4. V. G. Korpachev, S. P. Lysenkov, and L. Z. Tel', Patol. Fiziol. Éksp. Ter., No. 3, 78 (1982).
5. I. G. Lyudkovskaya, "Morphology and pathogenesis of changes in the brain and spinal cord during respiratory resuscitation after stroke," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences, Moscow (1984).
6. V. A. Negovskii, Essays on Reanimatology [in Russian], (1986).
7. N. I. Nezlina, Results and Prospects for the Development of Modern Reanimatology [in Russian], Moscow (1986), p. 80.
8. N. K. Permyakov, A. V. Khuchua, and V. A. Tumanskii, Postresuscitation Encephalophy [in Russian], Moscow (1986).
9. Z. Ya. Rublëva, Yu. I. Savulëv, and A. S. Pylaev, Zh. Nevropatol. Psikhiat., No. 7, 966 (1977).
10. Z. Ya. Rubleva, Functions of Neuroglia [in Russian], Tbilisi (1984), p. 56.
11. Z. Ya. Rubleva, Tsitologiya, No. 6, 652 (1985).
12. V. V. Semchenko, "Principles of structural changes in the cerebral cortex in the recovery period after short-term total ischemia," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences, Novosibirsk (1984).
13. Zh. V. Solov'ev and D. D. Orlovskaya, Zh. Nevropatol. Psikhiat., No. 7, 1040 (1977).
14. D. J. Malinska, W. Boellard, and W. Schlote, Acta Neuropath., 3, 222 (1984).
15. C. K. Petito and W. A. Pulsinelli, J. Cereb. Blood Flow Metab., 4, 194 (1984).

VASCULAR CHANGES IN MATURING GRANULATION TISSUE

Academician D. S. Sarkisov,* E. G. Kolokol'chikova, R. I. Kaem, and A. A. Pal'tsyn

UDC 616.003.92-005-091-07

KEY WORDS: granulation tissue; capillary; electron-microscopic autoradiography.

The morphology of granulation tissue has been studied in numerous investigations of wound healing during war and peace [1-3, 6-8]. In these investigations, conducted at the light-optical and electron-microscopic levels, the time course of structural changes in the cells and ground substance of granulation tissue during its maturation has been studied in fair detail. As regards the vascular network of granulation tissue, all that is known about it is that it undergoes considerable reduction during scar formation. However, the actual process of this reduction, i.e., how gradually the majority of these vessels of granulation tissue disappears, has not yet been studied.

The aim of the present investigation was to undertake an electron-autoradiographic study of granulation tissue at different stages of scar formation, paying particular attention to transformation of its capillary network during this process.

EXPERIMENTAL METHODS

Granulation tissue was studied in rats on the 20th and 40th days after wounding. A piece of skin with the underlying connective tissue, measuring 3 × 4 cm^2, was excised from the dorsal region of the animals under ether anesthesia. Material for histologic examination was embedded in paraffin wax and sections were stained with hematoxylin and eosin, with picrofuchsine by Van Gieson's method, and with toluidine blue.

For the electron-autoradiographic study pieces of skin measuring 1 mm^3 were excised and incubated at 37-38°C in medium 199 containing 20 μCi/ml of ^{3}H-thymidine (specific activity 21.6 Ci/mmole) for 1.5 h. At the end of incubation the material was washed to remove unin-

*Academy of Medical Sciences of the USSR.

Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 4, pp. 501-503, April, 1988. Original article submitted August 14, 1987.

