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STRUCTURAL MOBILITY OF LIVING MYELINATED NERVE FIBERS EXPOSED TO PROTEOLYTIC ENZYMES

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The structure of living myelinated nerve fibers exhibits considerable mobility [3, 6, 9]. All rapid morphological reconstructions have a significant effect on functional properties of the conductor and are involved in the development of the early stages of pathological demyelinizing processes [1, 4, 5, 8]. The attention of research workers is currently concentrated on the study of the role of proteolytic processes in the development of early structural changes in myelinated fibers and, in particular, in Ranvier nodes [2, 7, 12]. The view on "elimination," retraction of myelin at the nodes, which develops under the influence of proteolysis, separating the neurolemmocyte and axon in the early stages of demyelinizing processes [11, 13] and in certain functional changes [2, 5, 11], is under discussion. The mobility of other structures of the myelinated fiber under the influence of proteolytic enzymes has not been studied. This process must be analyzed first of all on living objects.

The aim of the investigation was to study the character of morphological changes in all structural components of the living myelinated fiber, developing under the influence of proteolytic enzymes, and to clarify the mechanisms of its reactive transformation.

EXPERIMENTAL METHOD

Experiments were carried out on single surviving myelinated fibers of *Rana temporaria*, isolated by mechanical dissection (on the method of isolation, see [3]). The fibers were placed in a microchamber with a continuous-flow system. The nodes, clefts, and perikaryon of the neurolemmocyte were studied. The zone of observation was not less than two nodes away from the zone of injury to the fiber. For proteolysis, a 0.2% solution of pronase E in Ringer's solution was used; this is the usual method for dissociation of neurons and glia in experimental physiology [4, 10]. Observations were made with the ordinary and phase-contrast

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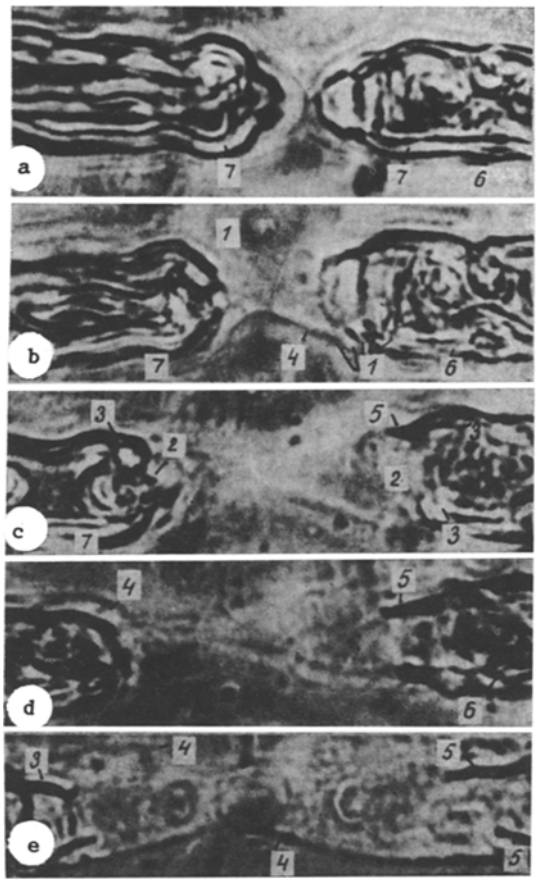

Fig. 1. Changes in Ranvier nodes under the influence of pronase. a) Initial state of structure, b) separation of myelin into layers and optical lysis of myelin cones (1) 5 min after beginning of action of pronase, c-e) further splitting into layers and destruction (2) of myelin after 30, 120, and 180 min respectively, 3) region of separation of myelin into layers, 4) outline of myelin sheath which has lost its contrast, 5) tapering ends of destroyed myelin segments, 6) clefts, 7) compact myelin. Intravital microscopy, phase contrast. Objective 40, ocular 10 ×.

microscopes over a period of 2-4 h. Serial photomicrographs were taken every 5-15 min on Mikrat-200 film.

EXPERIMENTAL RESULTS

The first structural changes in the nodes of Ranvier were observed 3-7 min after addition of pronase solution to the chamber (Fig. 1a, b). Two interconnected processes developed simultaneously: separation of the compact myelin into layers and enlargement of the so-called intersegmental cleft, or more precisely, the interval between areas of compact myelin of adjacent myelinated segments (Fig. 1a-f). The impression was obtained that the myelin was displaced away from the node. The same phenomenon has been interpreted by several investigators as movement (retraction, elimination) of myelin [5, 8, 13].

