Pathomorphological Signs of Damage to Capillary Endothelium in Skeletal Muscle after Traumatic Injury to or Replantation of a Limb

A. V. Volodina, N. S. Gurko, Yu. V. Kiprenskii, and O. M. Pozdnyakov

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Several months after an extensive crush injury to or replantation of a limb in rats, capillary endotheliocytes of its skeletal muscle are seen to undergo ultrastructural changes of both "dark" and "light" types. Destructive/degenerative changes of the "light" type are accompanied by intracellular edema and cytoplasmic homogenization and can eventually result in monocellular colliquative necrosis. Changes of the "dark" type are characterized by signs of functional overstrain in the endotheliocytes, succeeded by destructive/degenerative cytoplasmic changes, increased electron density of intracellular organelles, loss of distinct boundaries by the nucleus and intracellular structures, rupture of cell membranes, endothelial desquamation, and some other changes, which eventually lead to coagulation necrosis, followed by breakdown of the cell into small fragments.

Key Words: *trauma; skeletal muscle regeneration; microangiopathies; monocellular necrosis; colliquative necrosis; coagulation necrosis*

Ultrastructural evaluation of endothelium in the microvasculature following exposure to injurious agents is highly important because the destructive changes taking place in endotheliocytes underlie both vascular abnormalities and dystrophic (degenerative) processes of vascular genesis in the tissue concerned [3,5].

Experimental studies of endothelium in blood capillaries have demonstrated that endothelial cells are well able to adapt to the action of various pathogenic factors. The destructive changes in the ultrastructure of these cells are manifested, *inter alia*, in the emergence of "light" and "dark" endotheliocytes. "Dark" endotheliocytes are interpreted as cells in a state of hyperfunction [6]. Other investigators associate changes in the density of endothelial cell cytoplasm with stages of the cell cycle and have identified cells of a third type - "gray" cells [5]. An important sign of cell damage has been shown to be altered sorptive properties of the cytoplasm [2], and alterations in these properties have been used in describing necrotic and necrobiotic processes. However, it is sometimes difficult to discriminate between signs of functional stress experienced by the cells, the initial stages of their damage, and irreversible intracellular changes [4].

In the present study on rats, an attempt was made to provide an ultrastructural evaluation of the state of capillary endothelium when the pathological process in skeletal muscle had taken a chronic course after an extensive crush injury to or replantation of a limb. To this end, skeletal muscle capillaries were examined soon (up to 1 month)

Laboratory for Experimental Pathomorphology, Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

