THE INFLUENCE OF STH AND ACTH ON REPAIR PROCESSES IN WOUNDS

A. A. Voitkevich

Department of Histology (Head, Corresponding Member AMN SSSR Professor A. A. Voitkevich).
Voronezh Medical Institute
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The hypophyseal hormones, and in particular somatotropin (STH) and adrenocorticotropin (ACTH) influence different aspects of metabolism [3, 4, 5]. They are therefore evidently concerned in physiological regeneration and repair processes. It is known that the physiological action of these hormones is of many kinds: STH activates proliferation and growth, whereas ACTH whose action is mediated through the adrenal cortex accelerates the differentiation of cells of a number of tissues [1, 2]. It has already been established that STH generally accelerates the formation of granulation tissue and of the connective tissue capsule around abscesses produced by the injection of turpentine [8, 10]. The mitotic activity of several kinds of cell is increased, there is an accelerated formation of new blood vessels, and collagen formation is stimulated [6, 9]. The positive influence of STH on the proliferative processes and its local action on tissue has been demonstrated [7].

We have applied the usual histological methods and certain histochemical tests to study the effects of STH and ACTH on the development of the inflammatory reaction and the formaion of new tissue structures during the healing of wounds inflicted experimentally.*

EXPERIMENTAL METHOD

For these experiments we used 20 young white male rats whose mean weight was 110 g. They were divided into three groups: the first, or control group, consisted of five rats which received no hormonal preparations; the second, of ten rats which received 2 mg of STH per 24 hours; and the third, of five animals which received 3 units of ACTH daily. The preparations were given 3 days before the wound was inflicted, and treatment with them was continued to the end of the experiment. The wounds were made on the side of the body by means of a stencil and measured 1.5 × 1.5 cm. No stitches were made, and healing occurred by secondary intention. After 8 and 24 hours smear preparations were made of the exudate from the open wound. Each time three smears were made, and of these one, after special fixation, was stained in Romanowsky's azure II-eosin, the second was tested for glycogen, and the third for ribonucleoproteins.

At various times after the infliction of the wound (after 4, 8, and 10 days) we made drawings of the changing border of the open surface of the wound, by drawing the outline in Indian ink on cellophane. After 10 days the animals were killed, and small fragments were cut from the edge of the wound so as to include both regenerating tissue and the adjacent undamaged original skin. The material for histological examination was fixed in Bouin and embedded in paraffin. Sections were stained in hematoxylin-eosin, with van Gieson's picrofuchsin, in azure II-eosin, and in Heidenhain's azan; ribonucleoproteins were revealed by methylene green and pyronine; polysaccharides were determined by means of the PAS reaction and by Hale's method.†

EXPERIMENTAL RESULTS

The mean figures for the area of exposed wound surface showed that in rats treated with STH healing had been appreciably stimulated (Table 1).

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TABLE 1. Area of wounds (in mm^2) at various times during healing.

Conditions of experiment	Time (in days)		
	4	8	10
Control	135.8	43.2	25.7
Action of STH	118.4	19.1	5.0
Action of ACTH	145.7	64.4	27.3

Both four days after infliction of the wound, and at later times in the animals treated with STH the area of exposed wound surface was comparatively small, i.e. healing was accelerated. In the animals treated with ACTH the wounded surface was covered more slowly. In four of the ten animals treated with STH healing was complete after ten days, while in six of them the area had been reduced on average to a mere 5 mm^2.

Smears taken very early during the inflammatory reaction showed considerable differences between the different groups. The principal results of a count of the cells in the exudate (with an immersion objective) are shown in Table 2.

In the exudate of the control animals the usual reaction of the neutrophils was found 8 and 24 hours after infliction of the wound. Usually the nucleus was segmented, and contained 5 - 6 lobes (see Fig., a). In the early smears, besides the neutrophils there were occasional polyblasts whose number increased considerably during the next 16 hours. In the polyblasts the nucleus was round or bean-shaped, and eccentric; the homogenous cytoplasm formed a thin border. In the macrophages formed by differentiation from such polyblasts the nuclei were large and the cytoplasm was more extensive. The nucleus showed changes, being less compact, and the cytoplasm showed vacuolization, or it became foamy, and its edges scalloped. Basophilia of the cytoplasm was somewhat reduced. These macrophages were actively phagocytic, as could be inferred from the large number of different particles included in their cytoplasm.

The neutrophils of the exudate contained fine glycogen granules distributed quite evenly throughout the cytoplasm; the polyblasts were richer in ribonucleoproteins.

