GLYCOGEN CONTENT OF TISSUES DURING HEALING OF SKIN WOUNDS IN ALBINO MICE

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It is known that regeneration of any tissue, and in particular the healing of wounds, is associated with changes in metabolism which favor rapid growth of tissue. We thought it of interest, in this connection, to study changes in glycogen content in regenerating tissues, in particular of connective and epithelial tissues.

We found that glycogen can be regarded as an essential energy-producing and structural material, participating in morphogenesis; its presence is absolutely indispensable for growing tissues. Best [2], using a histochemical method, has shown that during healing of a corneal ulcer the young epithelial cells contain glycogen. The study of the glycogen content of tissues, and of its dynamics during processes of regeneration, have been approached exclusively by biochemical methods. Thus, D. E. Ryvkina [1], who determined chemically the glycogen content of regenerating tissues of albino rats, found that during healing of wounds the amount of glycogen in the granulation tissue and in the margins of the skin wound falls considerably, to a minimum on the 5th day after infliction of the wound, when the granulation tissue consists of immature, undifferentiated cells, and the growth of new epithelium is only just beginning at the margin of the wound. At later stages, when the process of differentiation has begun, the rate of growth falls, and the glycogen content gradually rises. The author hence concludes that regeneration is associated with a fall in the glycogen content of the skin, which is at a maximum at the earliest stage of the growth process.

It has been reported in the literature that the glycogen content of growing tissues falls as a result of glycogenolysis.

We have been unable to find any reference to histochemical studies of the glycogen content of tissues during healing of wounds.

We have studied the glycogen content of tissues during healing of wounds, applying both histochemical and biochemical methods.

EXPERIMENTAL METHODS

Skin defects 2 × 2 cm in size were produced aseptically on the backs of albino mice. The wound was covered with a gauze pad, attached by means of 4 ligatures. Tissue was taken from the wounded area and from its surroundings 1, 2, 3, 4, 5, and 6 days, 2 weeks, and a month after the operation, and the biopsy specimens were subjected to histological, histochemical, and biochemical study.

Sections for histological study were stained with hematoxylin-eosin and picrofuchsin. Histochemical study was done by A. L. Shabadash's method for glycogen, and biochemical studies by Golyandas' method in G. A. Cherkes' modification.

EXPERIMENTAL RESULTS

Our basic object was to ascertain what connection might exist between morphological changes at different stages of healing of skin defects in albino mice and the glycogen content of the affected tissues.

The skin of white mice contains normally an average of 7.7 mg of glycogen per g of tissue. The glycogen is seen histochemically in small amounts in the protoplasm of cells of the granulosum layer of multilayered flattened epithelium, in the form of small granules, in the fibrous connective tissue layer, distributed linearly along the fibers, and in the capillary endothelium. The epithelium of the hair follicles is rich in glycogen.

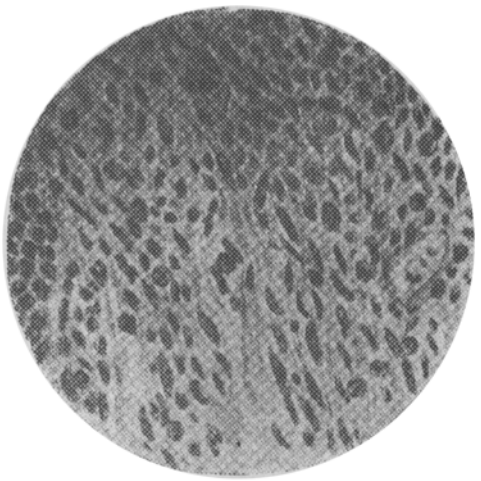

Fig. 1. Glycogen in granulation tissue.

Fig. 2. Glycogen in regenerating epithelium.

The glycogen content falls progressively during the first 3 days after infliction of the skin wound, to an average value of 2 mg/g. Histochemical investigation shows that this fall is at the expense of the hair follicles (which are the principal depot of glycogen in the skin of albino mice), whereas in the regenerating epithelium accumulation of glycogen is proceeding at the same time. Glycogen is to be seen in the form of granules or diffusely distributed in the cytoplasm of neutrophilic leucocytes, epithelioid cells, early fibroblasts, and histiocytes, in granulation tissue (Fig. 1). Extracellular glycogen may also be observed. Glycogen is present in considerable amounts in the cytoplasm of regenerating epithelium cells (Fig. 2); columnar cells contain less than flattened ones.

Thus our findings are, as far as the biochemical aspects are concerned, in consonance with those of D. E. Ryvkina [1]. The histochemical findings show, however, that the glycogen content of regenerating epithelium not only does not fall, but actually rises. The fall in glycogen content indicated by biochemical methods is ascribable to the absence of hair follicles (during the first 3 days), which normally have a high glycogen content, from the site of skin excision and repair.

The glycogen content of the skin wounds begins to rise on the 4th day after wounding, attaining an average value of 10.5 mg/g on the 6th day. Histochemical observation shows that this rise is due both to its continuing accumulation in regenerating epithelium cells, and, chiefly, to regenerating hair follicle epithelium. D. E. Ryvkina also noted accumulation of glycogen in regenerating tissues, at later stages.

The glycogen content returns to a normal average value of 7.9 mg 2 weeks after infliction of the wound. Glycogen is to be seen histochemically in large amount in the stratified flat epithelium, except in the cells of the basal layer. Newly formed fibrous tissue contains considerable amounts of glycogen, distributed along the fibers.

No further change is observed in the glycogen content, which had an average value of 7.8 mg/g a month after inflicting the wound. The histochemical picture is one of return to normal conditions. Glycogen is to be

seen in the newly formed epithelial cells and connective tissue, in small amounts, and in large amount in the epithelium of the newly formed hair follicles.

LITERATURE CITED

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