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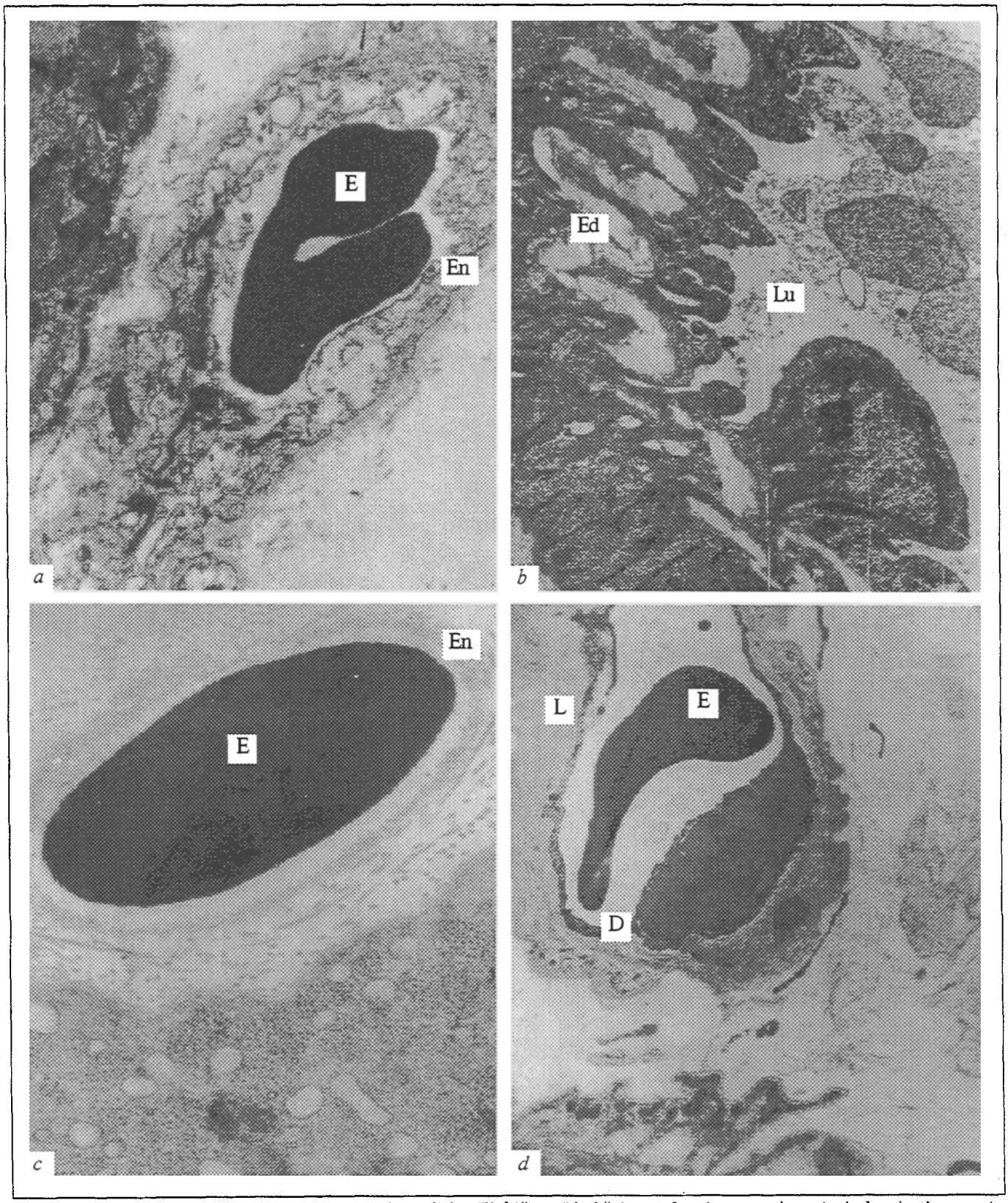

Fig. 1. Endotheliocytes undergoing degeneration of the "light" or "dark" type after trauma. *a*) acute ischemic damage to capillary endothelium, with intracellular edema of endotheliocytes. One month after trauma (×10,000). *b*) arteriole: subendothelial edema and desquamation and clasmatosis of endotheliocytes. One month after trauma (×7000). *c*) lysis of capillary wall; an erythrocyte is seen in the lumen. One month after trauma (×16,400). *d*) endothelial degeneration of the "light" and "dark" types. Eight months after trauma (×3200). *E*: erythrocyte; *En*: endothelium; *Ed*: subendothelial edema; *Lu*: lumen; *L*: degeneration of "light" type; *D*: degeneration of "dark" type.

and a long time (8 months) after the traumatic injury or replantation.

MATERIALS AND METHODS

Two groups of random-bred white rats weighing 300-360 g were used. In one group, an extensive traumatic injury to muscles of the saltatory complex was produced according to Cannon. In the other group, a hind limb was cut off in the region of the upper third of the femur (preserving blood flow in the central artery and central vein) and immediately replanted. After the trauma or replantation, skeletal muscle samples were fixed sequentially in a cooled Formol-sucrose solution and 1% buffered osmium tetroxide solution, and embedded in Araldite. Ultrathin sections were prepared and examined under a light microscope and then in a JEM-7A electron microscope.

