# ELECTRON-MICROSCOPIC STUDY OF CAPILLARY REGENERATION IN SKELETAL MUSCLE AFTER MECHANICAL INJURY

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UDC 616.74-001-07:[616.16-031:611.74]-003.93-076.4

KEY WORDS: regeneration, endotheliocyte, satellite cell.

The first signs of regeneration in an injured adult rat muscle begin to appear on the 3rd day after trauma, and the stages of formation of myoblasts and muscle tubes are well defined [2, 3]. Meanwhile, in extensive trauma, the muscle tissue defect is filled in the focus of injury as a rule by a scar. From this point of view it is interesting to study the causes preventing completion of the process of muscle tissue regeneration, and also ways and times of restoration of its other components, primarily the microvessels.

The aim of this investigation was an ultrastructural study of the blood capillaries in a traumatic focus in skeletal muscle.

## EXPERIMENTAL METHOD

Noninbred male albino rats weighing 300-360 g were used. Trauma was applied to the medial head of the triceps surae muscle by Cannon's method [5]. Material was investigated on the 1st, 2nd, and 3rd days after trauma. Pieces of the medial head of the gastrocnemius muscle were fixed successively in cold formol-sucrose solution and 1% buffered $OsO_4$ solution and embedded in Araldite. Ultrathin sections were examined in the electron microscope.

## EXPERIMENTAL RESULTS

On the 1st day after trauma destruction of blood vessels took place and many blood cells were extravasal in their situation. Endothelial cells of preserved capillaries were edematous, with rouleaux of erythrocytes in their lumen. On the 3rd day thrombi formed in the arterioles and capillaries. Only membranes of the endothelial cells were preserved in some microvessels. Meanwhile the structure of some microvessels was almost completely intact. By the 4th-5th day signs of irreversible damage were found: The endothelial cells had an electron-dense cytoplasm and a very compact nucleus. Cell organelles could not be distinguished in these endotheliocytes. Microvessels of this kind were usually located next to muscle fibers showing destructive changes. The cytoplasm of the satellite cells in the composition of these fibers also had very high electron density. All preserved capillaries showed signs of activation of their endothelial cells: numerous microvilli and evaginations on the luminal and basal surfaces of the endotheliocytes.

On the 8th-10th day, when muscle tubes and young muscle fibers were forming, distinct signs of regeneration of the blood capillaries could be seen. Formation of the new capillary wall usually took place within the boundaries of the preserved basement membranes of the destroyed vessels (Fig. 1a). It can be seen in longitudinal sections how narrow outgrowths of endothelial cells grew toward one another. Fragments of old endothelial cells can be seen between the basement membranes (Fig. 1c). The formation of a new layer of endotheliocytes on the old is clearly visible in Fig. 2c. As a result, the newly formed capillaries had duplicate basement membranes – a feature which could be used to identify them. Particular ultrastructural features of the young endothelial cells must be pointed out: the absence of any specialized transport organelles (micropinocytotic vesicles), the abundance of ribosomes and polysomes, the hypertrophied lamellar apparatus and, which must be specially mentioned,

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 0007-4888/85/0007-0972$09.50 

