

# THE EFFECT OF ADRENALIN ON THE PROCESS OF REPAIR IN NORMAL AND CASTRATED ANIMALS

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Adrenalin is one of the essential factors of the physiological basis of the adaptational and trophic reactions of the body. Various changes in the environment, in particular stimuli having a profound effect on the body, cause an increased secretion of adrenalin. At the same time the hormonal function of the adrenal cortex is stimulated.

Results obtained in our laboratory [2] on the effect of cortisone on the reparative process and on the inflammatory reaction preceding it suggested the value of an investigation of the action of adrenalin in similar experimental conditions. Since the effect of the adrenal hormones is to produce characteristic changes in the white cells of the blood, opposite in a number of respects to those resulting from castration, we carried out parallel series of experiments on intact and castrated animals. Extirpation of the sex glands is known to be followed by increased function of the lymphoid organs.

## EXPERIMENTAL METHOD

Experiments were carried out over a period of 2 years on 35 male white rats (average weight 230 g) and on 8 male guinea pigs (average weight 480 g). Nine rats were castrated 30 days, 10 rats — 60 days and 4 guinea pigs — 30 days before wounding. The animals of each species were divided into 4 groups. The first group of animals received no adrenalin (control); the second group were castrated; the third group of rats were injected with adrenalin; the castrated animals of the fourth group were injected with a 1:1000 solution of adrenalin in a dose of 0.1 ml per 100 g body weight, the injections beginning 3 days before wounding and continuing throughout the entire period of observation of the wounds. The daily dose of the drug was given as two intramuscular injections in the 24 hours; during the period of observation of the wounds the adrenalin was injected 2 hours before taking the impression.

Wounds were inflicted on all the animals on the lateral surface, by the standard method: a square piece of skin, measuring 1 cm<sup>2</sup>, was removed together with the subcutaneous cellular tissue from the white rats, and a piece measuring 4 cm<sup>2</sup> from the guinea pigs. The operation was carried out under general ether anesthesia. The wound was covered with two sterile towels, the bottom one being smeared with vaseline and applied to the surface of the wound, and the upper, dry towel was fixed to the animal's skin with collodion. Periodically the outline of the open wound surface was traced on cleaned cine-film.

Particular attention was paid to the activity of cell migration and to the numerical relationships and the morphological features of the various blood and tissue cells in the initial period of healing, i.e. at the time of development of the inflammatory reaction. For this purpose, at an early stage, when the wound surface was covered with a scab and was not yet epithelialized (4, 8, 24, 48 and 72 hours after formation of the skin defect), impressions of the wound exudate were taken. Accordingly the dressing was removed from each animal and a fat-free cover glass applied to the surface of the wound. The impressions were dried in the air, fixed in Nikiforov's mixture and stained by the Romanowsky method. In addition, 7 days after removal of the skin squares,

Number of Cells in the Wound Exudate and the Change in the Size of the Wound in White Rats under Different Conditions

Series of experiments	Number of neutrophils in 10 fields of vision		Polyblasts	Area of wound surface, mm <sup>2</sup>	
	after 2 days	after 3 days		after 9 days	after 15 days
Control	243	343	67	22	0
Adrenalin	119	168	5	33	8
Castration	73	117	3	46	9
Castration and adrenalin	100	146	22	99	30

biopsy specimens were taken of the young regenerating tissues together with the adjacent area of the edge of the wound. The tissues excised were fixed in alcohol and formalin and embedded in celloidin; sections were stained with hematoxylin-eosin.

### EXPERIMENTAL RESULTS

Observation of the wounds showed that the process of healing was retarded when adrenalin was given to both normal and castrated animals. The results of the experiments on white rats are given in the table.

Under the influence of adrenalin the process of healing was most severely retarded in the early period — up to the 4th or 5th day there was no indrawing of the wound edges. At later periods suppression of the process of repair by adrenalin was more pronounced in the castrated animals. For instance, 15 days later, the control animals showed complete epithelialization, but in the animals receiving adrenalin the area of the wound was still 8 mm<sup>2</sup> and in the castrated animals 9 mm<sup>2</sup>. Administration of adrenalin to castrated animals severely retarded healing — the surface area of the wound was 30 mm<sup>2</sup>, and complete healing of the wound took place only on the 21st day after it was sustained.

