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MORPHOLOGICAL INVESTIGATION OF GUNSHOT WOUND HEALING BY THE USE OF SEMITHIN SECTIONS

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The technique of semithin sections (STS) substantially widens opportunities for morphological analysis at the light-optical level. It is used at the present time to study organs and tissues: the nervous system [3], kidneys [4], hematopoietic tissue [8], the eye [2], liver, and gastrointestinal tract [7]. The STS method has not been widely used for the morphological study of wound healing, and the only references to its use for this purpose relate to work by Shekhter et al. [1, 5, 6].

EXPERIMENTAL METHOD

In the present investigation the STS method was used to study the cell composition of granulation tissue during healing of gunshot wounds. Experiments were carried out on 24 rabbits of both sexes weighing 2-2.5 kg. A perforating gunshot wound of the soft tissues of the thigh was inflicted on the animals by the standard method under thiopental anesthesia. The rabbits were killed by air embolism 1, 3, 5, 7, 9, 14, 21, and 28 days after primary surgical treatment (three animals at each time) and material was taken from the region of the wound for morphological investigation. Pieces of tissue were fixed in 2% OsO_4 solution, dehydrated in acetone, and embedded in Epon. STS 1-2 μ thick were obtained on an LKB Ultratome (Sweden). Polychromatic staining of the STS was carried out without removal of the resin in mixture of a 1% solution of methylene blue and a 1% solution of sodium tetraborate, followed by counterstaining with 2% fuchsin solution.*

EXPERIMENTAL RESULTS

The structure of single cells and relations between them were clearly revealed in the granulation tissue on STS. In the initial period of granulation tissue formation (3-7 days) various cells differentiated in it: fibroblasts, macrophages, and neutrophilic granulocytes. The fibroblasts were found in two forms. Cells of the first type, young proliferating fibroblasts, were fusiform, with large oval nuclei containing several nucleoli and with a peri-

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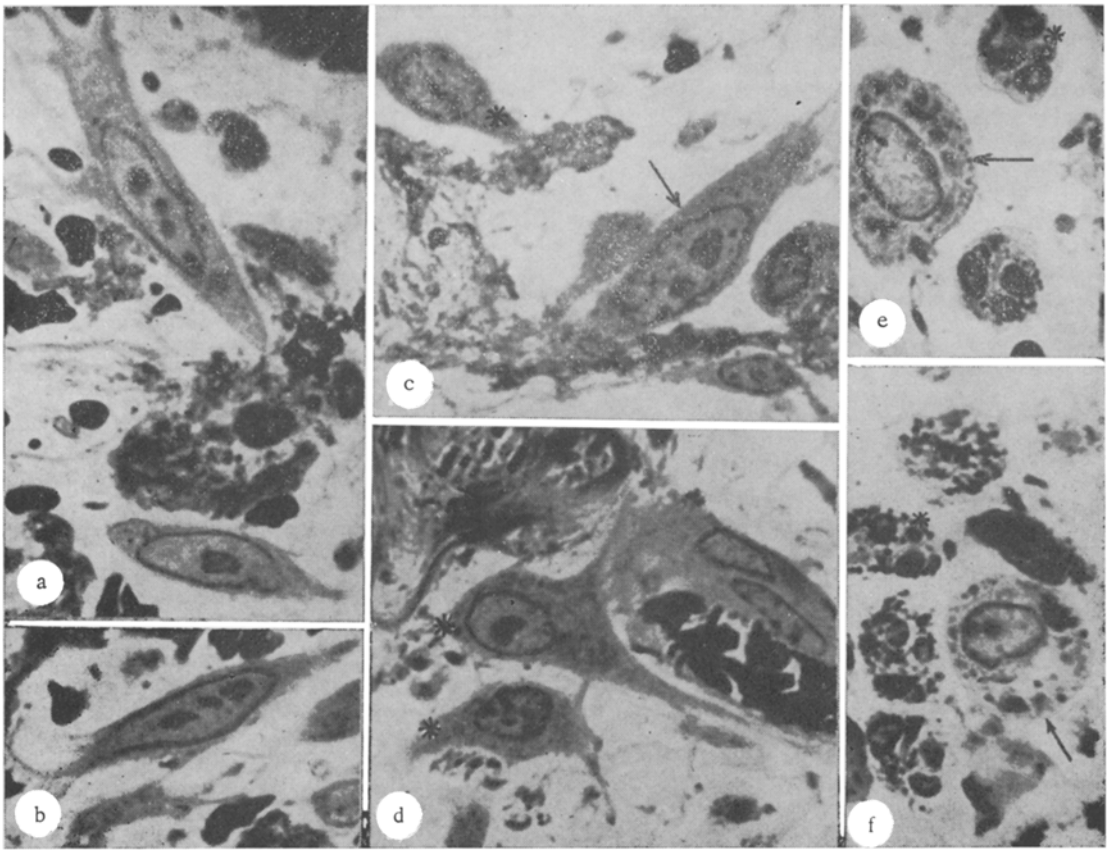