Under the influence of STH there was some increase of migration of cells into the granulation tissue which was forming. The main mass of cells in the exudate consisted of neutrophils and phagocytosing macrophages, and this condition was especially marked one day after the would had been inflicted. Many neutrophils were of the normal size and structure. Besides the typical neutrophils having a nucleus with 5-6 lobes, frequently cells with only slightly segmented nuclei were found. They were larger and their nuclei were usually ring-shaped with only very slight constrictions; their nuclei were less strongly stained than usual (see Fig. b). Under the same conditions, the macrophages had a pale nucleus and vacuolized cytoplasm. As a rule they were larger than the macrophages of the exudate of control animals (see Table 2).

Under the influence of STH the neutrophils accumulated increased amounts of glycogen, as could be seen from the dark purple stain of the granules. Frequently small portions of cytoplasm rich in glycogen were formed by constrictions. In the macrophages the glycogen was found mainly at the periphery of the cells. In the cytoplasm of the polyblasts and of the macrophages there was a strong reaction for ribonucleoproteins.

Under the influence of ACTH the migration of neutrophils into the exudate was somewhat reduced. The cell nuclei were clearly segmented; the cytoplasm of the neutrophils was somewhat enriched with glycogen, as was shown by the pale pink color of the cytoplasm. In animals treated with ACTH the migration both of neutrophils and of polyblasts into the damaged area was inhibited. The macrophages had a strongly vacuolized cytoplasm and their phagocytic activity was reduced.

TABLE 2. Total Number of Cells in 25 Fields of View

Conditions of experiment	Time after infliction of wound (in hours)						Size of cells (diam. in μ)	
	8			24				
	Neutrophils	Polyblasts	Macrophages	Neutrophils	Polyblasts	Macrophages	Neutrophils	Macrophages
Control	89	6	3	154	39	15	11.4	14.8
Action of STH	141	9	4	208	59	22	12.8	17.3
Action of ACTH	56	5	2	85	8	9	11.0	15.6

A microscopic study of material taken from control animals on the tenth day showed that at this time the granulation tissue showed a wide variety of cell types, particularly of fibroblasts and histiocytes, and that there was a well developed plexus of blood vessels running vertically. The main structural elements of the granulation tissue of the control animals were fibroblasts at various stages of differentiation (see Figure, e). The superficial layer of granulation tissue was extensively infiltrated with leucocytes. Between the cells there was a small amount of ground substance which was stained pale blue by Hale's method, and contained a fine reticulum of collagenous fibers. The height of the granulation tissue layer was on average 1330 μ. The adjacent portions of the original epithelium adjoining the wound were greatly hypertrophied and their thickness was 2-3 times greater than that of more remote portions of epidermis. The epithelium of the regenerating portion was stratified from the time of its origin, and was made up of 10-12 layers of young cells (see Figure, c).

In animals treated with STH the granulation tissue of the skin wound differed in its more abundant development, and its height which on average was 1425μ. In it there was a preponderance of horizontally disposed fibroblasts between which fine collagenous fibers and an amorphous substance could be seen. In the deeper layers of granulation tissue the number of the fibers was increased and in preparations treated by the van Gieson method it was seen that these fibers formed uniform clusters. The granulation tissue was comparatively weakly infiltrated with leucocytes. The neutrophils which were found in the upper layers of granulation tissue and in smear preparations of exudate had a larger diameter than that of the neutrophils in the controls. Numerous vessels surrounded by large weakly differentiated cells passed vertically through the layer of horizontally arranged fibroblasts (see Figure, f). In parts of the old dermis, at the junction with the damaged area, groups of mast cells could be found, chiefly round the blood vessels. They were usually oval in shape and contained a large number of basophil granules. Also they were larger than in the control group. From our results we conclude that among the various cell elements of connective tissue the most resistant to hormonal action are the mast cells.