Three facts must be noted. First, the distance from the end of the contrasted myelin to the nearest clefts (indicated in Fig. 1a-c) was appreciably reduced. This is more likely to be evidence of reduction of contrast (optical "lysis") of the myelin cones than of their

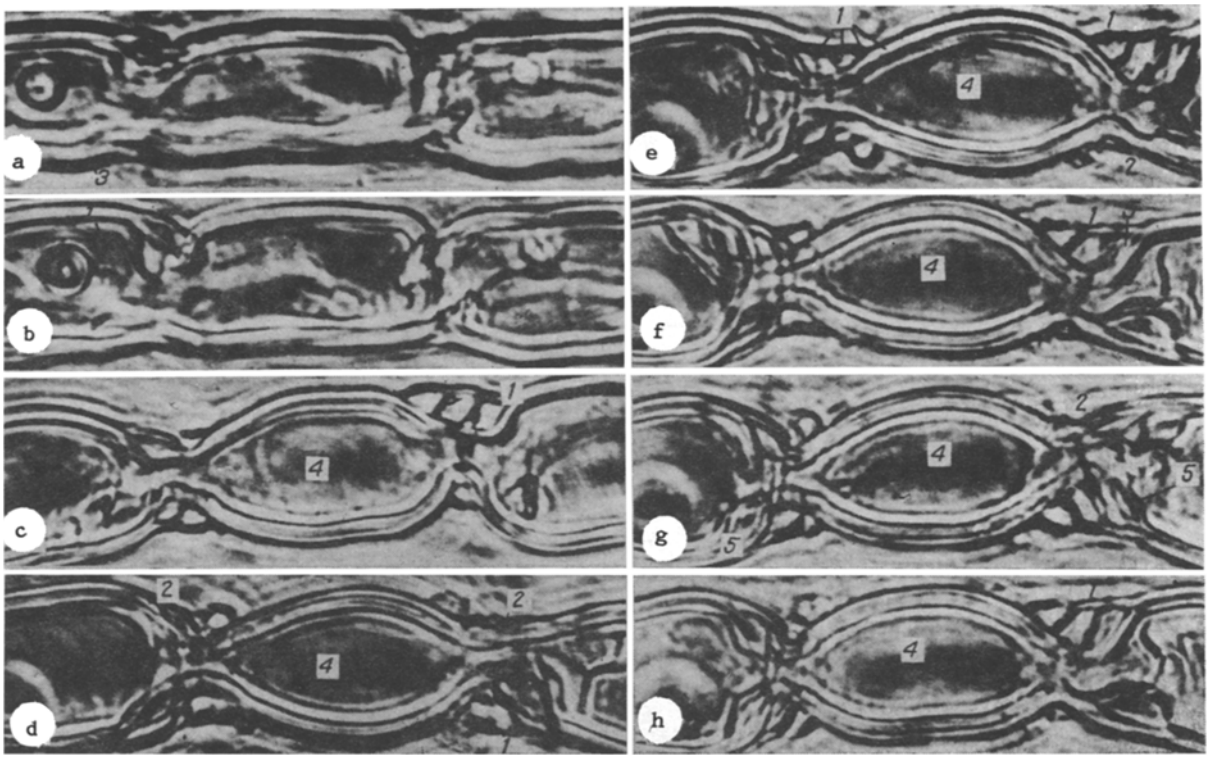

Fig. 2. Time course of separation (1) and fusion (2) of lamellar complex of myelin as a result of retractile activity of glioplasm of Schmidt–Lantermann myelin clefts, due to the action of pronase. a) Initial state of clefts (3), b-h) series of photographic frames reflecting time course of change in clefts 15-240 min after beginning of action of pronase, 4) varicosity of axon, 5) loss of contrast of myelin on separation into layers. Intravital microscopy. Objective 40, ocular 10 ×.

migration. Second, retraction of the myelin ought to have led to its accumulation at the ends of the segment and to a corresponding thickening of the myelin sheath, but experimentally, the opposite was observed (compare the indicator 5 in Fig. 1a-f). Third, the serial photographs show that not the myelin itself, but the sites of separation of the compact myelin into layers (arrows) are shifted in both directions from the cleft of the node, i.e., the process of separation of the myelin into layers spreads to new segments. The length of the compact (and therefore visible in the light microscope) myelin was reduced under these circumstances, and the region of separation of the myelin into layers was closer to the clefts (Fig. 1a-c). The stratified myelin lost its contrast (see indicator 4) and became difficult to see. It underwent apparent optic "lysis" and displacement of the zone of stratification created the impression of movement of the whole myelin. However, even in the most severely affected fibers, the poorly visible myelin (separated into layers and fragmented) was visible at its former site (Fig. 1b, e). Separation of myelin into layers is evidently the structural basis for demyelinization of the node.

The fact that the structural change in the myelin sheath is based, not on movement of the myelin, but on its separation into layers, also is shown by changes in the myelin clefts (Fig. 2). To begin with the reaction of the clefts is manifested only as a reduction in diameter of the fiber. Separation of the myelin into layers and a corresponding increase in size of the clefts become clearly visible 30-45 min after addition of pronase solution (Fig. 2b, c). No appreciable movement of the clefts in either direction was observed, but there was a clear increase in the degree of stratification of the compact myelin (compare Figs. 3b and 3h). The length of the clefts under these circumstances, despite cyclic fluctuations, gradually increased. Repeated stratification and fusion of the laminae of compact myelin (Fig. 3d, g) are evidence of the high lability of structure of the clefts. This process runs an extremely asymmetrical and even reciprocal course. When part of the cleft was swollen, causing maximal stratification of the lamellar complex of the myelin, in another part there was a proportional decrease in the volume of this structure, partial fusion of the laminae, and even restoration of the compact myelin (Fig. 2c, d, e, g, h). Sometimes this process took place every 15-30 min.

