Bulletin of Experimental Biology and Medicine, Vol. 126, № 11, November, 1998

PHARMACOLOGY AND TOXICOLOGY

Effect of Sodium Succinate on Histomorphological Changes of Autodermic Grafts in Rats

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Translated from *Byulleten' Experimental'noi Biologii i Meditsiny*, Vol. 126, No. 11, pp. 555-560, November, 1998
Original article submitted February 3, 1998

Sodium succinate was found to increase the viability of epithelial cells in autodermic transplants. It stimulated the proliferation of epidermal cells of the basal layer, enhanced the activity of succinate dehydrogenase, lactate dehydrogenase and adenosine triphosphatase, intensified the formation of keratin, and activated the ingrowth of blood vessels through the generation of functioning endothelial cells.

Key Words: *sodium succinate, autodermic graft, histomorphological changes*

Previously, we have found that under conditions of reduced circulation sodium succinate (SS) exhibited dermoprotective properties in different species of animals. It normalized the epidermal and blood concentration of histamine and 5-hydroxy-tryptamine, showed antitoxic activity, and increased microcirculation in the skin, brain, heart, and other tissues and organs [2].

The goal of the present work was to study the effects of SS on histomorphological characteristics of autodermic grafts in rats.

MATERIALS AND METHODS

Histomorphological changes in circular autodermic grafts of 25 mm in diameter and an area of 490.5 mm^2 were studied in 14 outbred male albino rats with 150-160g body weight. They were divided into two groups: the control and experimental. The rats of the experimental group were treated with SS for four days (single dose of 100 mg/kg i.p., daily). On the 5th day after transplantation the rats were killed by an overdose of diethyl ether, and several pieces of the skin graft were taken for investigation. One of the pieces was fixed in 10% formaldehyde, embedded into paraffin, and sectioned. The sections were stained by hematoxylin-eosin and azur II—eosin to study their morphology.

Another piece was sectioned in a cryostat to a thickness of 10 μm. To study the effect of SS on energy metabolism, the localization and activities of adenosine triphosphatase (ATPase, EC 3.6.1.3.), succinate (SDH, EC 1.3.99.1) and lactate dehydrogenases (LDH, EC 1.1.1.27) were determined. The given enzymatic complex is known to allow a proper assessment of the energetic state, as SDH belongs to the SDH system of mitochondrial enzymes and represents one of the crucial enzymes of the dicarboxylic acid (Krebs') cycle, LDH is active in the final stage of anaerobic glycolysis, and ATPase hydrolyses the high energy phosphate bonds releasing energy [1,3,5]. ATPase plays an important role in ionic transport. ATPase, SDH, and LDH activities were measured according to [8], [1] and [4], respectively.

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0007-4888/98/0011-1135$20.00

In parallel to histoenzymatic analysis we performed an electron microscopic study to assess the ultrastructural organization of the tissue elements. The third piece of skin to be used for electron microscopy was fixed in glutaraldehyde and post-fixed in 1% osmium tetroxide, then dehydrated through a graded series of ethanol solutions (from 50 to 100%), treated with propylene (or acetone) oxide (2 sessions, 20-30 min each) and immersed in Epon-Araldite (acetone and epoxy resin mixture) for 24 h. Then a sample was embedded in fresh Epon-Araldite in capsules and polymerized at 35, 45 and 60°C for 12h each. The prepared blocks were cut in a LKB 8801A ultratome. The semithin sections were stained with toluidine blue and viewed under a light microscope to select the regions for the detailed examination. The ultrathin sections (500-600 Å) were mounted on an uncoated electrolytic grid, stained with Reynold's lead citrate and uranyl acetate. The sections were examined and photographed in a

