REPRODUCTION OF EXPERIMENTAL ALLERGIC POLYNEURITIS WITH A PURIFIED MYELIN FRACTION

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Injection of purified myelin obtained from the bovine sciatic nerve by differential centrifugation and centrifugation in a sucrose density gradient, mixed with Freund's adjuvant, caused the development of experimental allergic polyneuritis in rabbits in more than 90% of cases. In their character, severity, and topography, the morphological changes were basically similar to those developing after injection of a suspension of nerve trunk tissues. The allergen responsible for development of the polyneuritis was shown to be present in myelin. The axoplasm and other connective-tissue components of the nerve trunk do not play an essential role in the development of periaxonal demyelinization.

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Experimental allergic polyneuritis (EAP) is usually produced by intradermal or subcutaneous injection of homologous and heterologous nerve trunk tissues. The latter, however, are considerably contaminated by axoplasm and connective-tissue structures, which may complicate the morphological picture observed. The problem of the extent to which the pathomorphological changes in EAP produced by purified myelin and by whole nerve trunk tissue are similar or different is therefore one of considerable importance.

EXPERIMENTAL METHOD

EAP was caused by injection of a myelin fraction obtained from the bovine sciatic nerve by differential centrifugation and centrifugation in a sucrose density gradient. The high level of purification of this myelin fraction from contamination by other subcellular particles was verified enzymologically and confirmed under the electron microscopw (Fig. 1a).

Since the process of obtaining purified myelin from nerve trunks is not familiar and presents certain difficulities, a few technical details of this procedure will be given. The bovine sciatic nerve was freed from fat and connective tissue and twice minced on a freezing microtome to obtaine a homogeneous mass. The myelin was isolated by a modification of the method used by Cuzner and co-workers [2] in the cold at 4°. A weighed sample of minced tissue was homogenized in 0.32 M sucrose solution (made up in 0.01 M tris-buffer pH 7.2-7.4) in the proportion of 1 g/10 ml in a knife homogenizer for 10 min. The resulting homogenate was transferred to centrifuge tubes, carefully balanced, and centrifuged at 1000 g for 10 min. The residue was discarded and the supernatant again centrifuged in a type MSE refrigeration centrifuge at 13,600 g for 30 min. The supernatant after this second centrifugation was discarded, and the residues from all the tubes were pooled and suspended in a glass homogenizer in 0.32 M sucrose solution in a ratio of 1 : 5, based on the initial weight of the tissues. This suspension was poured as a layer above 0.8 M sucrose solution in the ratio of 1:1 and centrifuged at 20,000 g for 1 h. A thick, opaque layer of myelin was formed at the boundary between the 0.8 M and 0.32 M sucrose solution; the myelin was carefully withdrawn by a pipet, aiming to take up as little sucrose as possible into it. A sample was taken from this fraction for electron-microscopic investigation. The resulting fraction of suspended myelin was diluted 1:10 with cold physiological saline and placed in an ice bath for 20 min, after which it was centrifuged at 20,000 g for 40 min. The precipitate of purified myelin thus obtained was used in the experiments.

According to reports in the literature, myelin is usually precipitated in distilled water, because a hypotonic medium liberates it more readily from contaminating membranous structures. However, our

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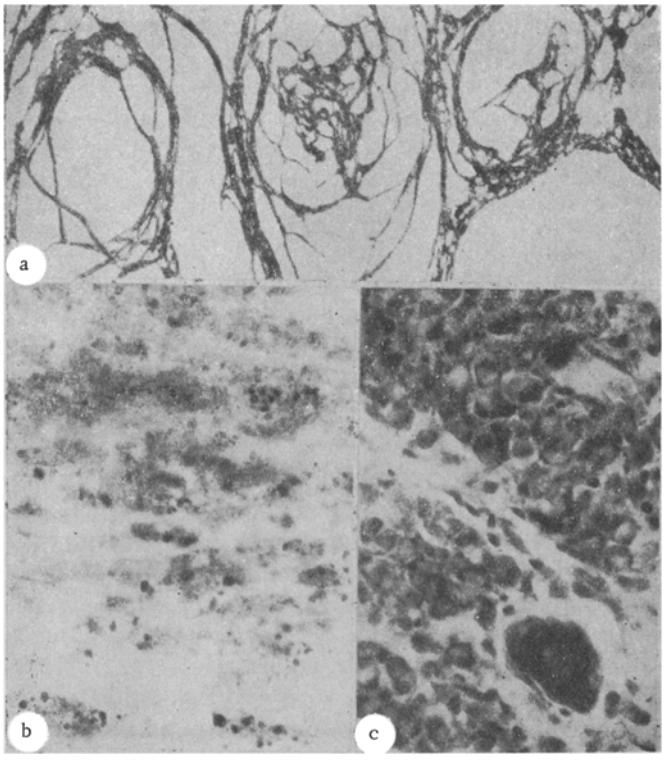

Fig. 1. Laminar structures of myelin isolated from bovine sciatic nerve (a), 8000 ×; osmiophilic granules of fragmented myelin in sacral sensory ganglion of spinal cord (b) in differnt stages of lysis (Marchi, × 120); c) collection of epithelioid cells closely connected with a blood vessel wall in the ganglion nodosum of the vagus nerve (Nissl, 500 ×).

observations showed that precipitation with physiological saline also yields an adequately purified fraction, and also gives a denser residue which can more easily be collected, thereby reducing the loss of myelin during its isolation. Investigations definitely showed that quantity of myelin obtained from fresh nerves was constantly higher than that isolated from frozen nerves, in agreement with the observations of O'Brien and co-workers [7].

