ELECTRON-AUTORADIOGRAPHIC STUDY OF INDOLENT WOUNDS

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In the many investigations devoted to the morphology of wound healing, the dynamics of the process and its cyclic nature have been sufficiently well studied, and each of the phases characterized by a definite cell composition and cellular interaction have been described in detail [1, 2, 4-9]. However, all these observations, both clinical and experimental, have as a rule been based on the study of normal wound healing. The study of wound healing in relation to the character of trauma and the conditions under which healing takes place (for example, wound sepsis) has shown that the particular features of each individual case are quantitative and not qualitative in character, and do not go beyond the boundaries of the generally accepted course of the process [4].

Our aim was to study the morphological and functional features of indolent wounds, which are common in clinical practice.

EXPERIMENTAL METHOD

Biopsy material was studied from indolent wounds (from 2 to 5 months after infliction) in patients aged 44-67 years. The wounds were due originally either to trauma (three cases) or to postoperative complications (excision of a phlegmon according to Lipton) – one case. As a rule the wounds were located in the soft tissues of the lower limbs, and the area of the lesion varied from 400 to 1200 cm^2.

Biopsy specimens from postoperative wounds treated by the open method, 10-15 days after the operation, served as the controls. Altogether three biopsy specimens were studied from patients aged 33-48 years.

The biopsy material was divided into two parts: one part for histological investigation, the other for electron-microscopic autoradiography. Material for histological study was embedded in paraffin wax and stained with hematoxylin and eosin and with picrofuchsine by Van Gieson's method.

Pieces of tissue measuring about 1 cm^3 were excised for electron-autoradiographic investigation and incubated at 37-38°C in medium 199 containing 20 μCi/ml of ^{3}H-thymidine (specific activity 21.6 Ci/mmole) or 100 μCi/ml of ^{3}H-uridine (specific activity 26.0 Ci/mmole) for 1.5 h. At the end of incubation the material was washed to remove unassimilated precursor with cold phosphate buffer, pH 7.4, fixed with 2.5% glutaraldehyde solution and 1% osmium tetroxide solution, and embedded in Epon-Araldite. Semithin sections were first prepared, mounted on slides, and covered with type M photographic emulsion. The semithin sections were exposed for 3 days. To develop the semithin sections, and also the thin sections later, D-19 developer was used. After development, the semithin sections were stained with toluidine blue and examined under the light microscope. Depending on the results of this analysis, regions for ultramicrotomy were chosen in the blocks from which the semithin sections had been taken. Electron-microscopic autoradiographs were prepared by the method described previously [3] and examined in the electron microscope. By arranging the thin sections on blinds, it was possible to study the experimental material in series of sections.

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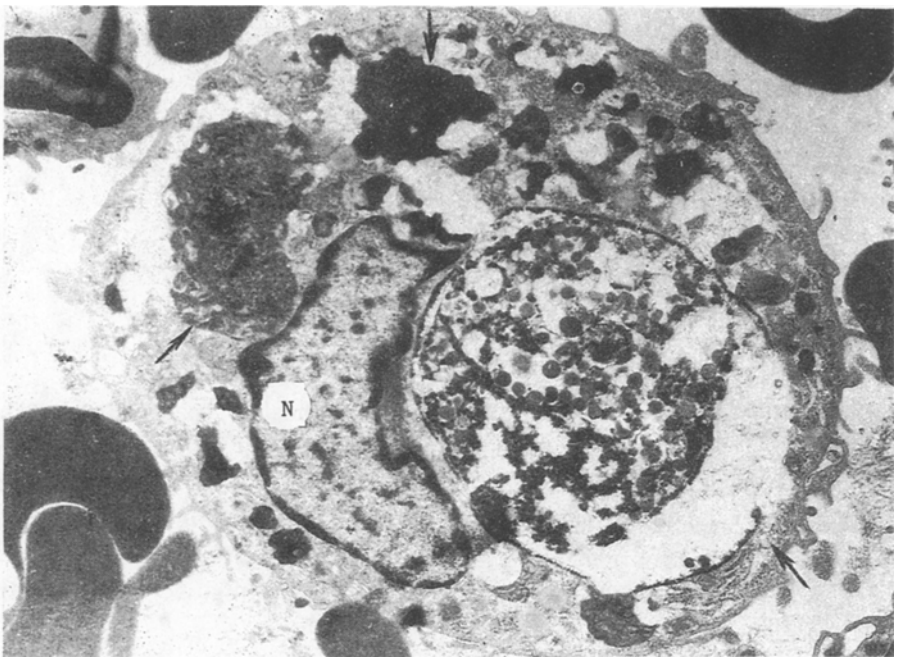

Fig. 1. Macrophage from indolent wound in state of active phagocytosis: many phagosomes with cell debris in cytoplasm (arrows). N) Cell nucleus. 8000×.