The results of visual examination agreed with the data obtained from study of the cell composition of the wound exudate in the initial period of healing. Impressions taken 4 and 8 hours after the wound was inflicted showed a well-marked inflammatory reaction in the animals under various experimental conditions. Later the differences in the microscopic picture of the wound exudate from animals of the groups under comparison were increased (see Table and Fig. 1).

In control animals 2 days after infliction of the wound a specialized leucocyte reaction was well developed — 243 neutrophils in 10 fields of vision. The neutrophils were fully differentiated; their nuclei were normally segmented and intensively stained. In each field of vision many polyblasts in various phases of maturation could be made out (in the first 24 hours — up to 67 in 10 fields of vision).

In impressions from the animals receiving adrenalin the number of migrating neutrophils was reduced to one half. The nuclei of the neutrophils were clearly segmented; the segments were differentiated from each other. The nuclei stained less intensively than in the control animals. Cells showing degenerative changes were found — with partial destruction of the nucleus and intensive vacuolation of the cytoplasm.

In castrated animals at the same period the number of neutrophils in the wound exudate was still further reduced by comparison with normal (73 neutrophils in 10 fields of vision). The segmented nuclei were palely stained. The solitary polyblasts, with vacuolated cytoplasm, showed little activity. Many lymphocytes, mainly small, were found.

The cytological picture of the wound exudate in the castrated animals receiving adrenalin differed from that in the foregoing series. With this combination of conditions the number of migrating leucocytes was higher than in the castrated animals not receiving adrenalin or in the animals receiving adrenalin alone. The neutrophils showed marked segmentation and were well stained. Polyblasts with clearly outlined cytoplasm were not

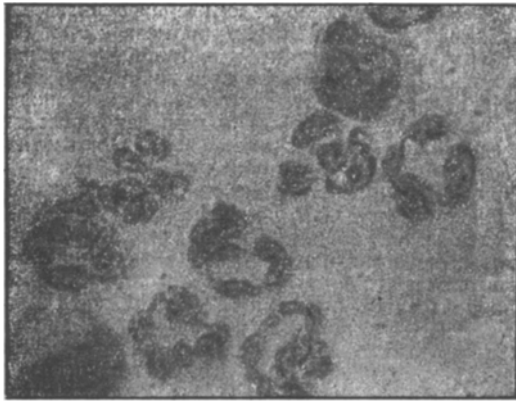


Fig. 1. Cells in the wound exudate of a white rat receiving injections of adrenalin. Magnification 1200.

found in every field of vision. The migration of lymphocytes characteristic of the castrated animals was suppressed by administration of adrenalin.

The development of an inflammatory reaction in the region of the wound was characterized at later stages in the experimental animals by a certain increase in the intensity of migration of neutrophils and by continuing hypersegmentation of the nuclei of the neutrophils during administration of adrenalin to normal, and particularly to castrated animals.

Analogous but less pronounced results were obtained in the small experiment on guinea pigs. The impressions taken here were confined to early periods, since progressive necrotic changes appeared in the region of the wound at later periods in the normal and particularly in the castrated animals.

A comparative study of the impressions of the wound exudates taken at different periods from the animals of the various series demonstrated certain distinctive features of the reaction of the neutrophilic leucocytes. In the animals receiving adrenalin, hypersegmentation of the nuclei of the neutrophils was clearly apparent. This effect persisted in experiments in two successive years. In Fig. 2 are shown the variation curves of the number of segments in the nuclei of the neutrophils in white rats: the observations made in 1957 showed a slight shift of the curve to the right, and those in 1958 to the left; the average number of segments in each case was close to the control value. When adrenalin was given, the shift to the right was clearly preserved in the experiments of the two successive years. The difference between the experimental and control values was biometrically significant. In the castrated animals receiving adrenalin, the difference from the control value was still greater. The lability of the nuclei of the specialized leucocytes towards strong stimulation was higher in the castrated than the normal animals.

