sidered to be a manifestation of "post-mortem microbe dissemination in the tissues", cannot be verified with certainly without taking into account the species and the biological properties of the microorganism.

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Muscle Spindle Ultrastructural Features in a Replanted Limb

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Muscle spindles are an important structure of muscle tissue participating in motor function coordination. Reports on the role of various components of spindles in the preservation of receptor intactness and receptor activity recovery in disease are contradictory [2-6]. An entire array of problems concerning the structure of the spindle capsule and its role in receptor viability preservation, reinnervation possibility, intercellular including axon-Schwann cell interactions, and specific features of the microcirculatory bed require a thorough ultrastructural analysis. The aim of the present study was to examine the ultrastructure of muscle spindle components in a replanted limb.

MATERIALS AND METHODS

Replantation of the right hind limb was carried out in white male rats weighing 270-320 g. The surgical technique has been previously described [1]. Specific features of the model used were the absence of prolonged thermal ischemia (blood flow in the main vessels was intact during surgery) and preclusion of the effect of muscle injury on the course of regenerative processes: muscle below the tissue crossing site were examined. M.ext.halucis longus was used to its small volume. Since the intrafusal fiber distribution is irregular, examinations of serial sections were carried out. The material for the investigation was collected under ether anesthesia one month after surgery (5 animals). The muscle fragments were fixed in cooled formol-sucrose solution and then in 1% OsO_4 buffered solution and embedded in araldite. Ultrathin sections were examined under a JEM-7A electron microscope.

RESULTS

Muscle spindles were found to survive one month after replantation. Some of them were rather well preserved. The majority showed signs of destructive degenerative changes of varying degree involving all the receptor components.

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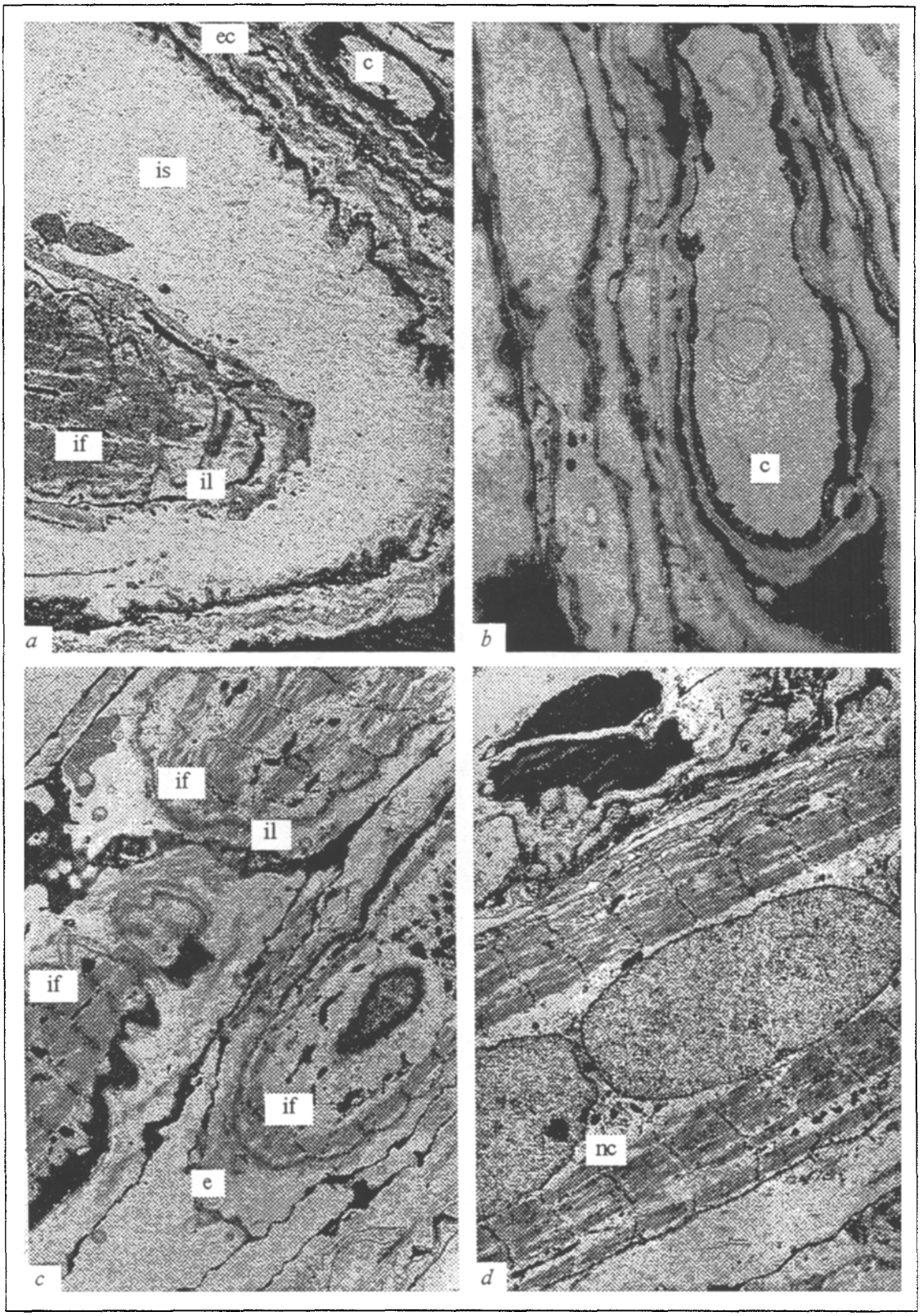