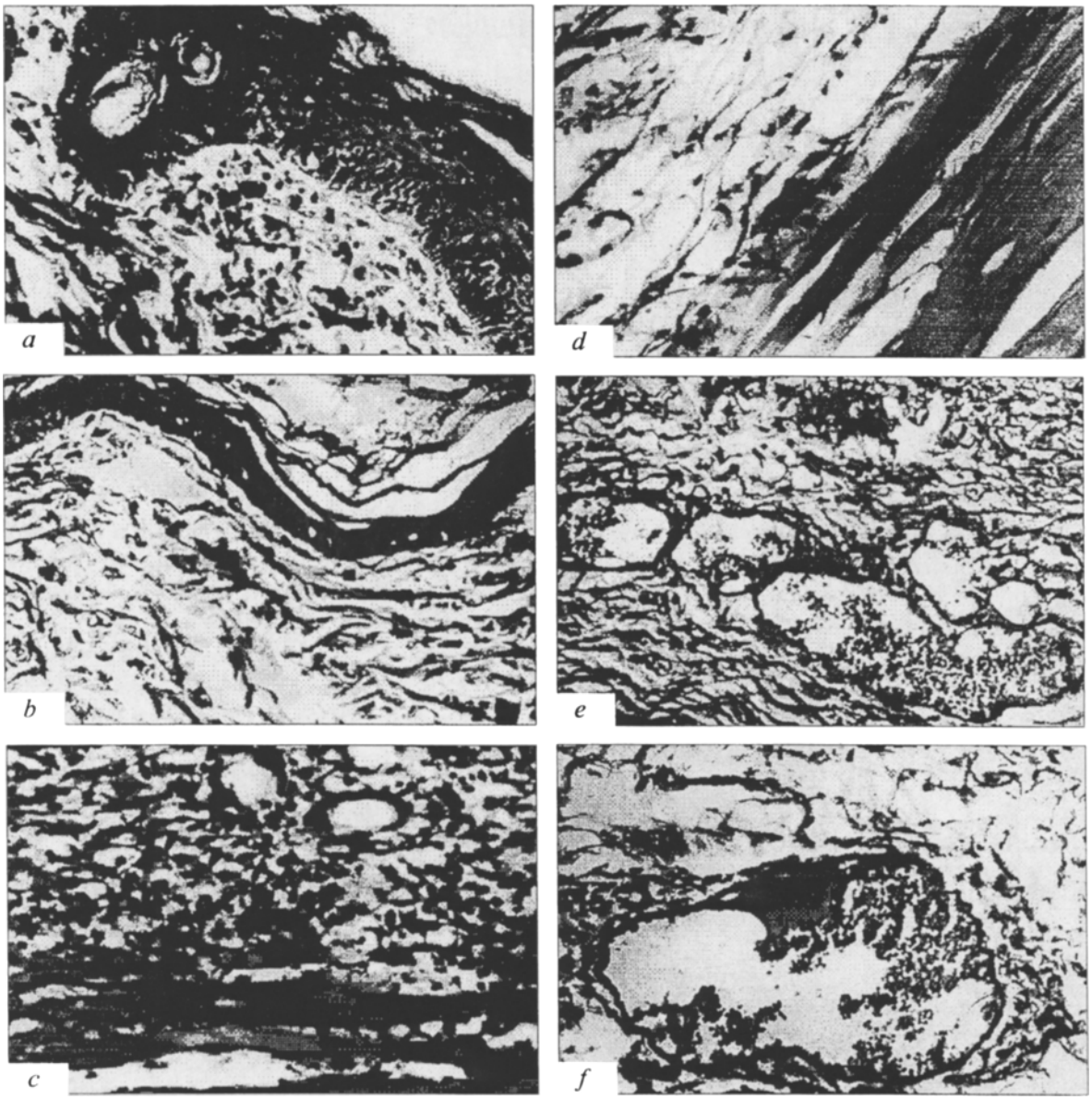

Fig. 1. The effects of sodium succinate on the morphology of autodermic grafts in rats. *a*) after treatment; cell proliferation in the basal layer of the epidermis and cellular infiltration in the dermis; *b*) control; thinned epidermis, the absence of infiltration in the dermis *c*) after treatment; the round cell infiltration of the connective tissue, vasodilatation with endothelium swelling, and well preserved muscular fibers *d*)control; insignificant infiltration of connective tissue, disordered tinctorial properties of muscular fibers *e*) after treatment; blood cells in dilated skin vessels; insignificant perivascular lysis of the connective tissue structures, a moderate round cell infiltration of the dermis; *f*) control, thrombs in dilated vessels, lytic changes in their walls, pronounced perivascular lysis of the connective tissue structures. Hematoxylin and eosin, ×200.