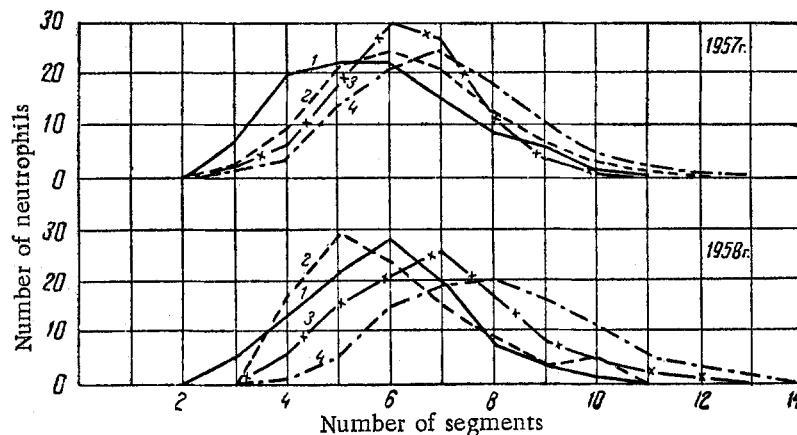


Fig. 2. Curves of variation of the number of segments in the nuclei of the neutrophils under different experimental conditions 24 hours after infliction of the wound (experiments in 1957 and 1958).

1) Control; 2) castration; 3) adrenalin; 4) castration and adrenalin.

The histological picture of biopsy of the wound margins corresponded to the results of the naked-eye study and to the cytological analysis of the wound exudate. Biopsy specimens taken from the control rats after 7 days showed that the wound was covered by a scab under which was a leucocytic barrier, rich in white blood cells. The dense, young granulation tissue was intensively vascularized; the cells in it were mainly fibroblasts at various stages of differentiation, together with abnormal histiocytes and white blood cells. In the animals receiving

adrenalin the scab on the surface of the wound was thicker than in the control experiments, and the leucocytic barrier beneath the scab was looser and contained relatively fewer blood cells. The granulation tissue was thinner than in the controls, and was arranged as a layer beneath the leucocytic barrier. Besides a large number of destroyed blood cells, this also contained a few young fibroblasts. When adrenalin was given to castrated animals the normal formation of granulation tissue was also retarded. Epithelialization, which at this period affected only the peripheral zone of the wound, was most marked in the controls and was extremely slight in the castrated animals. The small, wedge-shaped layer of young epithelium was thinner in the animals receiving adrenalin.

As a result of the administration of adrenalin, inhibition of the process of healing was thus observed in wounds of both normal and castrated animals, but it was more pronounced after castration. Under these circumstances there was a characteristic retardation of the formation of granulation tissue and of connective tissue, which did not favor epithelialization of the wound surface. The administration of adrenalin to normal animals inhibited migration of neutrophilic leucocytes, and this showed no recovery in the subsequent period of observation. After castration, the migration of neutrophils was considerably reduced in intensity in the white rats and the guinea pigs; administration of adrenalin stimulated to some degree the migration of neutrophils in castrated animals, but only at the beginning of the inflammatory reaction.

Some of the results which we obtained were in agreement with those of previous observations on the inflammatory reaction in castrated animals [1]. Suppression of the migration of leucocytes by adrenalin has been described earlier [4, 5], and this could be connected directly with a reduction in the permeability of the vascular endothelium. Our results further show a resemblance in the character of the changes in the wound exudate produced by the hormones of the medulla and the cortex of the adrenal glands [2]. They also support the view of interaction between the two sections of the adrenal gland and of the physiological synergism of their hormones [3, 6, 7].

#### SUMMARY

The process of wound healing is inhibited in administration of adrenalin to normal, especially to castrated white rats and guinea pigs. This is connected with a delay in the formation of granulation tissue and its development into connective tissue, as well as with retarded epithelialization of the wound surface. Administration of adrenalin to normal animals inhibits the migration of neutrophils in comparison with control. This is especially pronounced in castrated animals. The segmentation of the neutrophilic nuclei is increased under the effect of adrenalin. This reaction is also more intense in castrated animals.

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\* See English translation.

\*\* Original Russian pagination. See C.B. Translation.