Fig. 1. Ultrastructure of muscle spindles in a replanted rat limb. *a*) spindle capsule; c: capsule wall capillary; ec: muscle spindle exterior capsule. External leaflets (el) of the capsule are degeneratively changed, a fragment of intrafusal fiber (if) is seen, intracapsular space (is) characterized by increased electron density (×2700). *b*) capsule wall capillary: degeneratively changed endotheliocytes, foci of endothelium thinning visible, fenestra (shown by arrows), fragments of degeneratively changed endotheliocytes between multilayer membranes (×16,500). *c*) varying degree of destructive changes in intrafusal fibers (if), elastic fibers in intracapsulsar space (e) (×3200). d) Waller–type secondary degeneration of axon – fragmented lysis, lumpy degragdation and vacuolation of axoplasm (×2700). nc: nuclear–chain intrafusal fiber

The spindle capsule is known to be the key structure in receptor preservation and its role is comparable to that of the axonal perineural membranes [3]. The capsule is intact under conditions of replantation but the epineural epitheliocytes forming the outer coating are characterized by a high electron density and exhibit signs of destructive degenerative changes (Fig. 1, *a, b*). The capsule interior leaflets directly surrounding the intrafusal fibers are atrophic and represented by thinned perineural epitheliocyte sprouts with a high electron density. The degenerative changes in these leaflets are so pronounced that the intracellular structures are undetectable (Figs. 1, *c*, 2, *a*). Dystrophic changes involve the endothelium of the microvessels in the capsule exterior coating. These changes present as a marked increase of endotheliocyte electron density and impairment of their membrane integrity (Fig. 1, *b*). The ultrastructural changes in the capsule microvessels are similar to previously described vascular changes in replanted limb muscles and may be referred to nonspecific microangiopathies [1].

The intrafusal space is characterized by an increased electron density. Accumulations of elastic fibers, poorly differentiated cells in a state of mitosis, and free Schwann cells surrounded by multilayer basal membranes are detected in it. Degeneratively changed electron-dense Schwann cells are easily identified by the basal membranes; free spiral-rounded membranous complexes may be the rudiments of lysed Schwann cells (Fig. 2, a).

The atrophic internal membranes of the capsule, increased density of the intracapsular space, and the accumulation of finely dispersed material in this space indicate both impaired permeability of the capsular membranes from the interstitial space and impaired intactness of the capillary barrier due to endothelial coating injury (Figs. 1, *a*, 2, *a, b*). Impaired trophic supply of the capsule because of microvessel injuries and imperfect reinnervation processes appear to play the major role in the development of destructive degenerative processes in the capsular membranes.

The majority of intrafusal fibers retain their regular ultrastructure. Sarcomere size and disk width indicate that the fibers are in a state of relaxation. A certain relationship between spindle capsule cell structural integrity and intrafusal fiber state can be seen. Note that the fibers in the same capsule may have myofibrillar injuries of various degree (Fig. 1, *c*).

The data suggest an important role of spindle capsule intactness in receptor survival and structural integrity. It is an obligatory conditions, though not the only one.