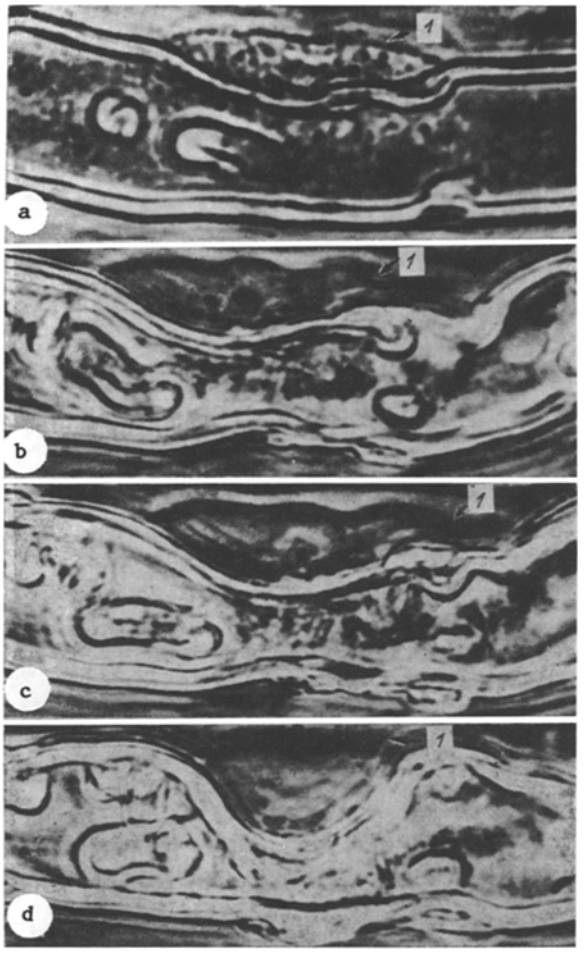

Fig. 3. Mobility of perikaryon (1) of neurolemmocyte under influence of pronase. a) Initial state of structure; b-d) frames of serial photography of perikaryon 30, 50, and 75 min after beginning of action of pronase; b, c) undulating mobility of surface and lengthening of neurolemmocyte; d) contraction and rounding of neurolemmocyte. Intravital microscopy, phase contrast. Objective 40, ocular 10 ×.

The clefts are known to be an accumulation of cytoplasm of the neurolemmocyte in the form of a single helical formation [3, 11]. It can therefore be tentatively suggested that this unique character of their mobility can be explained by the local contractile activity of the glioplasm. As was previously shown [3, 4, 6], separation of the myelin into layers is equivalent to its deinsulation, i.e., initial demyelinization of the fiber.

It must also be pointed out that a proportional narrowing of the axon was always observed in the region of swelling clefts. As a result, varicosities of the axon were formed. As will be clear from Fig. 2, varicosities of myelinated fibers are formed, not by swelling of the axon but, conversely, by its multiple compression.

The possibility of contractile activity of the glioplasm, observed in the clefts, was confirmed by simultaneous observation of the region of the perikaryon of the neurolemmocyte. In this zone, 15-30 min after addition of the pronase solution, intensification of the undulating motor activity of the glioplasm was observed, and was manifested on the photographs (Fig. 3b, c) as undulating deformation of the outline of the glioplasm and initial lengthening of the zone in which it was situated. However, the process ends with considerable retraction and rounding of the perikaryon (Fig. 3d).

The first ideas on retraction of myelin were evidently expressed by Müller-Mohnssen [9].

We have put forward an alternative hypothesis [3, 4]. According to our data, "widening" of the intersegmental cleft of the node is not true, but apparent. Myelin in the zone of the node, at least in the first stage, does not move away from the cleft but, breaking up into layers in situ and thereby losing its contrast, it becomes invisible in the light microscope. Away from the cleft, a process of stratification and destruction of the myelin clearly spreads. The following facts are evidence against any considerable displacement of the unfragmented myelin. First, in the paranodal zone there are many strong adhesive axoglial junctions, which keep the myelin sheath undamaged and ought to prevent its displacement. Second, the mass of myelin does not accumulate at the ends of the myelin segments. Finally, the forces which could shift the myelin along the axon are unknown.

Our investigations showed that proteolytic enzymes induce structural demyelinization, in which not only the nodes, but also the clefts, the perikaryon of the neurolemmocyte, and the axon participate. Enlargement of the intersegmental interval in the node is explained by the spreading of a process of stratification of myelin away from the cleft of the node, and this is accompanied by loss of contrast of the structures in this zone.

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