Fig. 1. Granulation tissue of gunshot wound in rabbit in initial period of its formation (3rd-7th days). a, b, c, d) Young (arrow) and undifferentiated (asterisk) fibroblasts; e, f) macrophages (arrow) and neutrophilic granulocytes (asterisks). Here and in Figs. 2 and 3, semithin section, methylene blue—fuchsin. Magnification 1000 ×.

nuclear distribution of chromatin, a relatively small volume of pale cytoplasm, and with few short cytoplasmic processes (Fig. 1a-c). Fibroblasts of the second type, undifferentiated (Fig. 1c), differed from the young fibroblasts in their smaller size, they were round or rather elongated in shape, and cytoplasmic processes were very poorly developed or absent. The nucleus, with large clumps of chromatin, occupied the greater part of the cell, leaving a narrow rim of basophilic cytoplasm. Fibroblasts with a perivascular distribution (Fig. 1d) became stellate in shape and tiny lipid inclusions could be seen in their cytoplasm.

Besides fibroblasts, other cells were constantly found in the granulation tissue: macrophages, with numerous phagocytosed particles in their cytoplasm, and neutrophilic granulocytes, many of them in a state of disintegration (Fig. 1e, f). Erythrocytes, single collagen fibers, and neutrophilic granules were identified in the ground substance.

In the proliferative phase of healing of the gunshot wound (9-14 days) numerous mature and young fibroblasts and also newly formed blood capillaries appeared in it. Mature fibroblasts, loosely arranged in the deeper layers of granulation tissue, were stellate in shape, with a fairly large volume of cytoplasm and with small, eccentric, round nuclei. Adjacent to them were a few thin, newly synthesized collagen fibers (Fig. 2a). In the superficial layers of the wound were blood capillaries with sharply hypertrophied endotheliocytes projecting into their lumen (Fig. 2b). Among the cells there were young fibroblasts, macrophage, neutrophilic granulocytes, and plasma cells.

After 3 weeks, in the period of maturation of the granulation tissue, the bulk of it consisted of a layer of densely packed mature fibroblasts, oriented along the wound surface. They were mainly elongated in shape, small oval nuclei, and with many lipid inclusions in their cytoplasm (Fig. 2c). The larger fibroblasts had weakly basophilic cytoplasm and were more frequently located close to newly formed blood capillaries with hypertrophied endotheli-

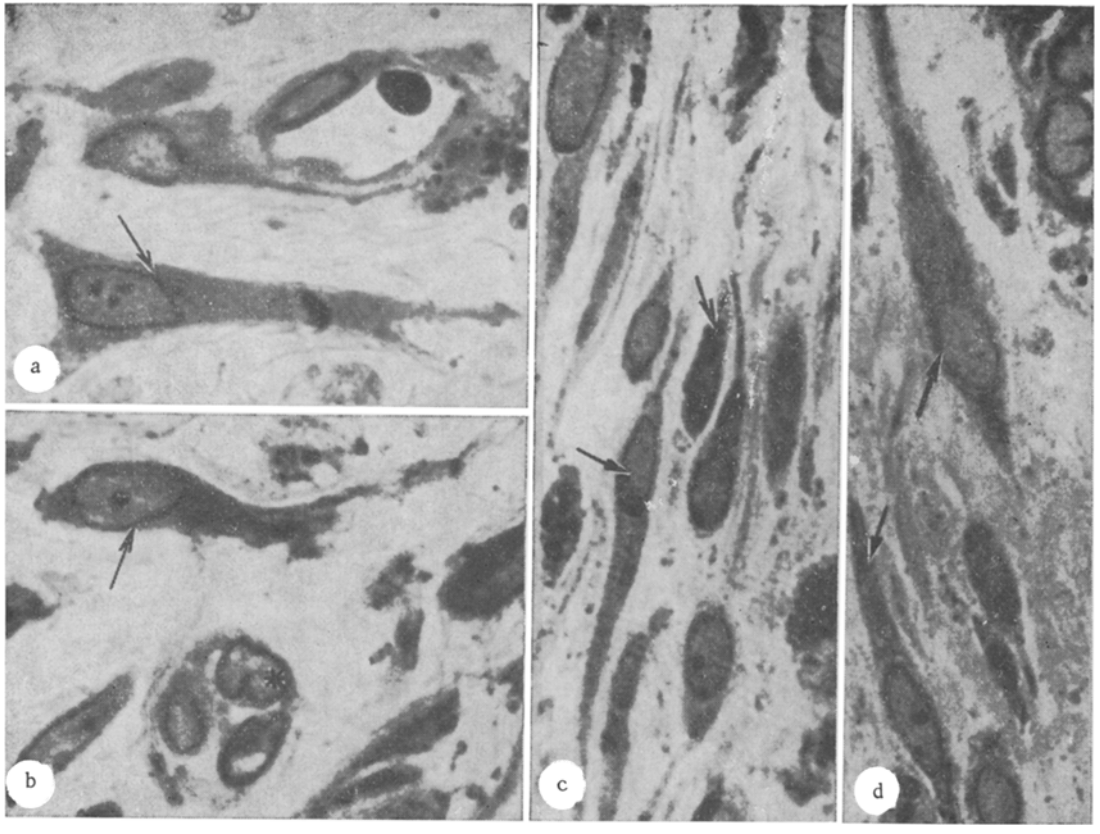

Fig. 2. Granulation tissue of gunshot wound in rabbit during period of proliferation and maturation (9th-21st day). a, b) Mature fibroblasts surrounded by a few thin collagen fibers (arrow); b) newly formed blood capillary with hypertrophied endotheliocytes (asterisk); c, d) numerous horizontally arranged mature fibroblasts with lipid inclusions in cytoplasm (arrow).