Investigation of nerve-spindle interrelationships in replantation is of particular interest, because full recovery of the structural and functional organization of a muscle spindle is possible only in the case of adequate reinnervation of the intrafusal fibers. Degenerative changes in the nerves connected with spindle cutting during surgery are mostly completed in the studied periods, and growth of numerous myelinized axons in the intracapsular space takes place. Light-optic examination showed that they branch from the same nerve stem and reflect the axonal sprouting phenomenon. Still, the reinnervation processes are incomplete. Axoplasm lumpy degradation and vacuolation are observed in axons with the usual electron density and normal structural organization. Filamentous degeneration phenomena are seen in some axons (Fig. 2, *b*). It is noteworthy that Schwann cells associated with sprouting zones penetrate into axial cylinders, divide the axoplasm into fragments, and phagocytize axon sites, leaving empty neural coatings. Such an ultrastructural picture is indicative of secondary degeneration of nervous conductors. Motor nerve endings may be found at polar ends of intrafusal fibers. They form solitary short postsynaptic folds. There are many empty axonal terminals in the presynaptic zone, this indicating the prevalence of intrafusal fiber denervation processes (Fig. 2, *d*). The detected sensory nerve endings represent as a rule structures with homogenous contents in which individual cell organelles, mitochondria, and vesicles can hardly be detected (Fig. 2, *c*).

In considering Schwann cell phagocytosis of intergrowing axons, we may assume that "foreign" axons are phagocytosed which sprouted after inadequate reposition of the cut nerve ends. Phagocytosis of intact axons resulting from disordered axon-Schwann cell interactions cannot be ruled out either. Both altered antigenic characteristics of intergrowing axon membranes and the characteristics of Schwann cells proper may underlie this phenomenon. Degenerative changes in young nerve structures aggravate the conditions for recovery of adequate neuromuscular interrelationships in the replant spindles.

The findings demonstrate that preservation of spindle capsule intactness alone does not solve the problem of long-term preservation of intrafusal fibers, for in many fibers we may find myofibril lysis, disorganization of intracellular organelles, and degenerative changes in the nuclei, this making it of independent interest to assess fiber preservation at the moment of innervation establishment.

Thus, many spindles are structurally intact one month after limb replantation. The ultrastructural characteristics of intrafusal fibers of sensory and motor nerve endings indicate the possibility in principle of muscle receptor functional recovery; however, the presence of muscle spindles with injured capsules,

