MORPHOLOGICAL STUDY OF HEALING OF BURNS COVERED WITH ACTIVATED CHARCOAL CLOTH

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The high detoxicating activity of activated charcoal adsorbents is responsible for their effectiveness when used as hemosorbents and for the treatment of infected wounds. Granulated charcoal, fibrous and felt materials, and charcoal cloth dressings of varied origin have been tested for application-sorption wound treatment [1, 3, 5, 6, 8, 9]. The therapeutic effect of charcoal sorbents depends on the degree of activity of their surface, formed as a result of structural-chemical modification of the original raw material, endowing them with biocompatibility, and also with ability to effect selective sorption of toxins and other biologically active substances. When burns are covered with charcoal materials, rapid and complete cleansing of the wounds from necrosis and infection, acceleration of epithelization, and successful skin autografting have been observved [2, 4]. Systematic studies of burn healing as a result of treatment with activated charcoal sorbents have not hitherto been undertaken.

The aim of this investigation was a morphological study of healing of experimental burn wounds, covered with activated charcoal cloth (ACC) and to carry out clinical trials of the material.

EXPERIMENTAL METHOD

Methods of infliction of thermal burns were developed in experiments on rats (16 animals) and the time course of healing of the wounds was investigated in rabbits (15 animals). Burns were injected on the rabbits on the shaved skin in the scapular region, by means of a disk continually heated to 180°C (the area of one burn was 12 cm^2), under anesthesia, with an exposure of 30 sec. The wounds were excised together with underlying tissues for a standard histological investigation for between 7 and 32 days of treatment after burning.

The experimental wounds were covered with ACC dressings [7], both alone and in combination with a layer of hygroscopic unwoven materials based on viscous or charcoal (not activated) fibers. The sorptive capacity of these two types of charcoal dressings with respect to the liquid media was estimated by a static gravimetric method and amounted to 7 ± 1 g water (blood) per gram of dressing materials or 0.4 g of liquid/1 cm^2 of dressing surface. Combining ACC with the hygroscopic materials, with a sorptive capacity of (15-17) g/g enhanced its effectiveness when used with solutions of drugs.

The experimental wounds were treated with Vishnevskii's ointment (control) and ACC or charcoal dressings soaked in nitrofurazone solution, with the property of specific sorption on charcoal fiber (experiment). The dressings were sutured to the skin, and covered with an aseptic dressing of gauze and wadding, and with replacement of the wadding by nonwoven threadless coarse hygroscopic linen (manufactured by the All-Union Research Institute of Nonwoven Textile Materials, Ministry of Light Industry of the USSR). One experimental burn and one control burn were inflicted on each animal. Dressings were changed every 2 days. The materials were sterilized by autoclaving.

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EXPERIMENTAL RESULTS

After infliction of the burns on the rabbits' skin a firm coagulation scab formed, and was removed on the 5th day, by using a combination of chemical and surgical necrectomy. The formation of a deep burn of the IIIb degree with a granulating surface was confirmed histologically. The wounds were covered with dressings.

On the 7th day after burning, the experimental wound, with even edges, was covered with a transparent secondary scab, which remained until the 17th day. The control wound, with uneven edges, was larger than before treatment with ointment, and the secondary scab contained areas of necrosis and bleeding. As a result of the atraumatic nature of the ACC, it could be completely detached from the wound surface without injury to it at all stages of treatment (in agreement with the results of measurement of the adhesive strength of ACC on a model with blood [7]), unlike gauze, which dried onto the wound.

Histological investigation on the 7th day showed that the floor of the experimental wound (Fig. 1a) was formed by a wide layer of granulation tissue, with marked congestion of capillaries. The young granulation tissue contained fibroblasts, a few leukocytes, and solitary lymphocytes. Sites of granulation were covered with erythrocytes or debris, densely infiltrated with leukocytes. Under the granulations was a layer of slightly edematous connective tissue with a marked fibroblastic reaction and with foci of collagen formation. The fibroblasts were mainly young, with pale, oval nuclei. In places the muscle tissue was in a state of necrosis and cloudy swelling, or was invaded by granulation tissue. The deeper muscles were unchanged. In the control (Fig. 1b) the floor of the wound was formed by a thin layer of granulations, densely infiltrated by polymorphs and covered with a layer of debris, fibrin, and numerous leukocytes. The connective tissue lying beneath the granulations was greatly edematous, with foci of hemorrhage, and infiltration of the depth of the muscles by leukocytes. In some places signs of destruction and myolysis of the muscle fibers were observed. Later, starting on the 10th day, the dimensions of the experimental wounds were appreciably reduced, and an increasingly wide zone of young epithelium was located around their periphery. Active peripheral epithelization and reduction of the wounds continued until they were completely healed (by the 37th day). By contrast with this, the wet control wound with coarse bleeding granulations was covered with a white deposit, and peripheral epithelization was observed only on the 20th day. Histological investigations showed these differences in more detail.