 0007-4888/88/0004-0604$12.50

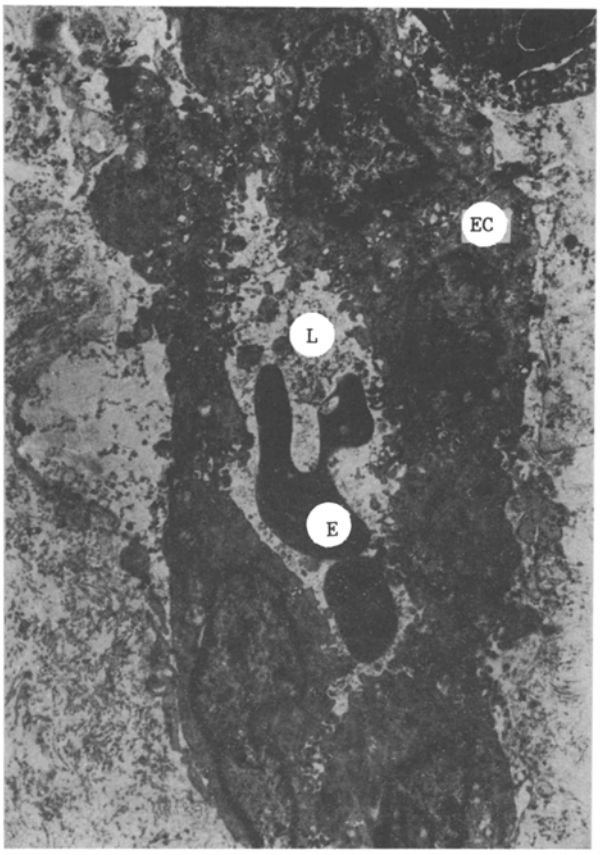

Fig. 1. Capillary in granulation tissue on 20th day after wounding. Here and in Fig. 2: L) lumen of vessel, E) erythrocyte. EC) endothelial cell. 4000×.

corporated precursor with cold phosphate buffer (pH 7.4), fixed in a 2.5% solution of glutaraldehyde and a 1% solution of OsO_4, and embedded in Epon. Semithin sections were first studied by light-microscopic autoradiography. After analysis of the semithin sections, regions for ultramicrotomy were selected. Electron-microscopic autoradiographs were prepared by the method described previously [4] and studied in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

Many small vessels, whose walls consisted of a layer of large endothelial cells, the basement membrane surrounding them, and with cells of pericyte type immediately outside it, were observed in the granulation tissue on the 20th day after trauma. The lumen of many of these vessels was slit-like (Fig. 1). Other vessels were seen with signs of destruction of the endotheliocytes and basement membranes, in the form of vacuolation of the cell cytoplasm, thinning of the cytoplasmic processes, disturbance of intercellular junctions, and the formation of large intercellular pores, of homogenization, and sometimes lysis of the cell nucleus, and thinning and fragmentation of the basement membrane. Fibroblasts, lymphoid cells, neutrophilic leukocytes, macrophages, and single collagen fibers were arranged in the intervascular tissue. Some fibroblasts were actively incorporating ^3H-thymidine, i.e., they were proliferating. At this stage of development of the granulation tissue its principal structural components were vessels of capillary type as described above.

On the 40th day electron-autoradiographic investigation of the granulation tissue revealed a different picture: its main mass consisted of various cells, mainly fibroblasts and macrophages, accompanied now by quite numerous bundles of collagen fibers, whereas considerably fewer vessels were observed than on the 20th day of the investigation. Some of them preserved their typical shape and structure, whereas others showed a unique kind of structural reorganization consisting of disturbance of the integrity of their wall, separation of its component cells and, finally, their rearrangement in a particular manner among

