2 motor axons; one of these fibres would innervate NB muscle fibres, while the other would innervate NC muscle fibres alone. We obtained no evidence for overlapping innervation of NB and NC muscle fibres. Two types of motor nerve terminal could be seen, one resembled the 'plate ending' of cat muscle spindles, the other was a fine single filament. The morphology of the ending, as seen in silver-stained specimens, was not specific to either NB or NC fibres although 'plate' endings were more common on NB fibres. Six cases of skeleto-fusimotor innervation were seen in which the motor nerve divided, sent one branch to skeletal muscle end-plates and the other branch to NB intrafusal muscle fibres.

The muscle spindle in the lumbricals of the rat would appear to be an elementary type of organ: it contains about 2 nuclear bag and 2 nuclear chain muscle fibres which do not branch, and the 2 types of muscle fibre receive separate motor innervation. The sensory innervation is also uncomplicated, 50% of the spindles possessing primary innervation alone. Skeletofusimotor innervation was found in some cases, but it is not possible to say, on the present evidence, how common this condition might be. However, it is possible to estimate, from the numbers of motor nerve fibres and the numbers of spindles in each muscle, that some form of α- and γ-motor-nerve sharing is likely in these muscles.

Zusammenfassung. Die Morphologie und Innervation der Muskelspindeln der Lumbricalmuskulatur im Hinterfuss der Ratte wird beschrieben. Eine typische Muskelspindel enthält zwei «nuclear bag» Fasern und zwei «nuclear chain» Fasern. Jede Spindel enhält 2 motorische Nerven, einer innerviert die «nuclear bag» Fasern, der andere die «nuclear chain» Fasern. 50% der Spindeln haben nur primäre sensorische Innervationen, die übrigen 50% haben eine primäre sensorische Endung und 1 oder 2 sekundäre Endungen.

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Migration of Lymphocytes Through the Endothelium of Venules in Experimental Allergic Neuritis

Intradermal inoculation of peripheral nerve tissue mixed with Freund's adjuvants in various mammals can produce a neuritis which is caused by immunologic mechanisms and called, therefore, experimental allergic neuritis or EAN[1]. In well-developed cases lesions which consist of perivenular collections of mononuclear cells and associated foci of demyelination are scattered throughout the peripheral nerves.

In this laboratory we have been for some time engaged in a light, phase, and electron microscopic study of the genesis of the inflammatory process of EAN in rats[2].

The light and phase microscopic studies show that, beginning 8 or more days after immunization, mononuclear cells may appear within the lumen of venules in the peripheral nerves, in perivenular areas and between nerve fibers prior to any recognizable alteration of myelin sheaths. Similar observations have been made in studies of early lesions in experimental allergic encephalomyelitis or EAE[3], a disease analogous to EAN but in which central nervous tissue is used as antigen and in which the lesions are confined mainly to the central nervous system. It has been concluded that in both diseases hematogeneous cells, presumably sensitized, are attracted to sites containing antigen, i.e. myelinated fibers, in the peripheral and central nervous system respectively. Here they subsequently in a hitherto unknown way will cause destruction of myelin. The mononuclear cells seem to be lymphocytes in the process of reaction i.e. 'transforming lymphocytes'[4]. It is not known why the lymphocytes in the first phase of the disease become arrested during their circulation, nor why they adhere to and migrate through the vascular wall and insinuate themselves between fibers in the peripheral nervous system.

During our electron microscopic studies of venular lesions in EAN, we have noted cells in cavities within the endothelium (Figure). We have concluded that these cells are sensitized lymphocytes, which at the time of sacrifice, were in the process of moving from the venular lumen towards the perivascular area. We further conclude that the normal route of migration of such cells in EAN is through rather than between the endothelial cells. These conclusions are based upon the following observations: (1) few if any cells are to be found in the lumen or in the perivascular areas of venules in peripheral nerves of normal animals or in animals killed during the first few days after inoculation, providing the vascular bed has been perfused according to the technique currently in use in this laboratory[5]. (2) In animals killed 8 days or more after inoculation venular lesions are noted frequently. In some specimens cells collect within but not outside a segment of a vessel; the lymphocytes are then attached to the inner side of the endothelium. In other animals cells are seen both within and without a vascular segment. Lesions of the former type predominate in animals killed earlier, lesions of the latter type in those killed later. (3) By cytologic criteria the 'intraendothelial' cells are of the same type as the intravascular lymphocytes which cling to the endothelial surfaces. (4) 'Intraendothelial' cells have been seen only in sites where there are intra and perivascular cells in the same or in an adjacent venular segment. (5) Degenerated or dead lymphocytic