Experiments were carried out on 43 male rabbits weighing 2.5-3 kg. Various doses of the neuritis-inducing mixture prepared from moist myelin and Freund's adjuvant were injected intradermally into the plantar pads of all four limbs. The animals received 2, 4, 8, 12, 16, or 22 mg myelin estimated as dry weight. The technique of inoculation with the allergen has been fully described elsewhere [1]. Material for histological examination was fixed in 10% neutral formalin, 96° alcohol, Orth –Mueller fluid, or 80° alcohol with acetic acid, and embedded incelloidin. Histological sections were stained by the Marchi and Nissl methods, and with hematoxylin-eosin, and impregnated by the Cajal–Favorskii method. All principal divisions of the peripheral and some parts of the central nervous system were investigated. Animals were sacrificed at various times: a few days before development of the disease, soon after appearance of the first signs of polyneuritis, and between the 1st and 97th day of the disease.

EXPERIMENTAL RESULTS

After a single injection of allergenic mixture containing 4-22 mg dry myelin together with Freund's adjuvant, signs of polyneuritis appeared in more than 90% of the animals: lowering of muscle tone and paresis of the hind limbs or all four limbs, together with weakening or complete disappearance of tendon reflexes. If the dose of myelin used was from twice to three times greater than the minimal effective dose (4 mg), a disease of about the same severity developed as when the minimal dose was given. Injection of a large dose of myelin led to the development of the disease in a more acute form.

Severe periaxonal changes in medullated fibers were found in the animals developing the disease in the sensory (spinal and Gasserian ganglia, ganglia nodosa) and sympathetic (superior cervical and stellate ganglia, ganglia of the celiac plexus) ganglia. Postmortem examination of rabbits a few hours after appearance of signs of the disease revealed advanced and widespread fragmentation of the myelin. From the very beginning this was seen as tiny granules impregnated black with osmium, but during the next 3-4 days it began to become paler and more grayish in color (Fig. 1b). The tiniest granules became dust-like in appearance.

The periaxaonal disease constantly caused marked proliferation and hypertrophy of the Schwann cells, whose cytoplasm contained most of the osmiophilic granules. From the 5th-6th day of the disease, myelin breakdown products were ingested also by hematogenous macrophages, in which they persisted for a long time and continued to be impregnated with osmium. In many periaxonal changes were found in most nerve fibers of the sensory ganglia. The changes were particularly marked and constant in the Gasserian ganglia and ganglia nodosa. Periaxonal changes in the spinal ganglia were essentially indistinguishable from those taking place in the Gasserian ganglion and ganglion nodosum, but they were usually less severe.

Soon after the beginning of myelin fragmentation, an inflammatory reaction developed in the affected parts of the peripheral nervous system, increasing in severity gradually but fairly rapidly. In the first 2-3 days of the disease it was manifested primarily by infiltration of the walls of the thin blood vessels and surrounding zones with lymphocytes and monocytes; a less marked infiltration with these cells also extended throughout the stroma of the ganglia. In some animals, at a rather later stage, epithelioid cells began to predominate among the collections of inflammatory cells, in which they were arranged in several rows around the blood vessels, and closely connected with their walls (Fig. 1). Despite the marked periaxonal and inflammatory changes, no severe disturbances were observed in the nerve cells of the sensory and a autonomic ganglia, and at the height of development of the inflammation, only the initial signs of hyperchromatosis of the nucleus and moderate chromatolysis, usually reversible in character, could be observed in them.

Comparison of the inflammatory reaction and the periaxonal changes showed that during the first few hours of development of polyneuritis, when severe myelin fragmentation occurred from the very beginning, the inflammatory infiltration was very slight in degree or absent altogether, becoming of significant intensity only at the height of the pathological process. Even with marked development of periaxonal and inflammatory changes, these two processes did not necessarily coincide topographically. Whereas the periaxonal disease was most marked at the point of ramification of intraganglionic nerve fibers, the inflammatory infiltration was most marked near the nerve cells of the ganglion.

The purified myelin fraction isolated from the bovine sciatic nerve produced a definite picture of experimental allergic polyneuritis in a higher percentage of cases (over 90%) than the neuritis-inducing suspension of whole nerve trunks (60-70%). In their character, severity, and topography, however, the morphological changes arising after injection of purified myelin were basically indistinguishable from those arising after injection of a suspension of nerve trunk tissues.

The results of the present investigation show that the allergen responsible for development of polyneuritis is present in myelin. This is in full agreement with results obtained by workers [4, 6] who obtained purified myelin by differential centrifugation from the central nervous system and used it to produce an allergic encephalomyelitis. Immunomorphological investigations in encephalomyelitis also confirmed the localization of an encephalitogenic allergen in myelin [3, 5, 8].

Comparison of the results obtained in this investigation with those published previously, relating to injection of purified nerve trunks with adjuvant shows that the presence of axoplasm and connective-tissue components of the nerve trunk as impurities does not play an essential role in the development of periaxonal demyelinization.

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