RESULTS

Shortly after the traumatic injury to the skeletal muscle (days 3, 5, 10, and 30), signs of intracellular edema preponderated in the endothelia of capillaries and other vessels of the microcirculatory bed. These signs included the emergence of vacuoles, local lysis of the cytoplasm, and diminished numbers of intracellular structures and micropinocytic vesicles (ultrastructural changes of the "light" type) (Fig. 1, *a*). In the arterioles, subendothelial edema and deep invaginations into their lumens were seen, together with signs of endothelial cell clasmatosis and endothelial desquamation (Fig. 1, *b*). In such situations, the edematous changes in capillary endotheliocytes may be irreversible and culminate in total cytoplasmic lysis. Around the preserved formed elements of the blood, a clear ring of finely dispersed material was observed (Fig. 1, *c*). Where the intracellular edema was only local and individual organelles and plasma membranes were preserved, such changes in endothelial capillaries may be interpreted as acute reversible ischemic changes (Fig. 1, *a*).

Later, signs of damage to the microvessels became more pronounced, and their walls came to be impregnated with plasma proteins. Eight months after trauma or replantation, large numbers of capillaries formed by heterogeneous populations of electron-dense cells were observed, and both the "light" and "dark" types of changes could be seen among the endotheliocytes, as illustrated in Fig. 1, *d*. The lumen of the capillary shown in this figure is delimited by "dark" endothelial cells containing large numbers of micropinocytic vesicles immured in an electron-dense cytoplasm. The cell nucleus is edematous and the karyoplasm appears structureless. We interpreted such cellular changes as necrobiotic changes of the "dark" type. Changes of another type were noted outside the inner layer of the capillary endothelium. Among greatly widened, dense basement membranes, homogenized and readily discernible remnants of what had been the endothelial layer were present; such degenerative changes were interpreted as being of the "light" type. Destructive/degenerative changes in endotheliocytes, which were accompanied by impregnation of microvascular walls with plasma proteins, eventually led to hyalinosis 8 months after trauma or replantation (Figs. 1, *d* and 2, *d*).

Reduced electron density of the cytoplasm has been associated with intracellular events resulting from hydration of the cells and accompanied by lysis of their membranes [4]. Changes of this kind have been referred to as monocellular colliquative necrosis [4]. An increase in cytoplasmic density caused by denaturation of cytoplasmic proteins is regarded as a sign of monocellular coagulation necrosis.

Whereas the state of edematous "light" cells can be readily assessed ultrastructurally, such assessment for "dark" cells may present problems. Figure 2 shows "dark" cells of two types: activated and degenerating (D_a and D_d). The capillary lumen is formed by four "dark" endotheliocytes in a state of hyperfunction (Fig. 2, *a*). Their cytoplasm was filled with ribosomes, polyribosomes, elements of granular endoplasmic reticulum, and small mitochondria, while their vesicular apparatus was represented by luminal, basal, and free vesicles. The intercellular junctions were tightly closed. The outside of the capillary was also surrounded by "dark" cells. However, the cytoplasm and karyoplasm of these cells had higher electron densities, their intracellular organelles were invisible, and the vacuoles alternated with areas of lysed cytoplasm. The cell nucleus had indistinct boundaries, the karyoplasm was homogeneous, and the intercellular borders were not defined. These were "dark" cells with irreversible changes characteristic of dry monocellular coagulation necrosis (Fig. 2, *a* and *b*). When the further fate of such cells was followed (Fig. 2, *b* and *d* and Fig. 3, *a* and *b*), the electron-dense cells with a coagulated compressed cytoplasm were found to be breaking down into small fragments and to undergo karyopyknosis and karyolysis. These events were best seen in longitudinal sections (Fig. 3, *a*). Subsequently, the capillaries formed by such cells disintegrated into fragments delimited by dense basement membranes.