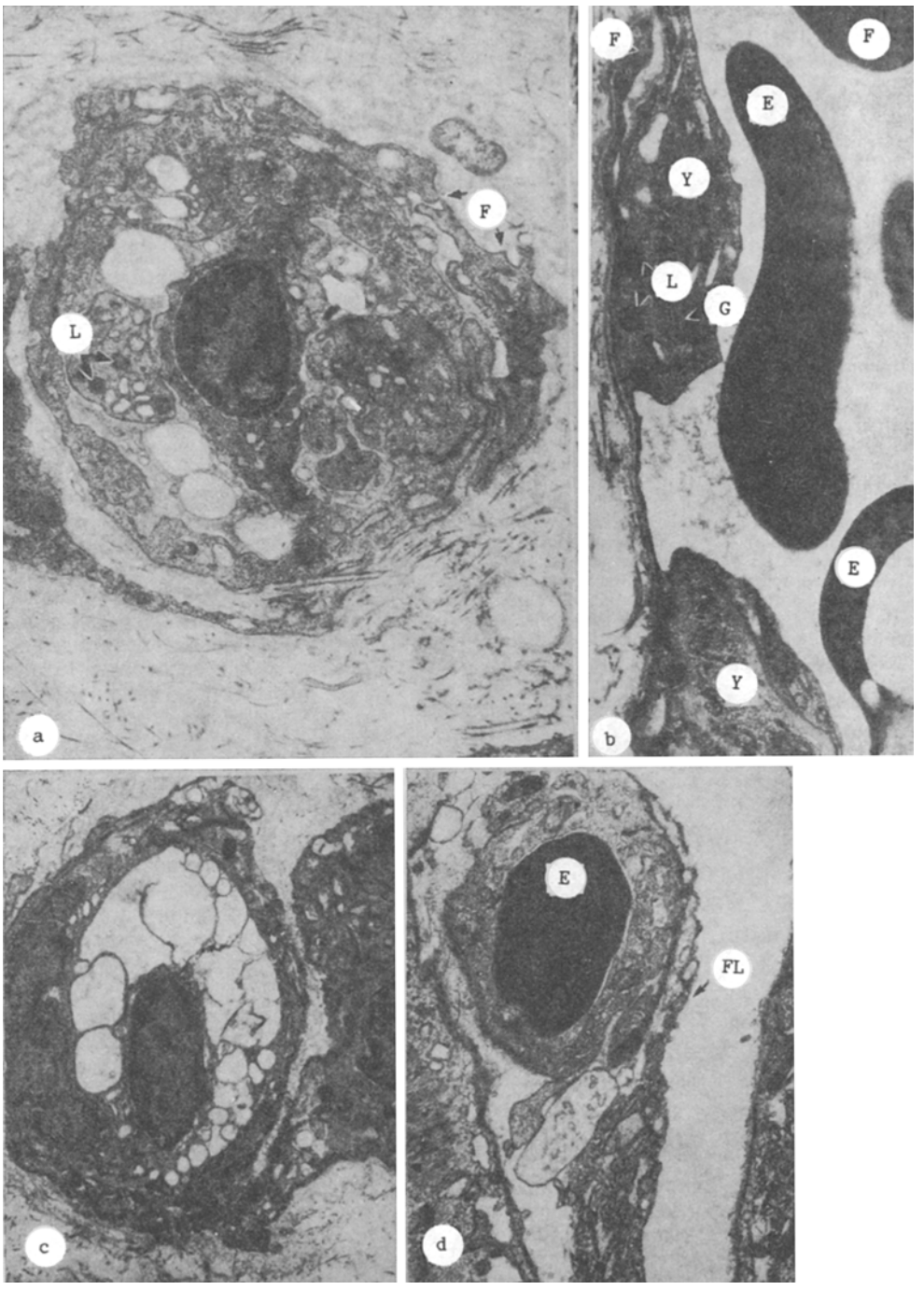


Fig. 1. Signs of regeneration of blood capillaries: a) regeneration of capillaries on boundaries of old vascular "sheath" — "growth bud." 31,500×; b) longitudinal section through regenerating newly formed capillary: E) erythrocytes, Y) young endotheliocytes, L) lysosomes, G) Golgi complex, F) fragments of disintegrated endothelial cells. 21,000×; c) formation of lumen in newly formed capillary. 21,000×; d) junction between fibroblast-like (FL) cell and newly formed capillary. 21,000×.

the presence of lysosomes (Figs. 1 and 2). Lysosomes probably play a role in the formation of the capillary lumen.