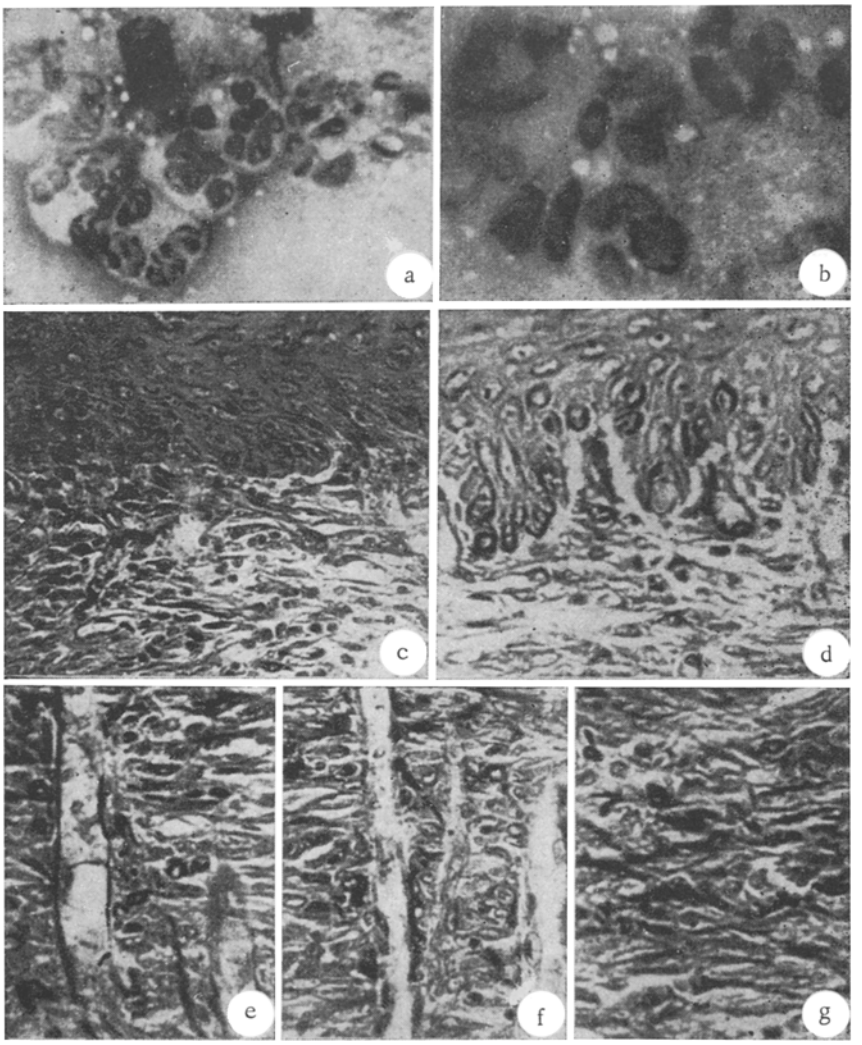
In the experiments with STH the characteristic response was an intense proliferation of the epithelium. The young epithelial layer extended further than it did in the control animals or in rats treated with ACTH. The young cells of the new epithelium were large, but they did not always retain their polarity nor were they always arranged in an orderly manner; this was true especially of the basal layer. In some animals the internal surface of the young epithelium covering the wound was uneven; from it small outgrowths passed into the young connective tissue (see Figure, d). The cells of the young epithelium were rich in ribonucleoproteins.

In rats treated with ACTH the granulation tissue was less well developed, and the chief alteration in the structure was in the superficial layers. There was some hypertrophy of the fatty tissue at the base of the wound and the layer of granulation tissue was thinner, having an average height of 665μ. In the granulation tissue fibrous structures preponderated, and the cells were relatively small (see Figure, g). As in the case of STH, but to a greater extent, in the deep layers of the granulation tissue homogenous accumulations of large number of fibers were found. In such granulation tissue, which developed under the influence of ACTH, no definite direction of the blood vessels could be made out. The epithelial layer in both the undamaged and the regenerating regions was thinner than in the control.

Thus STH influenced the proliferation of cells by stimulating the development of granulation tissue and the rate of formation of the epithelium over the wound. At the same time it somewhat restrained differentiation of cells such as leucocytes, fibroblasts, and the cells of the young epidermis. ACTH stimulated adrenal cortical function and in this way increased the differentiation of the young cells, and so hindered the development of granulation tissue, particularly in the superficial layers. As a result we found an abnormally early maturation of the young connective tissue. All these effects together in some cases resulted in the rapid development of a young epithelium which, however, proliferated to form a thin layer containing a small number of cells. In such animals stimulation of the recovery process in the wound was not fully effective.

SUMMARY

Albino rats were used in a study of the effect produced by the two hormones of the anterior pituitary lobe on repair processes in skin wounds. STH caused increased amounts of glycogen and RNP to accumulate in the young cells and retarded the differentiation of the blood and tissue cells concerned in the formation of granulation tissue; the result was an increased proliferation of the young epithelium. ACTH stimulated the differentiation and activity of the young cells with the result that the ratio of ground substance to cells was increased. Consequently the trophic function of the young epithelium was reduced, and the whole repair process was depressed.



Histological appearance of the wound exudate from the skin after treatment with various hormonal substances. First row) neutrophils of the exudate (24 hours, Romanowsky stain, magnification 1800×); a) control; b) action of STH. Second row) young epithelial layer (10 days, hematoxylin-eosin, magnification 480×); c) control; d) action of STH. Third row) middle zone of granulation tissue (10 days, Heidenhain's azan, magnification 480×); e) control; f) action of STH; g) ACTH.

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