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INTERMEMBRANE JUNCTION FORMATION DURING MYELINATION

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KEY WORDS: membrane; intercellular junctions; myelination.

The generally accepted hypothesis, due to Geren [8], explaining the formation of the myelin sheath by continuous spiral rotation of the lemmocyte around the axon, and its variants [5, 14], has recently been supplemented by new data to show that an important role in myelin formation is played by cooperation between the neuron and glial cell [9, 11-14]. The formation of the main and intermediate dense lines of the myelin sheath is accompanied by fusion of the inner and outer layers of the lemmocyte membrane. The question arises whether this process resembles that of the formation of intermembrane junctions. The important role of intercellular junctions in ontogenetic development and function of organisms is well known [2, 6]. Intercellular (glio- and axo-glial) junctions also play an important role in activity of the nervous system [4, 10].

The aim of this investigation was to study membrane junctions of a peripheral myelinated nerve fiber in the course of its myelination.

EXPERIMENTAL METHOD

The tibial nerves of 10 rats aged 5-10 days were used as the test object. Material was fixed in 2.5% glutaraldehyde solution in phosphate buffer and postfixed in 1% OsO_4. After dehydration of the nerves in alcohols they were stained with a saturated solution of uranyl acetate and embedded in Araldite. Longitudinal ultrathin sections were examined in the JEM-7A electron microscope.

EXPERIMENTAL RESULTS

In all preparations of developing nerve fibers studied an unusually large number of Ranvier nodes was observed at different stages of their formation (Fig. 1). Investigation of the Ranvier nodes revealed virtually all types of intercellular junctions: continuous, septate, gap, tight, and desmosome-like. Long processes of the lemmocyte (terminal loops filled with glioplasm), of unequal thickness, could be seen in the paranodal region. The intervals between these processes (intercellular gaps) were inconstant in size, and where neighboring processes widened the gap was narrowed (Fig. 2). In these narrow areas aggregation of the intercellular contrast substance was usually observed. If the gap was filled comparatively uniformly with the material, the picture resembled a continuous junction. If the aggregates in the gap were discretely arranged, in the form of separate masses, the picture corresponded to that of a septate junction. These septate and continuous junctions could be both glio-glial and axo-gilal (Figs. 1 and 2). In some cases considerable narrowing of the intercellular gap was observed or even complete fusion of adjacent membranes. These regions corresponded in structure to gap and tight junctions. Simultaneously with aggregation of electron-dense material was observed in these same places in the inner surface of the glial membrane of the loops (Fig. 2). Aggregation of the juxtamembranous electron-dense material on both sides of

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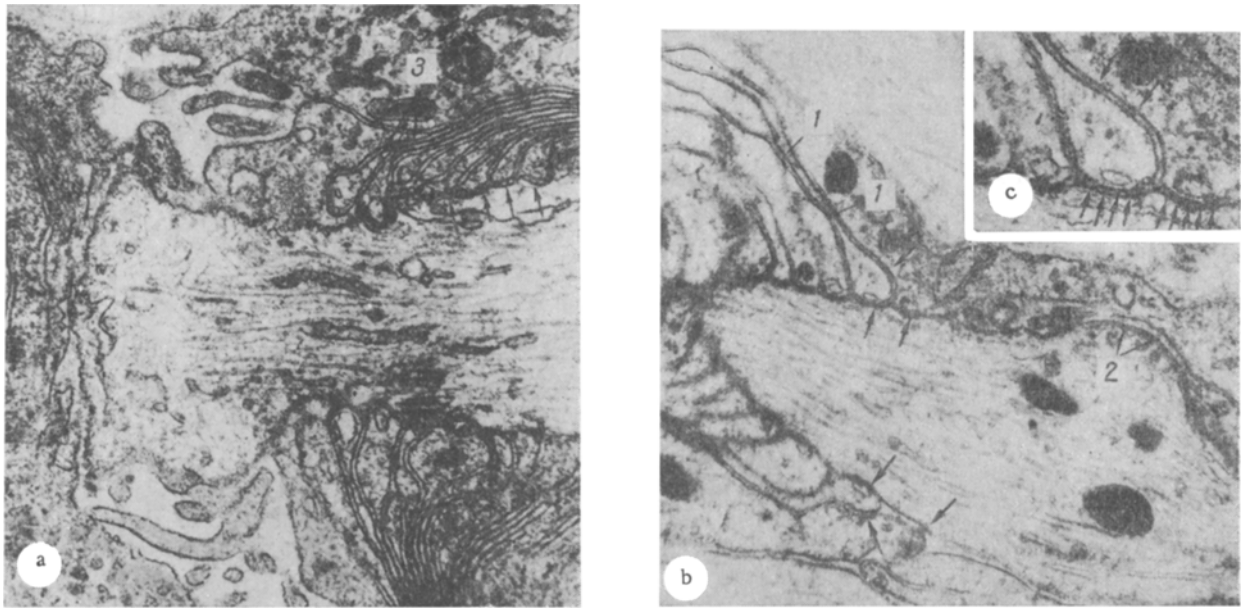