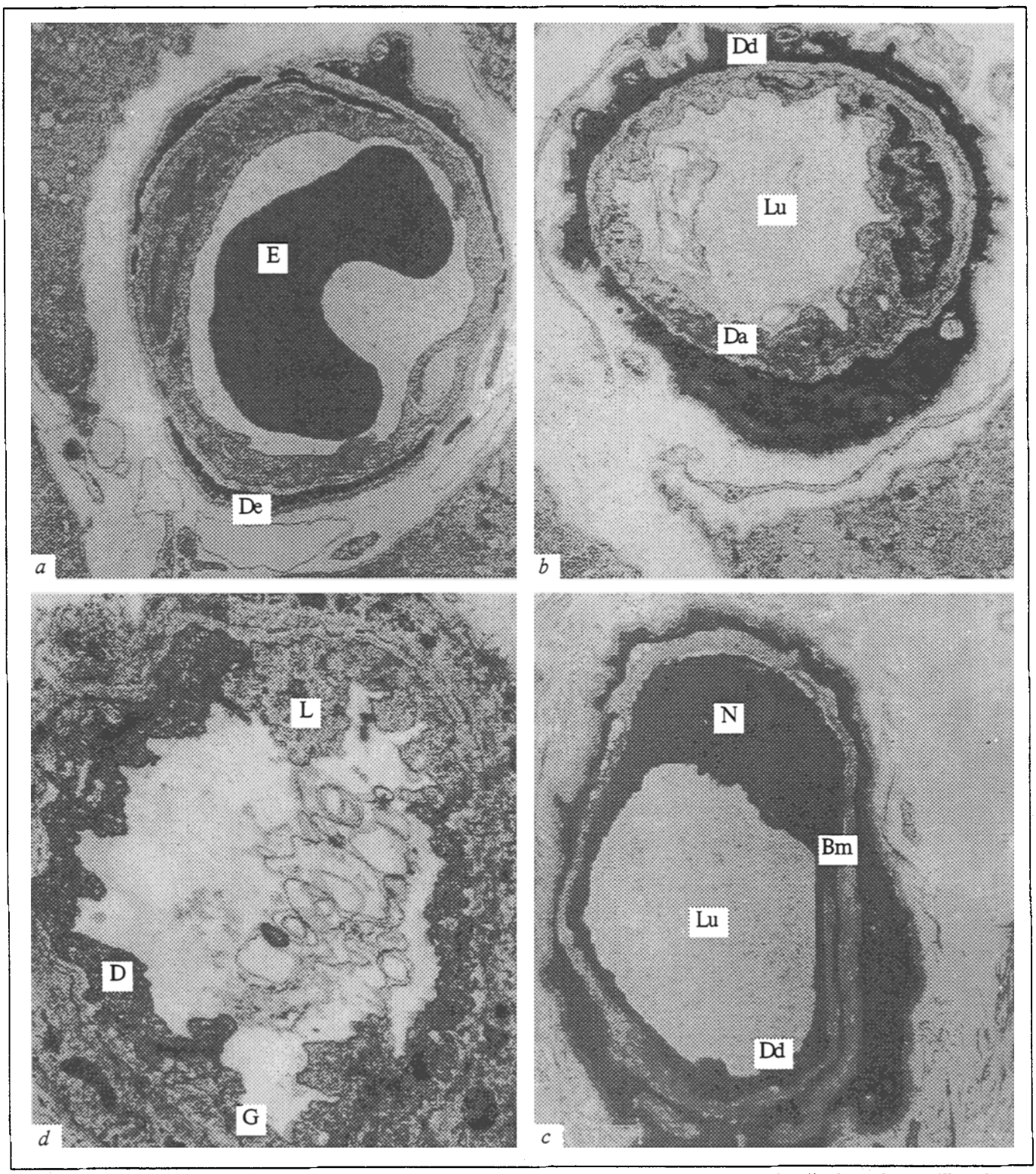

Fig. 2. Reactive changes of the "dark" type in endotheliocytes. *a*) "dark" activated endothelial cells form the capillary lumen. Electron density of the endothelium is determined by the large number of intracellular organelles (ribosomes, polyribosomes, micropinocytic vesicles). One month after replantation (×7500). D_e: degenerating fragments of "dark" endotheliocytes. *b*) "dark" activated cells (D_a) and "dark" degenerating (D_d) cells (monocellular coagulation necrosis of endotheliocytes); the cytoplasm and karyoplasm of "dark" degenerating endotheliocytes are structureless. One month after replantation (×3000). *c*) life–cycle stages of endotheliocytes: "dark" (*D*), "gray" (*G*), and "light" (*L*) cells. The endothelium produces invaginations into the arteriolar lumen. Day 10 after trauma (×7000). *d*) endothelial degeneration of the "dark" type (D_d). The nuclear boundary (*N*) is hardly noticeable, and a few vesicles are seen to be "immured" in the cytoplasm. *BM*: cleaved electron–dense basement membrane. Eight months after replantation (×7500). For other designations see Fig. 1.