EMB-100AK electron microscope at an accelerating voltage of 70 kV and an electron-optic magnification from 5000 to 30,000.

RESULTS

Morphological examination on the 5th day after transplantation showed that the epidermis of the autodermic grafts from SS-treated animals looked more viable than that in control rats (Fig. 1, *a*, *b*). All its layers were clearly seen. Proliferative processes occurred in a germinative layer. Intradermal and muscular fibers maintained their structure. At the same time, the typical feature of autodermic graft morphology was a pronounced dilation of blood vessels and round cell infiltration (Fig. 1, *c*, *d*). However, they exhibited neither thrombosis, nor fibrin clots, which were common for the preparations of the control group (Fig. 1, *e*, *f*). Sections stained with azur II—eosin were rich with tissue basophils located around dilated blood vessels. The vast majority of the vessels were free from blood cells but had a swollen endothelium. The count of tissue basophils was significantly lower in the control transplants, where stasis and throm-bosis were a frequent phenomena.

The treatment with SS significantly increased the activity of SDH in the transplanted skin. It was most clearly seen in the cytoplasm of epidermal cells located in the basal and spinous layers, in hair follicle and oil gland cells, in vascular endothelial cells and dermal cellular elements. In the control transplants high activity of this enzyme was determined in hair follicle cells. The basal and spinous layers showed a moderate activity, and low activity was typical of the vascular endothelium (Fig. 2, *a*, *b*).

Similar regularities were revealed when studying the LDH histochemical reactions. It should be noted, however, that in the cells of hair follicles and dermal glands both in the experimental and control rats this enzyme showed only moderate activity.

Analysis of ATPase activity revealed that in SS-treated animals it was relatively low in the epidermis and high in all the cellular elements of the dermis. In the control animals, the ATPase activity was moderate in all the studied structures (Fig. 2, *c*, *d*).

The treatment with SS favored the maintenance of the epidermal cells' ultrastructure. Thus, the