Fig. 1. Varieties of Ranvier nodes of developing nerve fibers: a) longitudinal section; b) oblique section; c) terminal loops of gliocyte (fragment of b). 1) Local "inside-out" junctions formed between inner surfaces of gliocyte membranes; 2) adhesion of submembranous electron-dense material of axoplasm; 3) frontal formation of main sense lines (inside-out junctions) of gliocyte. Arrows indicate septate and other intermembrane axo-glial and glio-glial junctions. Magnification: a, b) 45,000; c) 90,000.

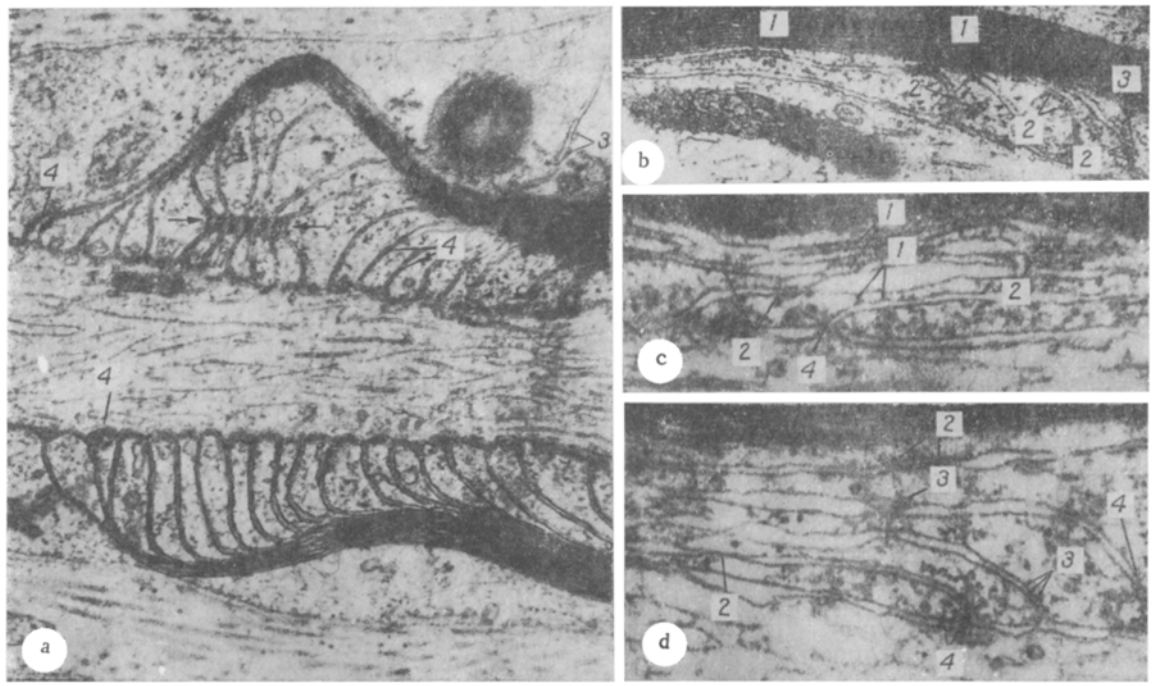

Fig. 2. Formation of local glio-glial intermembrane junctions during myelination: a) serial desmosomal junctions; b, c, d) local junctions of inner (1) and outer (2) surfaces of gliolemma, adhesion of aggregates of glioplasm with gliocyte membrane (3), other types of junctions (4). Arrow shows direction of retraction of dense aggregates of glioplasm in a serial junction. Magnification: a) 60,000; b, c, d) 70,000.