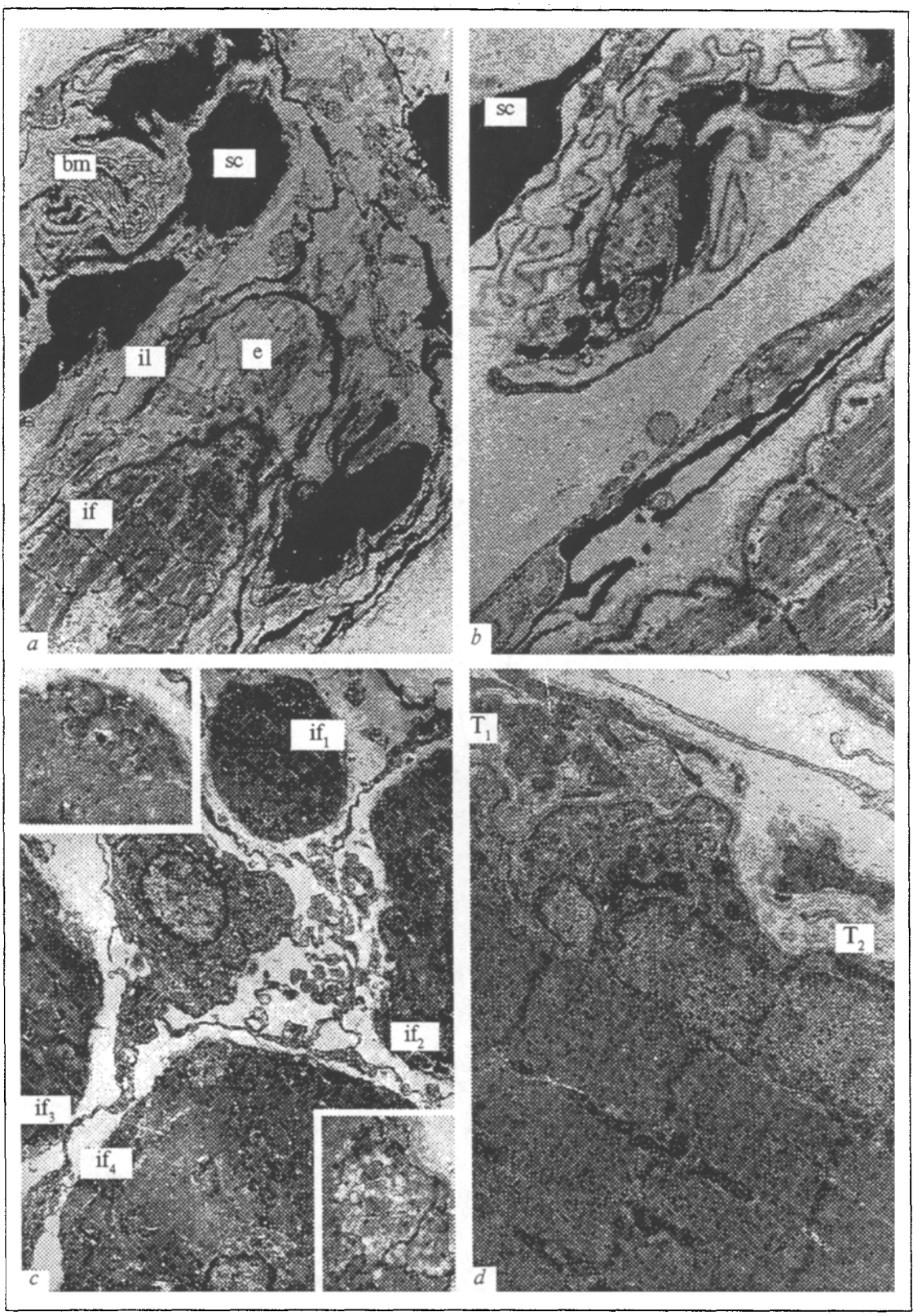

Fig. 2. Reinnervation of intrafusal fibers. *a*) free degeneratively changed Schwann cells (sc) in intracapsular space, bm: complexes of spiral–coiled basal membranes, if: intrafusal fiber, atrophy and impaired intactness of capsule internal leaflets (il), e: increased number of elastic fibers in intracapsular space (shown with arrows). ×2700. *b*) secondary degeneration of axon, sc:Schwann cell. ×7500. *c*) transverse section: sensory nerve endings on intrafusal fibers (if_1, if_3, if_4 (×3000)); sensory nerve endings in the upper left corner (if_1 fragment shown with an arrow (×7000); in lower right corner: destructively changed sensory ending on if_2 fiber (fragment shown by arrow) (×7000). *d*) zone of motor end plates. Processes of intrafusal muscle fober innervation. T_1: ultrastructural arrangement of terminal close to the norm. T_2: final stage of axon terminal phagocytosis by Schwann cells. Schwann cell process in synaptic fissure (shown by arrow). (×11,500).

degenerative changes in the internal membrane neural epithelium, and detected changes in the microvessels and intrafusal fibers make the possibility of the preservation of many receptors doubtful. The prevalence of destructive degenerative changes in regenerating neural structures and impaired axon-Schwann cell interactions impede the formation of normal neuromuscular and sensory contacts already limited due to the random nature of the reinnervation processes.

The pattern of muscle spindle structural arrangement in a replanted limb suggest a marked limitation of the processes of recovery of adequate functional activity in many muscle receptors, which may be responsible for the observed abnormal sensitivity of the replanted limb and for failure of its satisfactory functional restoration [5].

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Ultrastructural and Electron-Cytochemical Alterations of Large Intestine Cells in Viral Diseases

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Research on virus particles and viral inclusions is being successfully carried out using cell culture [4-6]. However, the study of virus-induced alterations *in vivo* has not still received sufficient attention, even though it is an essential step in the investigation of the morphological features of viral effects on cell ultrastructure. We have not found any published data dealing with cell ultrastructure, virus particles, and viral inclusions in various populations of large intestine cells in HIV infection.

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It is common knowledge that viruses do not express metabolic activity their own. But the formation of viral inclusions can markedly change the cellular metabolism [2,9]. For example, the activity of the main metabolic enzyme adenylate cyclase (AC) adequately reflects the processes of virus-induced cell alterations. This is true, specifically, for the RNA-containing viruses such as HIV (retroviruses) and the influenza virus (orthomyxoviruses).

The aim of this investigation was to reveal the salient features of the ultrastructural and cytochemical alterations in various large intestine cells in HIV infection. Considering the well-known fact that diseases of the large intestine are often induced by respiratory

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