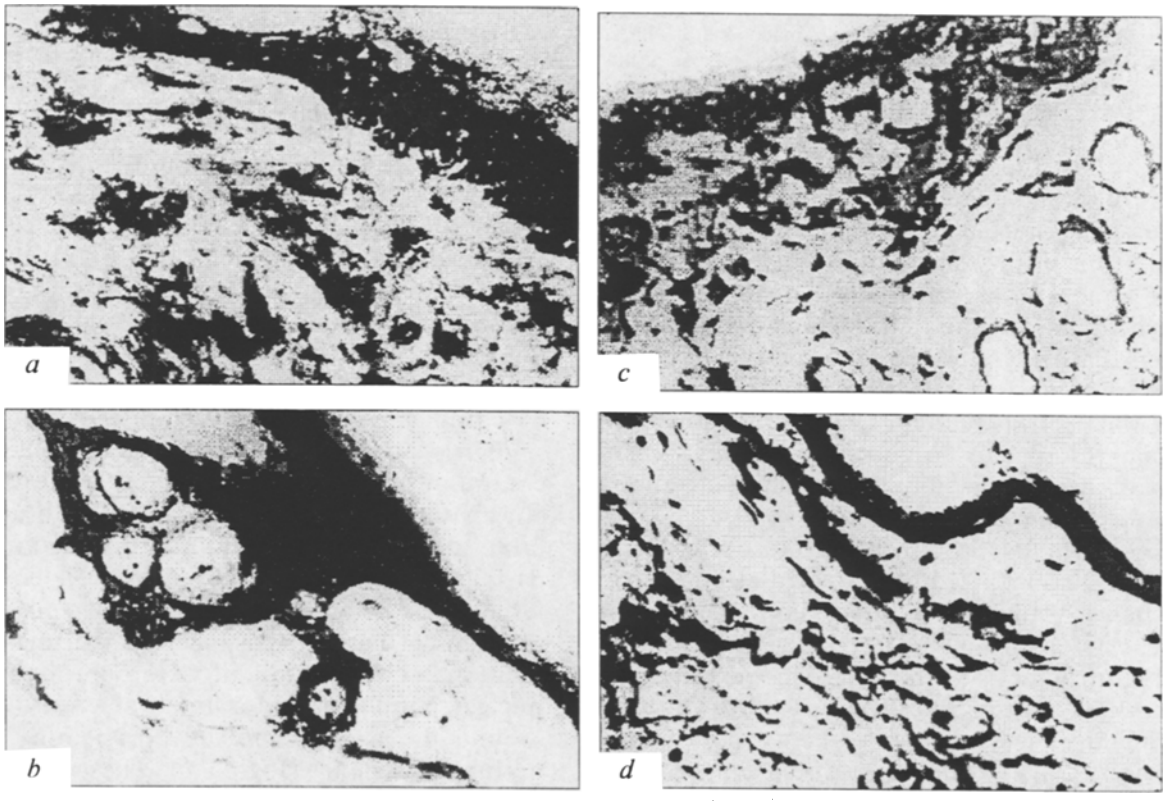

Fig. 2. The effect of sodium succinate on SDH and ATPase activity in autodermic grafts in rats. *a*) after treatment; high cytoplasmic activity of SDH in the cells of the basal and spinous layers of epidermis, hair follicles, oil glands and blood vessels' endothelium; *b*) control; high cytoplasmic SDH activity in the hair follicle cells, moderate activity in the basal and spinous epidermal layers, and low activity in vascular endothelium. *c*) after treatment; high ATPase activity in the epidermal basal membrane and dermal vascular endothelium, moderate activity in the vascular walls; *d*) control; moderate and high activity of ATPase in the epidermis and dermis. *a*, *b*) Berston's reaction; *c*, *d*), Wachstein and Meisel's reaction, ×200.

