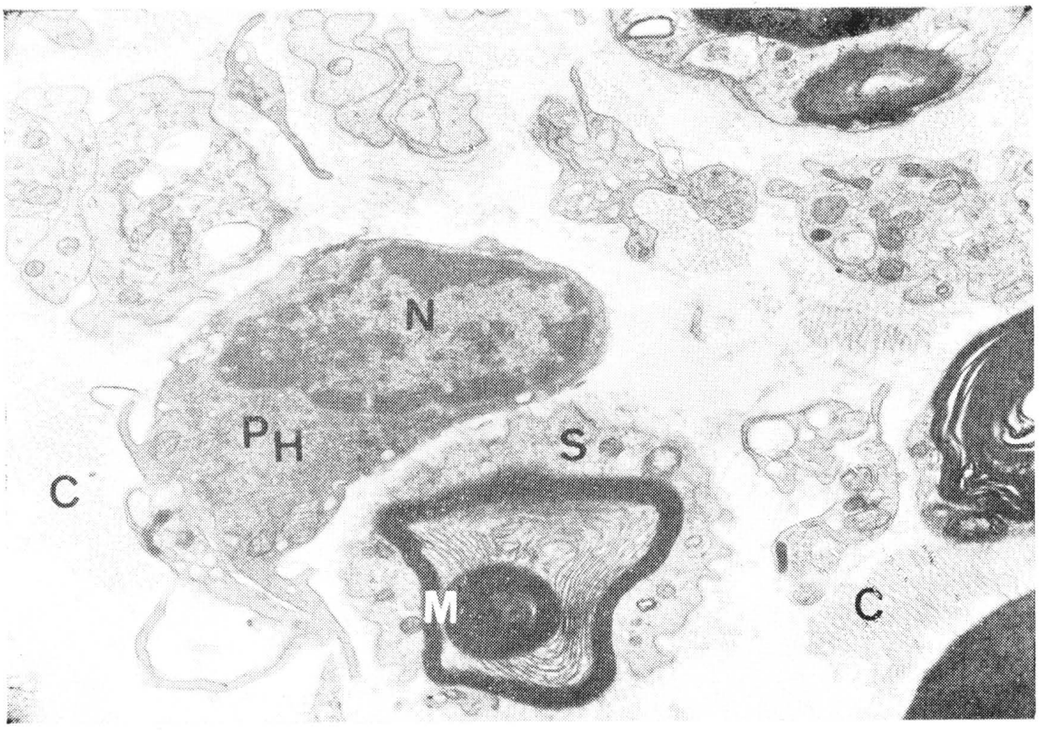

Fig. 7 — A phagocyte with long processes is near to a nerve fiber in degeneration. This fiber shows almost no axon. The myelin lamellae is in dissolution and the contour of the Schwann cell cytoplasm appears irregular although its basement membrane is well defined. Several unmyelinated axons and parts of others Schwann cells occur nearby. Upper right is part of another myelinated fiber. X 16,000. 15th day. C = collagen, Ph phagocyte, N = nucleus, M = myelin, S = Schwann cell cytoplasm.