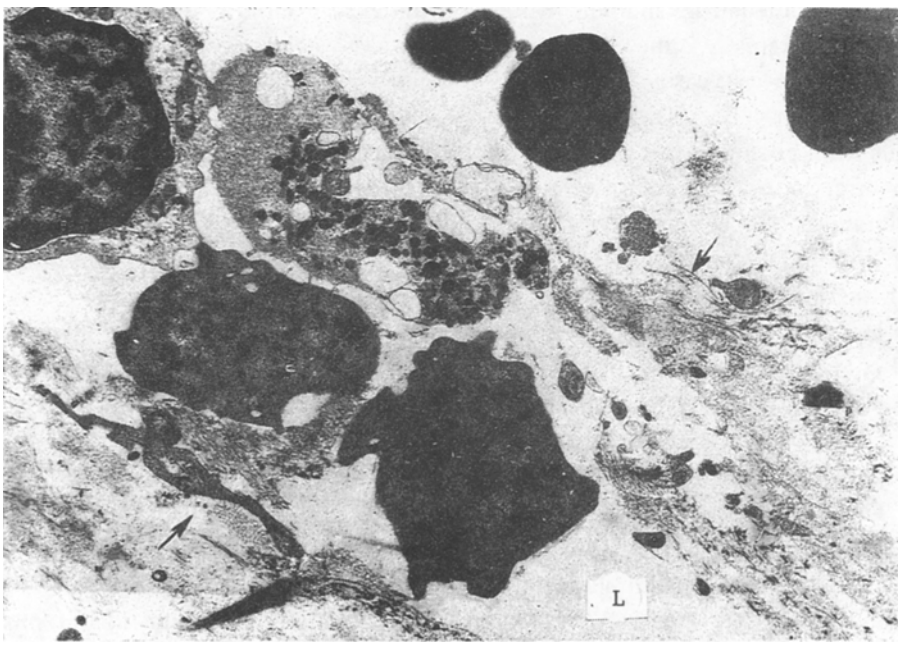

Fig. 2. Destroyed blood vessel in indolent wound. Destruction and lysis of vascular wall (arrows). Blood cells in lumen (L) of vessel. 5000×.

EXPERIMENTAL RESULTS

Analysis of the material showed that the morphological picture of the indolent wound in each patient had its own individual features, although at the same time, something common could be identified in all cases.

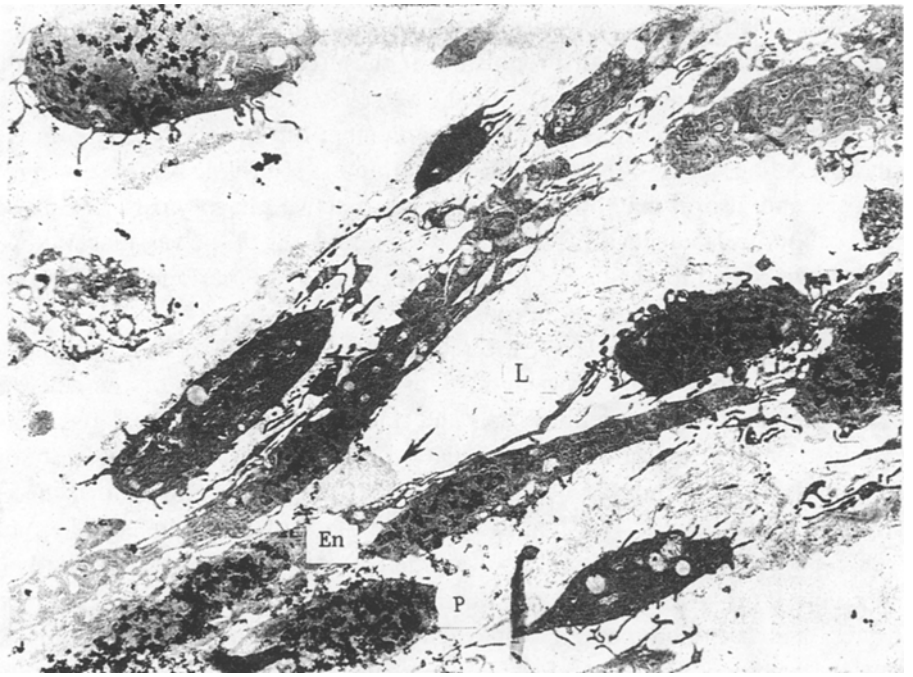

Fig. 3. Blood vessel of granulation tissue in normal wound. Endotheliocytes (En) and pericytes (P) incorporate ^{3}H-uridine intensively (black grains of silver). Erythrocyte (arrow) in lumen of vessel (L) ×2500.