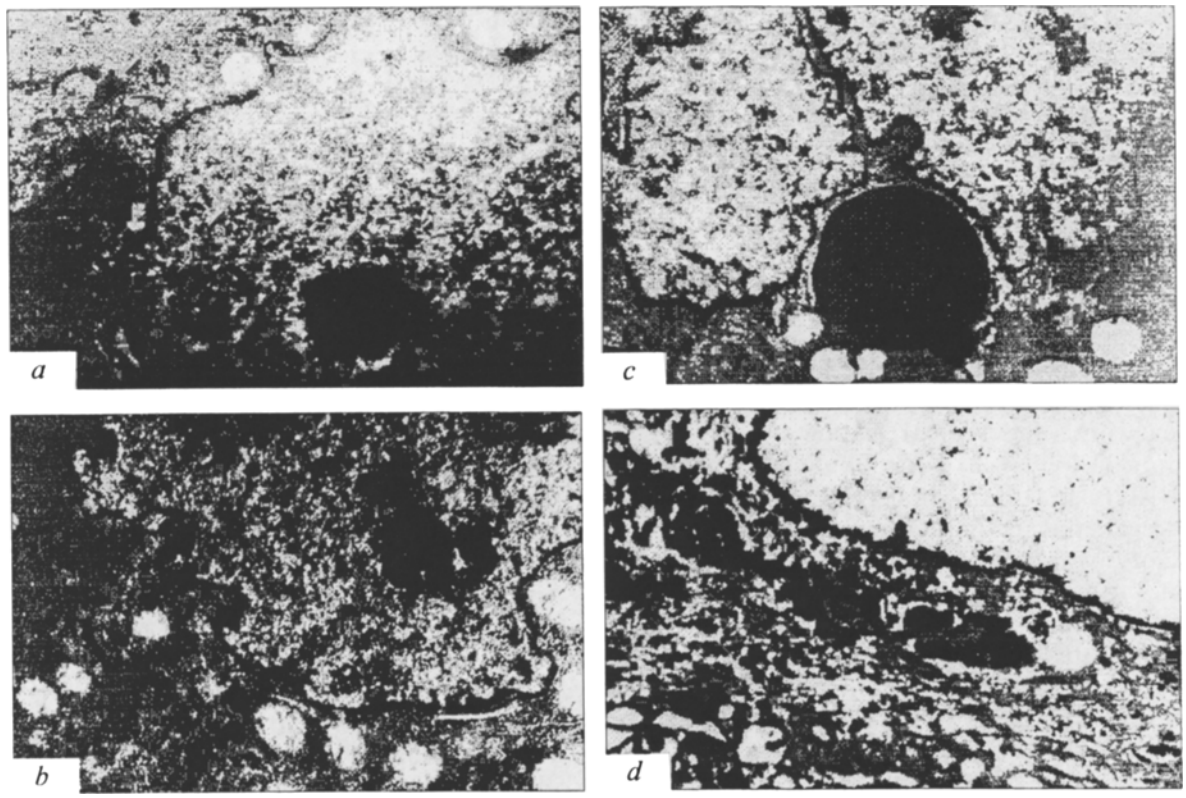

Fig. 3. The effects of sodium succinate on the ultrastructural organization of autodermic transplants in rats. *a*) after treatment; the nucleus with nucleoli and dispersed heterochromatin, mitochondria with an electron light matrix, ribosomes; *b*) control; increased electron density of the cytoplasm and nucleus, changes in the compactness of the nucleolus; *c*) after treatment; dispersed nuclear chromatin, condensation of cytoplasmic matrix, the presence of keratohyalin in the granular layer cells; *d*) after treatment; electron light nucleus, numerous aggregates and fibrillar structures in the superficial cells of the granular layer. Magnification: *a*, *b*) ×11,000, *c*) ×11,500, *d*) ×11,800.

germinative layer cells showed nuclei with dispersed heterochromatin and a well pronounced compact nucleolus. The nuclear membrane often formed a tooth-like line. The mitochondria with badly-contrasted cristae and electron light matrix were often located near its margins. The cytoplasm contained numerous desmosomes, tonofibrils, and fragments of granular endoplasmic reticulum (Fig. 3, *a*). Considering these ultrastructural characteristics as close to normal [6], it can be concluded that SS treatment maintains the viability of the germinative layer of the autodermic graft.

The germinative layer cells in the control transplants were characterized by increased electron density of their cytoplasm. The process of condensation of nuclear heterochromatin and partial disintegration (altered compactness) of the nucleolus were observed (Fig. 3, *b*). These processes can be indicative of the development of degenerative processes in the autodermic graft due to its lowered trophism.

Epidermal cells with minor alterations of ultrastructure, such as condensation of the cytoplasmic matrix, were observed in the granular layer of the autodermic grafts of the SS-treated rats. These cells also demonstrated nuclei with dispersed heterochromatin and electron dense granules of keratohyalin (Fig. 3, *c*). An electron-light nucleus was typically revealed in more superficially located cells. Osmiophilic keratohyalin aggregates and fibrillar structures were abundant in their cytoplasm (Fig. 3, *d*). These ultrastructural characteristics indicate that the function of keratin formation is retained.

Cytoplasmic condensation and homogenization of cytoplasmic structures occurred in cells of a similar location in the autodermic transplants of the control rats. The electron-dense aggregates of heterochromatin were observed (Fig. 4, *a*). Shrunken cellular nuclei contained numerous electron dense particles which can be attributed to hypoxic manifestations. At the same time, some cells exhibited the signs of nuclear lysis, such as the nucleus clarification, condensation of heterochromatin at the nuclear periphery, and a broad electron light space around the nucleus probably originating from the perinuclear edema (Fig. 4, *b*). These ultrastructural changes might be due to dystrophic processes and developing hypoxia.

In the connective tissue underlying the autodermic graft in SS-treated rats we observed the indications of the blood vessel formation, such as