On the 17th day, in the experimental wound (Fig. 1c) further development and maturation of the granulation tissue took place. The clean granulations contained only single lymphocytes and virtually no leukocytes; only in the surface layer were there solitary leukocytes and remains of debris, which formed a demarcation barrier in places. In the deep layers the clean granulations were organized. No inflammation was present in the underlying muscle tissue. In the peripheral zone of the wound the granulations were covered by layers of young squamous epithelium, in which the appendages were still absent. Organization of the granulations was observed. In the control the floor of the wound was formed by a layer of necrotic muscle fibers, infiltrated by neutrophilic leukocytes. Beneath them in some places there were remains of adipose and connective tissue in a state of edema, necrosis, and leukocytic infiltration. Marked signs of chronic inflammation and intensive fibroblast formation were observed in the underlying adipose and muscle tissues, with the development of granulation tissue and early sclerosis of the granulations, leading subsequently to the formation of a coarse burn scar (Fig. 1d).

On the 32nd day, the maturing granulation tissue in the experimental wound (Fig. 1e) was covered by a layer of epithelium, and only in the center of the wound was epithelization absent. In the substance of the young scar, fragments of separate charcoal fibers, evidently shed by the cloth, were seen, but they were surrounded by only a mild giant-cell reaction, and the granulations were organized. In the control (Fig. 1f), the sclerotic granulation tissue with foci of chronic inflammation was covered by stratified squamous keratinizing epithelium.

It was thus confirmed morphologically that when experimental burn wounds were covered with ACC and the experimental dressings, the inflammatory reactions quickly subsided, the wounds did not contain purulent secretion, peripheral epithelization took place actively, and the wounds healed with the formation of a soft, delicate scar.

The results of this experiment were confirmed by clinical trials of the materials. ACC and the experimental dressings were used at the All-Union Burns Center on 15 patients with burns