the gliolemma apparently took place. Frequently the submembranous aggregates came to resemble specializations of hemidesmosomes or desmosomes. Frequently the formation of serial desmosomes was observed. In that case a retractile effect of the submembranous aggregates was exhibited. Each glial loop in this place appeared greatly compressed, and the gaps between the retracted areas were widened.

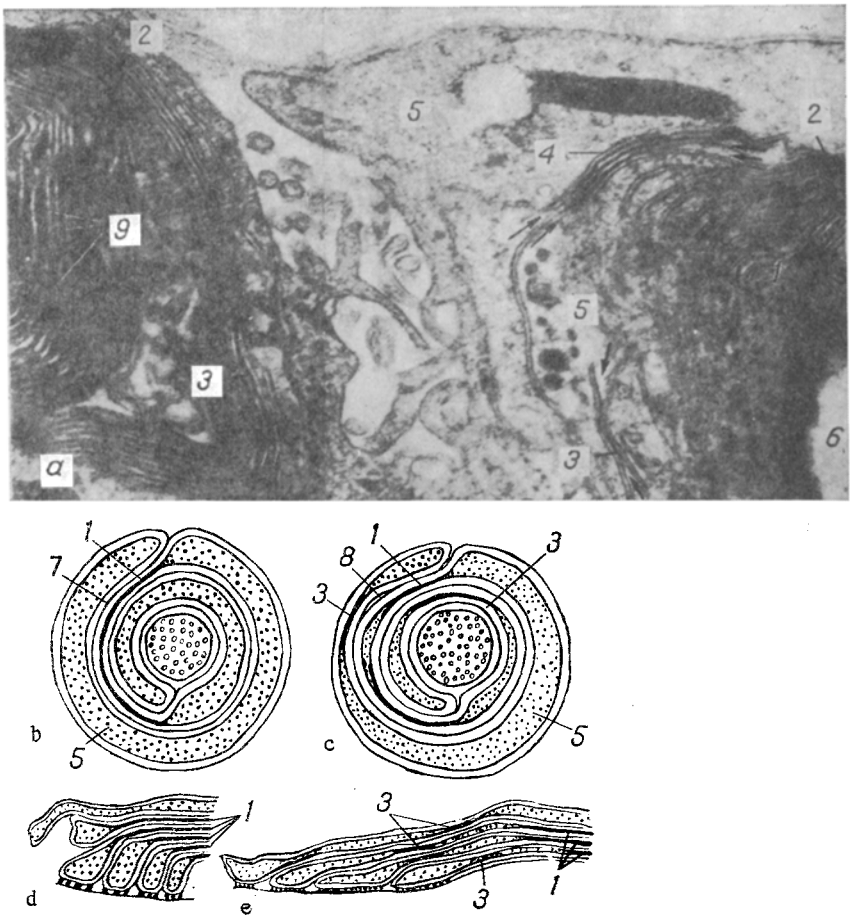

Fig. 3. Formation of main dense lines (1) of complact myelin (2) from discrete junctions (3) of glial membranes, connected by their own inner (the wrong side) surfaces. a) Formation of single junctions and of serial junction loci (4) at some distance (in front of) compact myelin (30,000 ×); b) scheme showing formation of continuous main dense lines; c) scheme summarizing data on formation of main dense line by fusion of discrete scheme of formed myelin in node; d) scheme of formed compact myelin; e) scheme of formed myelin in node; 5) cytoplasm of glial cell; 6) axis cylinder; 7) intermediate dense line; 8) its splitting up; 9) unevenness of thickness of glial laminae. Arrows indicate points of constriction of laminae of myelin, turning into membrane junctions.