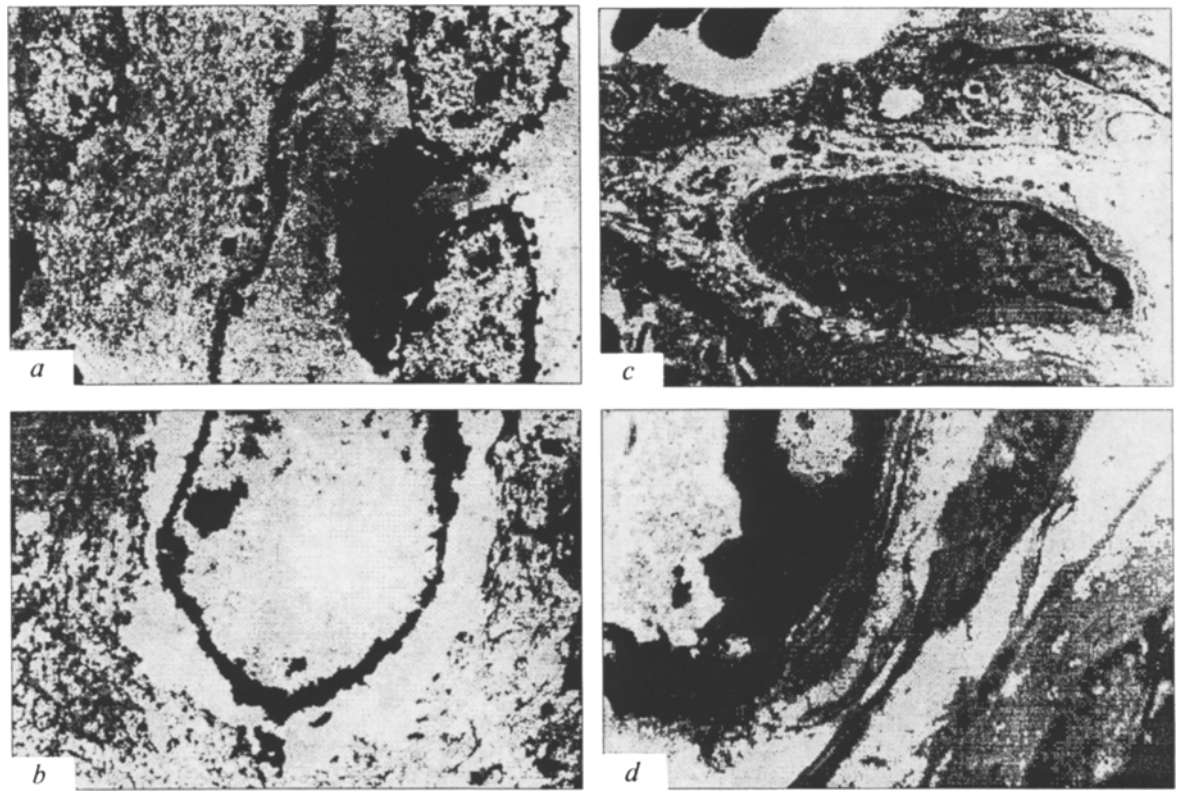

Fig. 4. The effects of sodium succinate on the ultrastructural organization of autodermic transplant cells in rats. *a*) control; homogenization of the cytoplasmic structures, nuclear electron dense particles and cytoplasmic keratohyalin aggregates in the superficial cells of the granular layer; *b*) control; nuclear lysis with peripheral condensation of heterochromatin and pronounced perinuclear edema; *c*) after treatment; endothelial cells from the bed of the autodermic transplants with a high content of nuclear heterochromatin and numerous cytoplasmic ribosomes and pinocytotic vacuoles; *d*) control; endothelial cell with an electron dense cytoplasm and the signs of cytoplasmic disintegration; *a*) ×9000; *b*) ×10,500; *c*,*d*) ×8500.

endothelial cells with numerous cytoplasmic projections. These cells had extended nuclei with a high content of heterochromatin. The ribosomes and pinocytotic vacuoles were abundant in their cytoplasm (Fig. 4, *c*). The fibroblasts and histiocytes had no abnormalities in their ultrastructural organization. The connective tissue contained a large number of active macrophages, neutrophils, leukocytes, and other cell elements.

In the control rats the count of newly-formed and functionally active endothelial cells in the same tissue was significantly lower than in SS-treated animals. Some of these cells showed condensation of their nucleus and cytoplasm (Fig 4, *d*). The signs of cytoplasmic disintegration, such as the presence of perivascular lytic zones probably arising from edema were revealed. The walls of blood vessels contained cells with numerous electron light vacuoles and lipid granules, which is indicative of the development of destructive and dystrophic changes.

Thus, the treatment with SS enhanced the viability of epithelial cells. It increased cell proliferation in the epidermal germinative layer, maintained the high activity of SDH, LDH, and ATPase, providing energy metabolism, stimulated the formation of keratin, and activated the ingrowth of blood vessels and generation of functionally active endothelial cells, thus intensifying energy metabolism in the autodermic transplants and accelerating their taking in.

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