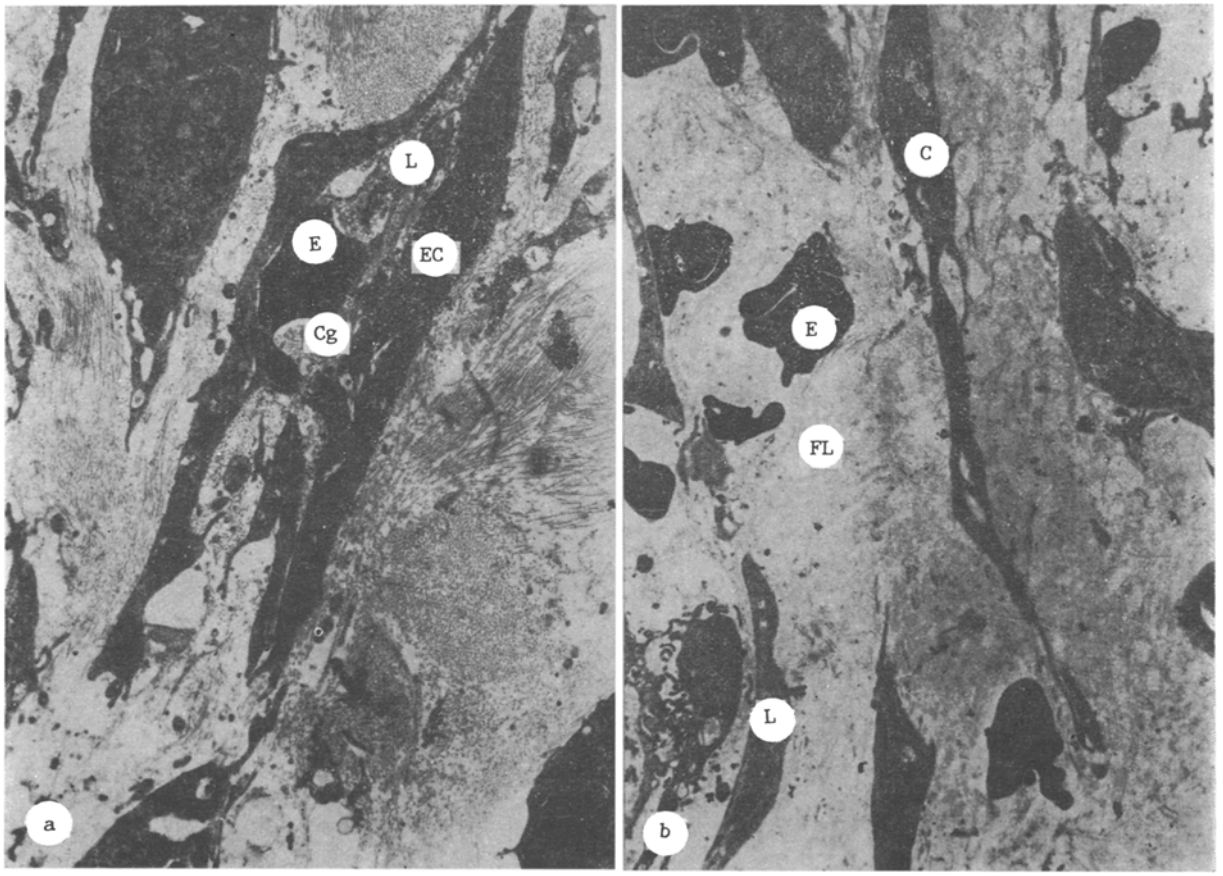

Fig. 2. Granulation tissue on 40th day after wounding. a) Loss of integrity of vessel wall with growth of collagen fibers into its lumen. Cg) Collagen; b) preservation only of general outline of former vessel, its cells separated by connective tissue. FL) Former lumen of vessel; C) cell in wall of former vessel.

the other cells and fibrous structures (Fig. 2). All phases of this gradual "disappearance" of the vessel, starting with disturbance of contact between its endothelial cells and ending with a group of cells lying independently, can be traced in serial sections. The character of the mutual arrangement of these independent cells, and erythrocytes usually present among them suggests on resonably firm ground that this was a vessel whose wall had become transformed so that its component cells were apparently dissociated and converted into interstitial tissue cells. Incidentally, active incorporation of ^{3}H-thymidine was frequently observed in endotheliocytes, pericytes, and also in separate cells from groups which, it may be assumed, the vessel was formed previously. DNA synthesis in these cells is evidence of their readiness to divide.

On the basis of the above description it seems that the reduction of the number of vessels during maturation of granulation tissue, a well known fact for a long time, can be attributed not simply to their disappearance, but to the separation of the cells forming their wall and their distribution among other cells lying freely between the vessels. Some of these cells, pericytes for example, evidently differentiate into smooth muscle cells [9] and fibroblasts, which subsequently become the cells of the future scar tissue.