Capillaries could also be formed *de novo* close to young muscle fibers or muscle tubes. The endotheliocytes of such capillaries have a high nucleo-cytoplasmic ratio, their lumen as a rule is slit-like, and the electron density of the nucleus and cytoplasm is almost equal (Fig. 2a, b). Capillaries of this kind formed tight junctions with cells of fibroblast type (Fig. 1d). The morphological similarity of the young endothelial cells, pericytes, and

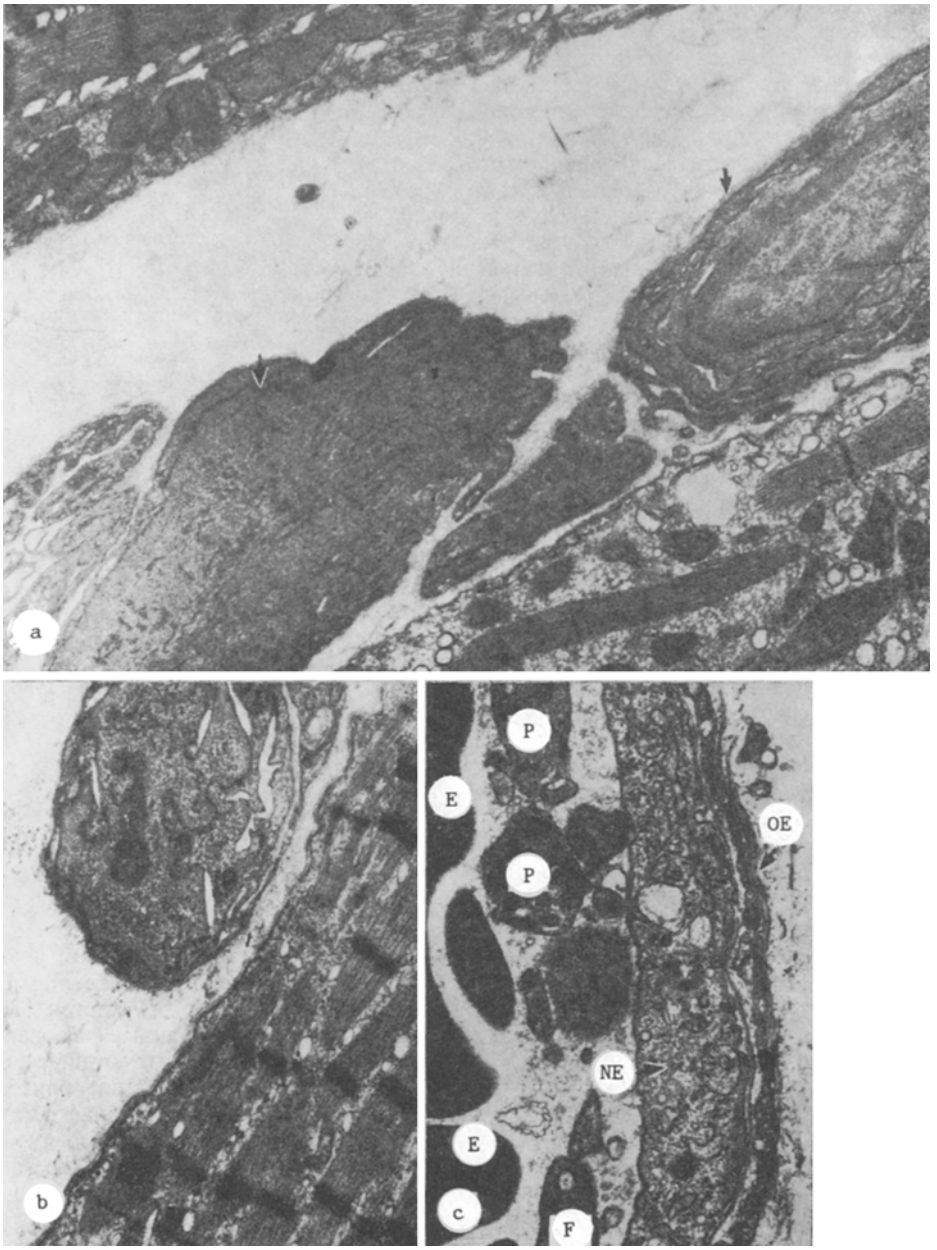


Fig. 2. Structure of newly formed capillary: a) undifferentiated cell in interstitial space (arrow on left), newly formed capillary (arrow on right). 31,500×; b) newly formed capillary. 15,000×; c) new layer of capillary endotheliocytes, remains of old endothelial cell beneath it, between basement membranes. 28,000×. NE) New endotheliocytes, OE) old endotheliocytes, P) platelets, E) erythrocytes.

fibroblast-like cells, which were found in the interstices, must be mentioned. The individual stages of incorporation of these undifferentiated fibroblast-like cells into the composition of the capillary wall could be traced, i.e., they occupied the place of a pericyte. Often the pericytes formed a continuous ring around the endothelium, and formed finger-like junctions, which normally are rarely observed. However, junctions of this kind have been described previously for the early stages of postnatal development [1].

Thus on the 8th-10th day after trauma, parallel with regeneration of muscle fibers but taking place a little later, intensive restoration of the blood capillaries was proceeding. The preserved basement membrane of the destroyed vessels acted as a framework around which the new layer of endothelium was formed. A similar picture has been described in patients with diabetes mellitus [7].

The results suggest that the "growth bud" of the capillary is a multicellular formation. Lysosomes present in the young endothelial cells probably participate in the formation of the lumen of the vessel. Capillaries also form *de novo* in the zone of injury. Undifferentiated cells are evidently their source. The character of restoration of microvessels is further evidence of the simultaneous course of injury and repair processes at the tissue and cellular levels.

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# FLUORESCENCE SPECTRA OF SIF CELLS IN RAT NERVE GANGLIA


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UDC 612.89.014.445