Aggregation and retraction of material of the glioplasm are well marked in Fig. 2a and strictly localized. However, small submembraneous associations of electron-dense material of the same process, in contact with each other, fuse to form a junction-like structure, outwardly indistinguishable from gap and tight membrane junctions, but formed by approximation, not of the outer surfaces, but of the inner surfaces of the membrane (inside-out junctions, Fig. 3), much more frequently in places of narrowing of the glial loops. The formation of these "inside-out" membrane junctions does not differ in principle from the formation of intermediate dense lines of myelin (Fig. 3b, d), although they are arranged in the form of scattered loci on peripheral outgrowths of gliocytes, at a distance from the continuous zone of compact myelin formation (Fig. 3c, e). It can be postulated on this basis that the main dense lines of myelin are formed not only by frontal circular coiling of the mesaxon, but also by fusion of many unconnected local "inside out" membrane junctions. Intermediate dense lines of myelin are also evidently formed by fusion of local junctions formed previously as a result of approximation of the outer (the right side) surface of the gliolemma of neighboring loops. All these facts suggest that myelin can be regarded as a system of two giant intermembrane junctions formed as a result of fusion of ordinary local (right side out) and inside out junctions.

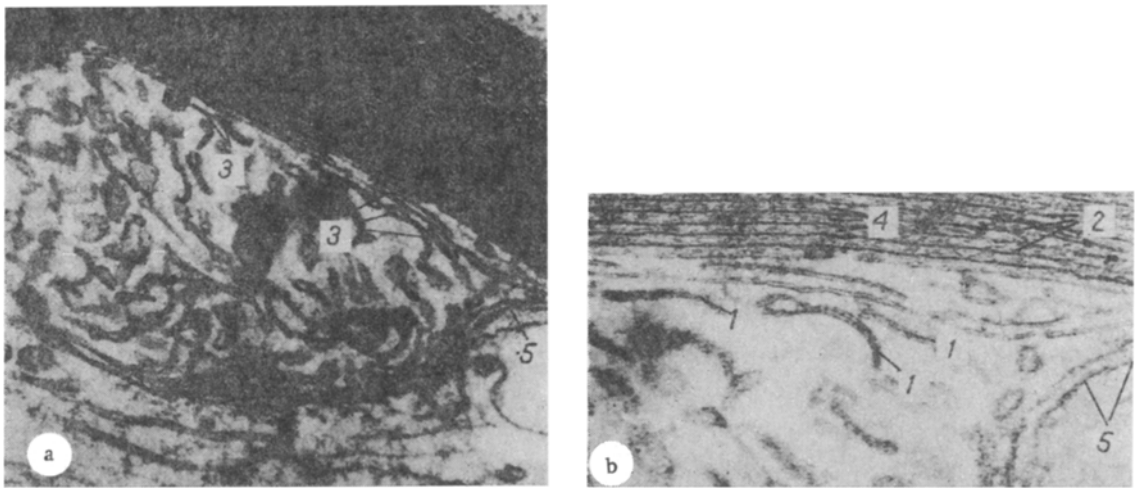

Fig. 4. Formation of structures with junctions of inner surfaces of membranes (main dense lines): a) general view of invagination of glioplasm into region of axon (33,000×); b) details of structure of functions (fragment of 4a, 92,000×). 1) Main dense line ("inside-out" junction); 2) main dense line of compact myelin; 3) membrane structures imbedded into myelin from within; 4) intermediate lines; 5) axolemma.