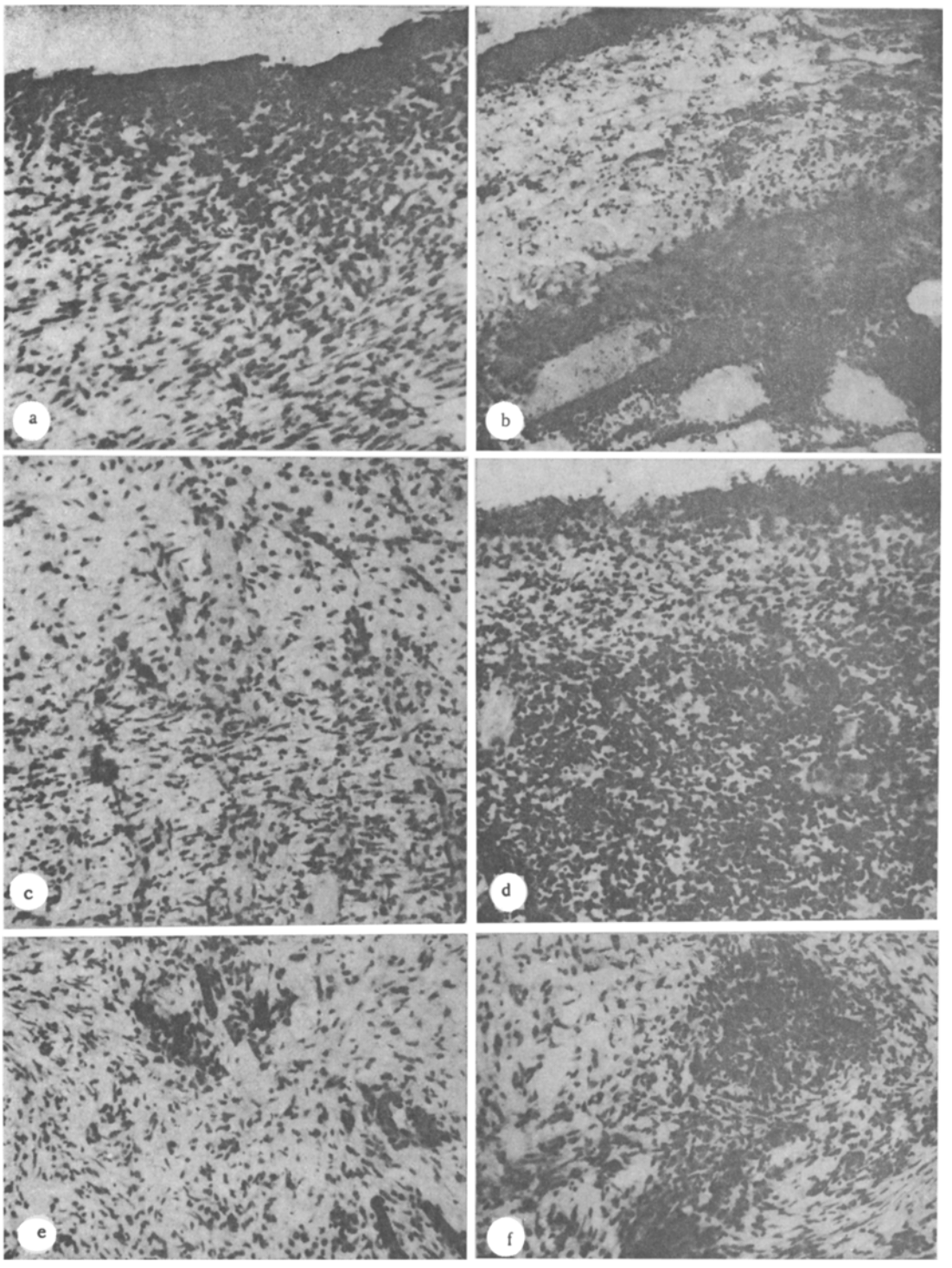

Fig. 1. Morphological picture of burn wound: a and b) 7th day after burning (experiment and control, respectively); c and d) 17th days after burning (experiment and control, respectively); e and f) 32nd day after burning (experiment and control respectively). Sections stained with hematoxylin and eosin. 140 ×.

of the II and IIIa, b degrees, with an area of involvement of between 2 and 40% of the body surface; burn wounds with an area of between 1 and 7% of the body surface were covered with charcoal materials. More than 30 dressings were carried out. The use of charcoal materials on burned patients considerably improved healing of burns of the II and IIIa,b degrees compared with the use of ointments and with conventional wound exudate-drying dressings. They could be kept on surface burns until the appearance of epithelization, or they could be changed after

5-7 days, and when indicated for use together with solutions of drugs, with daily redressings, the state of deep burn wounds could be improved and the wounds prepared for skin autografting. With early covering of burn wounds with charcoal materials, the local use of strong antibacterial agents could be reduced. Several clinical cases could be cited.

Thus ACC and dressings based on it, in combination with necrolytic and antimicrobial agents, led to cleansing of burn wounds from purulent secretions, improved preoperative preparation of deep burn wounds, and accelerated the epithelization and healing of superficial burns.

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ORIGIN OF LIPOFUSCIN GRANULES IN HYBRIDOMA CELL CULTURE

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Lipofuscin granules (LG) are lipoprotein-pigment inclusions which accumulate in the cells of various organs and tissues of man and animals during natural aging [15] or in pathology [7]. This phenomenon in pathology is particularly marked in genetically predisposed diseases of premature aging: the ceroid lipofuscinoses [5]. Investigations of LG accumulating with age in situ has not shed light on their genesis or functinoal role in the cells [5, 7, 10, 15]. Attempts have been made to create a model of their appearance in cells of young animals [3, 9], but this likewise has not yielded any basically new results. LG formation has recently been demonstrated in certain organotypic and cell cultures [12, 16]. However, ideas on the mechanisms of the intracellular genesis of LG still remain in dispute. Some workers consider that the precursor of LG is one of the intracellular organelles, for example mitochondria [16], whereas others associate their formation with a complex series of biochemical reactions taking place in several cell organelles [14]. Accumulation of LG in a culture of retrovirus-transformed hybridoma cells as found previously, and as a result of this it became possible to study LG on a relatively simple and convenient cell model.

We have studied the ultrastructure of LG in hybridoma cells. This paper gives details of their structure, and provides evidence in support of LG formation from the endoplasmic re-

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