As regards the distinguishing features, in some patients, for example, most of the cells in the wound were fibroblasts. They had a well-developed rough endoplasmic reticulum, which occupied nearly all the cytoplasm. Quite a high proportion of the fibroblasts had widely dilated tubules of the rough endoplasmic reticulum, and often part of the cytoplasm of these fibroblasts was occupied by large cavities. Myofibroblasts also were found, and differed from fibroblasts in their larger size and the bundles of myofilaments in their cytoplasm. These cells showed signs of commencing destruction: large cavities in the cytoplasm, absence of any clear cell boundaries, indistinctness of some structures of the cell. Many plasma cells also were seen, and as a rule they were grouped near the fibroblasts; plasma cells, moreover, often incorporated ^{3}H-uridine. RNA synthesis in their nuclei indicated their functional activity.

In other wounds fibroblasts were not different from those found in the normal wound, i.e., a wound healing without complications. Incidentally, both fibroblasts and other wound cells, macrophages for example, often contained many fat droplets.

A common feature of indolent wounds was the large number of macrophages found in them, and containing large phagosomes, which were either empty or contained cellular debris (Fig. 1). Quite often erythrocytes could be seen in the cytoplasm of the macrophage. Contact between fibroblast and macrophage was frequently observed.

Leukocytes also were present in large numbers in these wounds, and like fibroblasts, they also often made contact with macrophages. Cellular associations of fibroblast, macrophage, and leukocyte also could be observed.

Vessels found in indolent wounds were few in number. They usually consisted of an accumulation of many cells, as a rule around a collapsed lumen. If the lumen was not narrowed, however, it was packed with erythrocytes forming sludge, or with destroyed erythrocytes and leukocytes.

A noteworthy feature was the collagen of the wound, which often was not formed into bundles. Under high power, disturbance of the characteristic periodic cross-striation was observed in individual collagen fibers, and sometimes this regular striation and granularity of the structure were absent.

Destructive processes were characteristic of the indolent wounds studied. Here were found not only destroyed cells, but also destroyed vessels (Fig. 2). Erythrocytes or leukocytes were frequently visible in the lumen of these vessels. Often neutrophilic leukocytes or their granules were found close to the destroyed cells, and sometimes neutrophilic granules were found at the periphery, in the cytoplasm of a destroyed cell.

The main difference between indolent and normal wounds was the different functional activity of their cells. In the normal wound, high functional activity was characteristic of fibroblasts, macrophages, and cells of the vascular wall – endotheliocytes and periocytes: as a rule a high proportion of these cells incorporated ^{3}H-uridine intensively, i.e., they synthesized RNA (Fig. 3). Some fibroblasts and cells of the vessels were able to incorporate ^{3}H-thymidine. Functional activity of fibroblasts in the indolent wound was not high. The number of fibroblasts taking up ^{3}H-uridine also was small. In addition, the intensity of labeling was low. Intensive incorporation of ^{3}H-uridine was observed in some macrophages with no evidence of phagocytosis, and also in macrophages in a state of active phagocytosis, i.e., possessing large phagosomes with digestible contents. Leukocytes labeled with ^{3}H-uridine were rare. Cells of the vascular wall were functionally the most active. Endotheliocytes and pericytes often had signs of RNA synthesis, and occasionally of DNA synthesis, in their nuclei, the latter reflecting the proliferative process in these cells.

This investigation thus showed that delay of the regenerative process and its stretching over a longer than usual period of time, is manifested morphologically by a number of particular features of the cells and fibers of granulation tissue. In the first place, synthetic activity of the fibroblasts and cells of the capillaries was at a significantly lower level than in a normally healing wound. Delay of maturation of granulation tissue was accompanied by dystrophy and destruction of the wound cells, including cells forming the wall of the small blood vessels, and as result of this, the appearance of many macrophages phagocytosing cellular debris. Fibroblasts were in a state of active collagen production, as shown by the dilated tubules of the rough endoplasmic reticulum. However, the collagen produced was evidently imperfect, for there were few fibers, they were thin, and their cross-striation was disturbed or absent. Many plasma cells were seen, and also many leukocytes and lymphocytes, evidence of an immunologic reaction of the wound. All these features of the granulation tissue of indolent wounds are a local reflection of general changes in reactivity taking place in the patient.

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