KEY WORDS: autonomic ganglia, cytofluorometry, SIF cells.


Determination of the type of mediator synthesized by the so-called small, intensively fluorescent (SIF) cells, can make a definite contribution to the elucidation of their function. To investigate this problem, besides other methods, fluorescent histochemical methods have been used and, in particular, various modifications of the method of condensation of amines with paraformaldehyde. The principal mediator for the SIF cells of several ganglia is considered to be dopamine, whereas in some SIF cells the content of noradrenalin, and also of adrenalin, has been determined [7].

Meanwhile evidence is accumulating of the existence of a subpopulation of SIF cells containing serotonin [6, 8]. Since the maximum of fluorescence of paraform-induced fluorophores (serotonin derivatives) lies in the yellow region of the spectrum, the discovery of "yellow" SIF cells is considered to be confirmation of their serotoninergic nature. Several workers have described an increase in the number of "yellow" SIF cells in animals with age and have postulated an age change in the type of mediator characteristic of some SIF cells [1]. However, it must be pointed out that the data of fluorescence analysis do not always allow different types of amines to be differentiated because of the closeness of the spectral characteristics of their paraform-induced fluorophores, and also because of the properties of visual perception, leading to the "red shift" with an increase in concentration of the fluorophore in a cell under observation [5].

The aim of this investigation was to study fluorescence spectra of SIF cells of various ganglia of old animals under the conditions of standard processing by one of the accepted "aqueous" methods of monoamine detection [4], and also to compare the curves obtained with the emission spectrum of lipofuscin granules, accumulating with age in cells of autonomic ganglia.

## EXPERIMENTAL METHOD

The test objects were nerve ganglia from male rats aged 24 months. SIF cells in ganglia in the lumbar portion of the sympathetic trunk and of the great pelvic ganglion, and nerve


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(Presented by Academician of the Academy of Medical Sciences of the USSR, V. V. Kupriyanov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 100, No. 7, pp. 114-115, July, 1985. Original article submitted November 16, 1984.