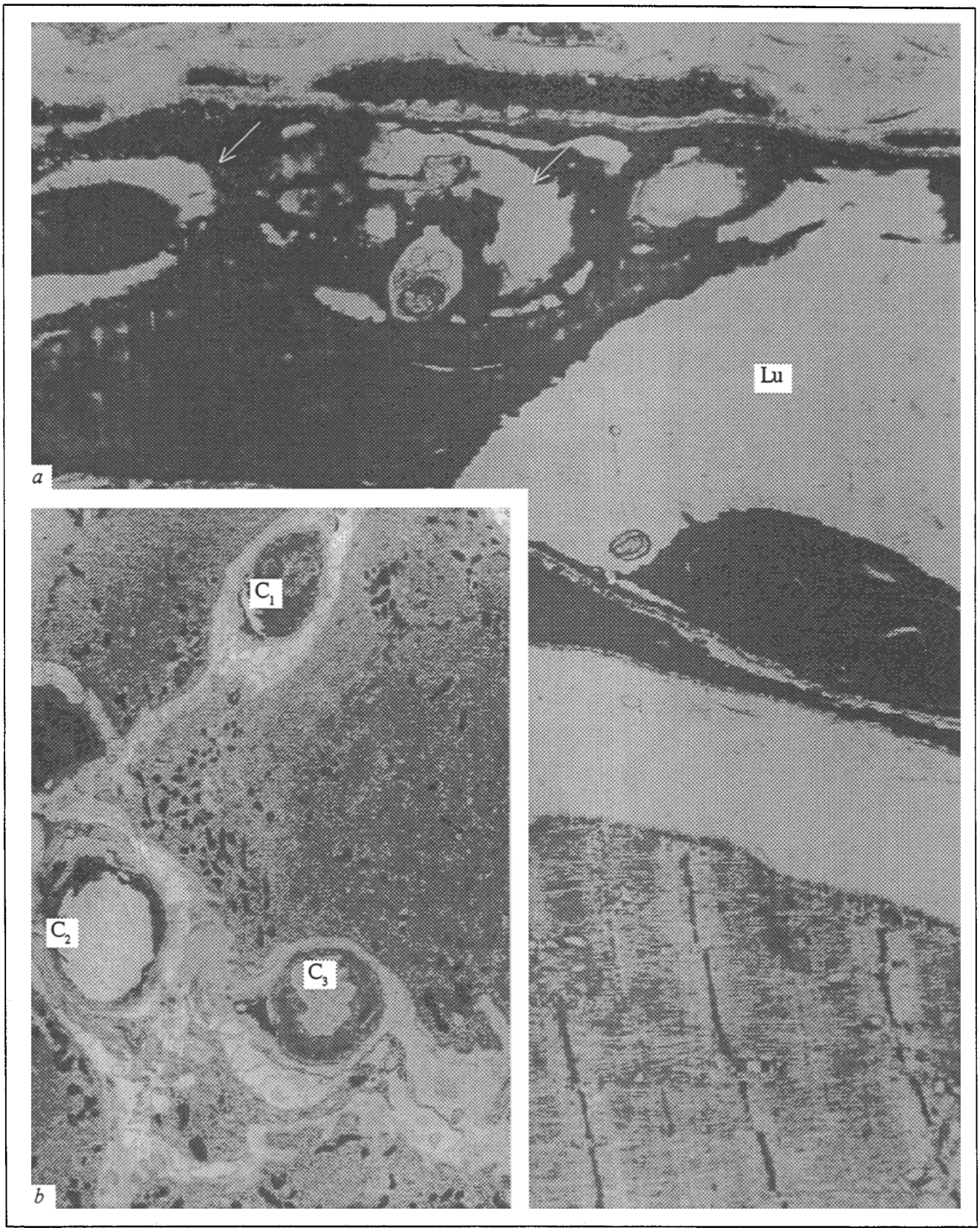

Fig. 3. Coagulation necrosis of microvessel wall. *a*) longitudinal section, showing breakdown of degenerating "dark" endotheliocytes (arrows) into fragments, accompanied by karyolysis. *Lu*: lumen. One month after replantation (×7500). *b*) capillary structure in muscle fibers a long time (8 months) after replantation. C_1: capillary lumen formed by "dark" endotheliocytes in a state of hyperfunction; C_2: capillary lumen formed by two (dark and light) cells; C_3: capillary necrosis, with the lumen closed by membrane complexes. One month after replantation (×2700).

That such fragments were remnants of a former microvessel could only be deduced from the occurrence of erythrocytes in close proximity to the fragments of degenerating endotheliocytes and from the presence of hardly recognizable micropinocytic vesicles in these fragments. A consequence of the coagulation necrosis undergone by endotheliocytes was complete destruction of capillaries or vascular fragments in the microcirculatory bed. However, their destruction was usually preceded by renewal of the endothelial lining (Fig. 2, *a*, *b*, and *d*). Endothelial renewal in skeletal muscle and other tissues is a well-known phenomenon and has been described previously [1], but the capacity for renewal appears to be limited. Following damage to the skeletal muscle, not more than three renewal cycles could be observed in transverse sections. Changes of the "dark" type in endotheliocytes may therefore reflect both a state of functional stress and one of the stages of degeneration, and it should be noted that functional stress may culminate in degeneration of the "dark" type.

Normally, "dark" and "light" cells alternate in capillaries. The arteriolar lumen shown in Fig. 2, *c* is formed by endotheliocytes differing in electron density. The ultrastructural organization of these cells indicates that they are in a state of hyperfunction (the plasma membrane produces deep invaginations, and ribosomes, polyribosomes, and multiple vesicles are well defined in the cytoplasm). In the event of prolonged exposure to a pathogenic agent, there occurs an increase not only in the