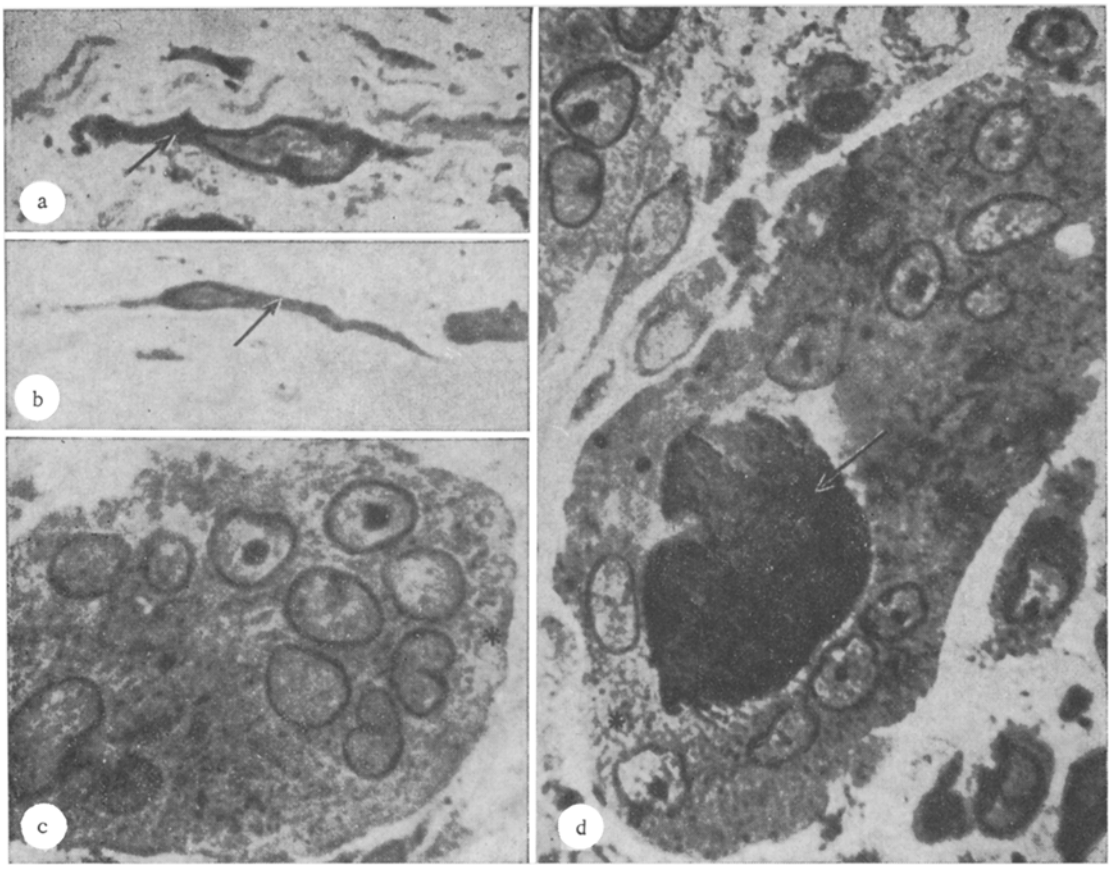

Fig. 3. Granulation tissue of gunshot wound in rabbit during period of scarring (28th day). a, b) Single fibrocytes in fibrous layer (arrow); c, d) multinuclear foreign body giant cells (asterisk); d) large particle of foreign material (arrow) inside giant cells.

ocytes (Fig. 2d). By the 28th day, a few fibrocytes with narrow, flattened nuclei, poor in chromatin, could be identified among the well formed bundles of collagen fibers (Fig. 3a, b). Multinuclear foregn body giant cells were often seen in the layer of horizontal fibroblasts and in the fibrous layer. They were distributed singly or in groups together with macrophages and fibroblasts. From 10 to 20 nuclei could be counted in the cytoplasm of the giant cells, and they often contained large particles of foreign material (Fig. 3d).

By using the STS method in a morphological study of gunshot wound healing it was thus possible to identify the different cell forms of the granulation tissue clearly and to characterize their cytological features and reactive changes. This technique is valuable on its own account and is a substantial addition to the ordinary histological methods of studying granulation tissue and enables the process of wound healing to be investigated more fully at the light-optical level.

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