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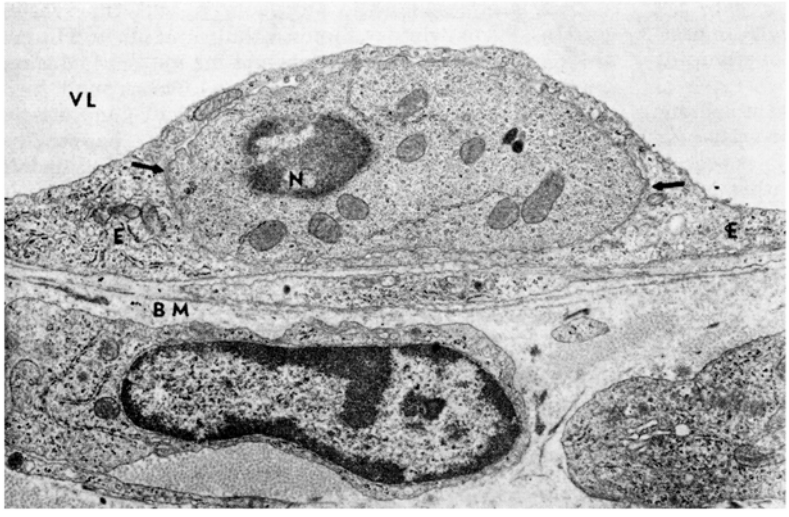

Lymphocyte (arrows) is situated within membrane-lined cavity of endothelial cell (E) in venule of sciatic nerve in rat with experimental allergic neuritis. Lymphocyte is surrounded by a mostly well-defined membrane; its nucleus (N) has been cut tangentially and the cytoplasm contains mitochondria, many ribosomes but scant endoplasmic reticulum. Below basement membrane (BM) is another cell which presumably already has migrated through the vascular wall. VL = vascular lumen. × 7,600.

cells are occasionally seen in perivascular or interstitial areas but never within the endothelial cells or in the vascular lumen. (6) Cells of the type under consideration have not been seen between endothelial cells in the course of this study. (7) Serial sectioning has shown that the cells may be confined in toto within endothelial cells.

Even in instances with vigorous inflammation only a limited proportion of lymphocytes are seen within the vascular wall itself, suggesting that the transit time is short.

In acute inflammation of the classic type polymorphonuclear leucocytes as well as true monocytes migrate through the intercellular junctions[6]. It has been noted that in inflammatory responses lymphocytes behave differently from the polymorphonuclear leucocytes and the monocytes but their mode of penetration has previously been unknown[6]. The observations reported here show that in the allergic inflammation of EAN cells of lymphocytic origin reach the interstitial spaces by migration directly through the cytoplasm of endothelial cells of venules and in a manner exactly comparable to that seen when lymphocytes pass from blood to lymph in the postcapillary venules of the lymph-nodes during their normal circulation[7]. Lymphocytes probably pass through the endothelial layer in an analogous manner in the inflammatory lesions in the central nervous system of rabbits which have been inoculated with herpes simplex virus[8] and during tuberculine reactions in guinea-pigs[9].

Thus, several studies on migration of lymphocytes from venules towards perivascular areas indicate that these cells in normal as well as in pathological conditions can leave the circulation by passing through rather than between the endothelial cells. It is conceivable that this mode of migration constitutes a characteristic biological attribute of the circulating lymphocyte.

In EAE the situation may be different since it has been stated or implied that monocytic cells in this disease traverse the wall of the venules in the central nervous system by passing between the endothelial cells[10]. More studies are required to settle the question of cell migration conclusively in this condition.

Our studies to date have shown that the 'intraendothelial' lymphocytes are encased by well-defined membranes, that they are situated in cavities which in turn are surrounded by membranes, and that the 2 membranes are separated by a cleft. Consequently, the migrating lymphocyte can tentatively be considered as situated outside the endothelial cell cytoplasm.

The migration of lymphocytes through the endothelial cells can be looked upon as a form of emperipolesis[11]. An electron microscopic study of emperipolesis of lympho cytic tumor cells within macrophages has shown that here also the lymphocytic cells are separated from the cytoplasm of the host cell by a well-defined membrane[12,13].

Zusammenfassung. Bei der experimentellen allergischen Neuritis verlassen die Lymphozyten das zirkulierende Blut der Venolen der peripheren Nerven, indem sie eher durch die als zwischen den Endothelzellen austreten. Der Durchtritt durch das Zytoplasma der Endothelzellen ist ein Beispiel von Emperipolesis.

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[11] Emperipolesis literally means 'inside round about wandering'. It refers to the unique capacity of normal and leukemic lymphocytes to enter other cells and move freely within their cytoplasm. Both host and guest cells may retain their cytologic integrity. The concept was introduced by J. G. HUMBLE, W. H. JAYNE and R. J. V. PULVERTAFT, Br. J. Haemat. *2*, 283 (1956). See also: O. A. TROWELL, Int. Rev. Cytol. *7*, 236 (1958); R. J. V. PULVERTAFT, Proc. R. Soc. Med. *52*, 315 (1959).
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