numbers of "dark" endothelial cells in the capillaries but also in the number of vessels formed solely of such cells, and these then show signs of functional overstrain (occasional vacuoles, membrane inclusions, a somewhat dilated perinuclear space, damaged mitochondrial cristae). Such signs are succeeded by margination of large chromatin granules in the nucleus, further expansion of the perinuclear space, and by compression and homogenization of the cytoplasm; as a result, the endothelial cells become more osmiophilic (D_d cells in Fig. 2, *d*).

The morphological signs of endothelial cell death described for a skeletal muscle after trauma or limb replantation are similar and they also resemble those characteristically observed in other pathological states of skeletal muscles and in cells of other tissues. We, for example, saw such changes in microvessels when examining a sympathectomized skeletal muscle or skeletal muscle biopsy material from a patient with thymoma (unpublished data).

To sum up, two degenerative types of endotheliocytes - "light" and "dark" - were identified in skeletal muscle a long time (8 months) after traumatic injury to or replantation of a limb. The destructive/degenerative changes in endotheliocytes of the "light" type involved the development of intracellular edema, which is a characteristic feature of acute tissue damage. In endotheliocytes of this type, the cytoplasm sometimes undergoes total lysis of the colliquative necrosis type. Such changes appear to be of an ischemic nature and are reversible in most cases if the cell membrane and nucleus are preserved. Once the pathological process has assumed a chronic course, the damaged skeletal muscle comes to contain an increasing number of "dark" endotheliocytes with destructive/degenerative changes, and the process may culminate in coagulation necrosis and fragmentation of the cells. After limb reimplantation this outcome is more likely and more conspicuous.

An increase in the number of "dark" cells in the capillaries of a skeletal muscle indicates progression of the destructive/degenerative process of vascular genesis in the muscle.

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