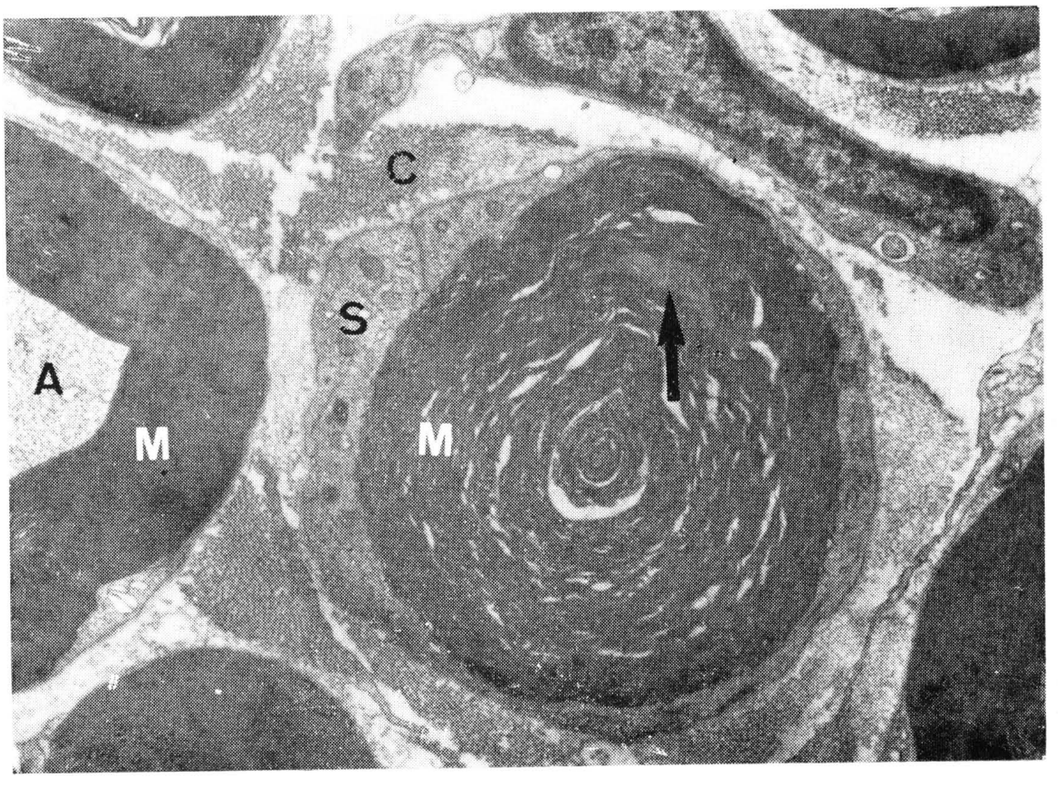

Fig. 8 — Within a Schwann cell cytoplasm is a large mass of myelin in dissolution with almost no axon (arrow). Parts of myelinated fibers are seen nearby. X 17,000. 9th day. A = axon, C = collagen, M = myelin, S = Schwann cell cytoplasm.

central nervous system by way of the nerve pathway and the segment of the central nervous system first invaded corresponds to the site of intramuscular inoculation", although some authors believe that those symptoms are due exclusively to an ascending myelitis instead of a manifestation of peripheral nerves involvement. To avoid general infection some authors have carried out neurectomy prior to the inoculation and this sparing effect of neurectomy suggests that the virus travels to the CNS via peripheral nerves. In 1963 Dean et al. through experimental models calculated the rate of propagation of the viruses. They have estimated it in about 3mm per hour. Kaplan et al. (1962) and Dean et al. (1963) have also suggested that local anesthetics injected intramuscularly proximal to the site of exposure has a sparing effect due to the anesthetic action on the metabolism of the inoculated tissue.

Although all these theories of axonal spread have been popular for many years, nobody has ever shown the rabies viruses in peripheral nerves and how they move within nerves has remained a perplexing problem. The main purpose of the present work was to detect the virus particles along the neural routes (axons, Schwann cell cytoplasm, myelin sheeth or tissue spaces occurring between the nerve fibers). Unfortunately no evidence of the virus particles was found in the neural structures, but their effect on the axons are remarkable. It is possible that, after inoculation, the viruses looses their capsules and replicate once in the axoplasm. If this happened, by electron microscopy the viral RNS molecules would become undistinguishable from the rest of the axoplasm content and throughout the axoplasm these dangerous "invaders" would reach the medula by active reproduction and, later, upper parts of the CNS. This behaviour would explain the sparing effect of neurectomy and anesthetics injected proximal to the site of expossure. The evidence of striking axonal degeneration along with secondary demyelination may explain the signs or the acute progressive paralytic course of the disease. These findings contrast with the theory that the paralytic course of the disease is due to exclusively an ascendig myelitis. From what was observed both processes may take part in the pathogenesis of the paralysis.

RESUMO

Ultraestrutura de nervos periféricos de ratos inoculados com virus da raiva.

Alterações ultraestruturais bastante significativas foram encontrados em nervos periféricos de ratos inoculados com virus da raiva. Tais achados se caracterizam por degeneração axonal com desmielinização secundária. Os achados são precedidos em cerca de 24 horas por sinais clínicos correspondentes. Contudo, os nervos examinados não apresentaram partículas com as características das do virus da raiva. É possível que após a inoculação os virus percam suas cápsulas e as moléculas de ARN que os constituem se confundam com o conteúdo axoplasmático, tornando-se indistinguíveis pela microscopia eletrônica. Só dessa forma poder-se-ia explicar a ação deletéria das partículas virais nos axônios e consequente tramitação centrípeta em direção à medula

e partes mais altas do sistema nervoso central, sem serem detectados pela microscopia eletrônica. A degeneração axonal encontrada, com consequente quadro de polineurite, mostra que os sinais periféricos não são exclusivamente de uma mielite ascendente. Ambos os processos podem estar envolvidos.

REFRENCES

1. DEAN, D. J.; BAER, G. M. & THOMPSON, W. R. — Studies on the local treatment of rabies-infected wounds. Bull. WHO. 28:477, 1963.

2. DEAN, D. J.; EVANS, W. M. & McCLURE, R. C. — Pathogenesis of rabies. Bull. WHO. 29:803, 1963.

3. JOHNSON, H. N. — Rabies Virus. *In* Viral and Rickettsial Infections of Man. Ed. Horsfall, F. C. & Tamm, I. 4th Edition. 1965.

4. KAPLAN, M. M.; COHEN, D.; KOPROWSKI, H.; DEAN, D. J. & FERRIGAN, L. — Studies on the local treatment of wounds for the prevention of rabies. Bull. WHO. 26:765, 1962.

5. REYNOLDS, E. S. — The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell. Biol. 17:208, 1963.

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