It must be pointed out in particular that transformations of small vessels of this kind were described previously by the writers during an electron-autoradiographic study of normal human dermis [5]. This suggests that there exists a hitherto unknown phenomenon of transformation of blood vessels of capillary type, which is common both to the normal dermis and to reparative processes involved in its healing after trauma. The essence of this phenomenon now postulated is that the small vessels of the dermis are continuously "disintegrating" and entering into the composition of the interstitial tissue cells, while at the same time new vessels are formed, thus making possible physiological regeneration of the cells and fibrous structures which are the basis of skin.

LITERATURE CITED

1. N. N. Anichkov, K. G. Volkova, and V. G. Garshin, The Morphology of Wound Healing [in Russian], Moscow (1951).
2. I. V. Davydovskii, Gunshot Wounds in Man [in Russian], Vol. 1, Moscow (1952).
3. M. I. Kuzin and B. M. Kostyuchenka (ed.), Wounds and Wound Infection [in Russian], Moscow (1981).
4. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, Electron-Microscopic Autoradiography of the Cell [in Russian], Moscow (1980).
5. D. S. Sarkisov, E. G. Kolokol'chikova, and A. A. Pal'tsyn, Byull. Éksp. Biol. Med., No. 5, 730 (1987).
6. C. Lundberg, D. Campbell, B. Agerup, and H. Ulfendahl, Scand. J. Plast. Reconstr. Surg., 16, 123 (1982).
7. M. H. McGraft and J. M. Emery, Ann. Plast. Surg., 115, 105 (1985).
8. R. Ross, Biol. Rev., 43, 51 (1968).
9. D. E. Sims, Tiss. Cell, 18, 153 (1986).

SKELETAL MUSCLE MORPHOLOGY IN ALLOXAN DIABETES

G. P. Titova and N. V. Khotenashvili

UDC 616.379-008.64-092.9-07:616.74-091

KEY WORDS: alloxan diabetes; skeletal muscle; morphology.

Many new facts on diabetes have been discovered in recent years, on the basis of which it can be concluded that diabetes mellitus is a polypathogenetic disease, in which an absolute or relative insulin deficiency gives rise to functionally expressed morphological disturbances of many organs and systems [2, 3].

Among patients with ischemic lesions of the lower limbs the largest group consists of diabetics, in whom the incidence of gangrene is 40 times greater than in patients with atherosclerosis alone. Diabetic gangrene of the lower limbs is not only, and not so much a manifestation of atherosclerotic changes in major blood vessels, as the result of a lesion involving vessels of the microcirculatory bed [1, 4, 5].

In the few morphological studies which have been made of skeletal muscles in diabetes, microangiopathies have been found, with a lesion of the vessel walls and of their basement membranes. These changes are particularly marked in patients with insulin deficiency in a state of decompensation, with which is associated the development of mechanical changes in vascular permeability and dystrophic and sclerotic changes in the tissues [6-8].

The aim of this investigation was to study the structural basis of the skeletal muscular lesion in animals on an experimental model of alloxan diabetes (AD).

EXPERIMENTAL METHODS

Experiments were carried out on 12 mongrel dogs of both sexes weighing 9-22 kg. Diabetes was produced by a single intravenous injection of 10% alloxan (80 mg/kg). The development of diabetes was confirmed by determination of the blood sugar by the Hagedorn–Jensen method. After the clinical data showed development of long-lasting AD, with a duration of 2, 3, 4, and 5 months, the animals were killed by intracardiac injection of hexobarbital. Pieces of skeletal muscle from the rectus abdominis muscle and a superficial thigh muscle were taken for morphological study.

The pieces of muscle for light-optical investigation were fixed in 10% neutral formalin and embedded in paraffin wax; sections were stained with hematoxylin and eosin, by Van

Laboratory of Pathomorphology with Electron Microscopy Group, N. V. Sklifosovskii Moscow Emergency Aid Research Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 4, pp. 504-506, April, 1988. Original article submitted July 17, 1987.