The formation of membrane junctions during myelination is found not only in the zone of the Ranvier node, but also in the central zones of the myelin segment. Here thickenings (invaginations) of the inner layer of the glioplasm, located between myelinand axon, are frequently found (Fig. 4a). These invaginations are usually filled with vacuole-like structures. Some of the vacuoles are so compressed that they become pear-shaped or even racket-shaped (Fig. 4b). Under these circumstances part of the inner surface of the vacuole becomes duplicated, comes together, and fuses to form a five-layered tight membrane junction resembling an "inside out" junction of the main dense line. The racket-shaped and lamellar five-layered structures, located in the immediate vicinity of the inner surface of the myelin, approach it by a distance equal to the intercellular gap, and come to lie parallel to the plane of the myelin, so that the impression is created that they gradually become incorporated into the compact myelin from inside (Fig. 4). Since the closest inner membranes of the lemmocyte are often fragmented, some idea may be obtained of the way in which racket-shaped and laminar structures with ready-made main dense lines participate in myelin formation through their incorporation (by the self-assembly principle) [1, 3, 5, 7].

It was shown previously that the different varieties of intercellular junctions can be regarded as different stages of approximation and fusion of the membranes [2, 4]. An important role in this phenomenon is played by increased adhesion and aggregation of the protein material of the membrane itself, and also the juxtamembranous proteins of the cytoplasm and intercellular substance. In the present investigation intercellular junctions were studied during myelination. Their abundance, and also the presence of small isolated zones of myelination, consisting in fact of serial intercellular junctions, suggest that the mechanism of formation of intercellular junctions plays an important role in the formation of the myelin sheath. Compact myelin is probably formed not only by continuous winding of the Schwann-cell membrane, but also by an interrupted mosaic of formation and fusion of local intercellular junctions. If this really is so, the whole complex pattern of myelin formation can be represented as one single giant complex membrane junction.

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CHARACTER OF PHAGOCYTOSIS IN INTRAVITREAL HEMATOMA

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KEY WORDS: phagocytosis; intravitreal hematoma; generalized phagocytosis; late focal phagocytosis.

In the study of phagocytosis in hemophthalmia the problem of the ways of penetration of phagocytes into the vitreous body (VB) has been studied in more detail in recent years [6, 12-16]. However, the role of phagocytosis in the morphogenesis of intravitreal hematoma, the formation of which is inevitable in some forms of hemophthalmia, still remains unstudied [5].

Accordingly, the aim of this investigation was to determine the characteristics of phagocytosis of a hematoma in this situation, allowing for the specificity of its surrounding medium, viz. VB.

EXPERIMENTAL METHOD

Experiments to create a model of hemophthalmia were carried out on 74 rabbits (144 eyes) of the albino and Chinchilla breeds, of both sexes weighing 1.5-2.5 kg. Under local anesthesia (1% procaine solution), autologous blood (0.1 to 1.2 ml), taken from the auricular vein, was injected subconjuctivally into the VB by the retrobulbar route. The sclera was punctured in the flat part of the ciliary body after preliminary paracentesis. The animals were decapitated at various times (from 1 h to 3.5 years) after single or repeated (two to four times) injection of blood, and the eyes were quickly enucleated and fixed in 10% formalin solution, which was injected into VB. Before fixation the VB was removed and investigated cytologically by reactions for glycosaminoglycans (GAG, with toluidine blue) and lipids (with Nile blue). After fixation the eye was cut into three blocks and treated histologically in the usual way and then embedded in celloidin and paraffin wax. Sections were stained with hematoxylin and eosin, Sudan black, and Nile blue and the histochemical reactions of Perls, Hailey and Seitelberger, Brachet, and Gomori were used. Material for electron microscopy was fixed in a mixture of paraform and glutaraldehyde and then postfixed in 1% OsO_4 solution. Ultrathin sections were examined in the UMV-100K and Tesla BS-500 electron microscopes.

EXPERIMENTAL RESULTS

On the 2nd day after injection of the blood separate round mononuclear cells with bean-shaped nucleus appeared around the hematoma. On the 3rd-5th day the number of cells around the hematoma and in VB was increased somewhat. By this time single cells had already penetrated into the depth of the fibrin film of the hematoma. Zones of translucency of fibrin were visible around these cells (Fig. 1a), evidence of participation of phagocytes in fibrinolysis. Whole erythrocytes (Fig. 1b) or their derivatives were found in the cytoplasm of individual macrophages on the 3rd-6th day. Evidence of the phagocytic activity of these cells was given by their positive reactions for alkaline phosphatase and their marked pyroni-

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