CHANGES IN THE FUNCTION AND STRUCTURE OF THE ADRENAL CORTEX AFTER AUTOTRANSPLANTATION

V. K. Kulagin and D. Ya. Shurygin

Department of Pathological Physiology (Head, Corresponding Member AMN SSSR Prof. I. R. Petrov) and Department No. 1 of Therapeutics (Head, Prof. V. A. Beier) of the Order of Lenin S. M. Kirov Military Medical Academy, Leningrad

(Presented by Active Member AMN SSSR N. G. Khlopin)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 9, pp. 108-112, September, 1960

Original article submitted August 29, 1959

Besides its own special interest, autotransplantation of the adrenals has attracted our attention because by means of this method the reactivity of the animal body can be studied during changes in the functional state of the adrenal cortex. It is now known that after autotransplantation the medullary substance of the adrenals rapidly degenerates, but the cortex, while undergoing degenerative changes, soon begins to regenerate [1-3,6,7, 10]. Degenerative changes arise primarily in the zona fasciculata and zona reticularis, and regeneration begins in the cells of the subcapsular layer [4]. Regeneration of the cortex is complete in a month [5].

As a result of autotransplantation the medullary substance of the adrenals is thus irreversibly lost, whereas the cortex is present in different functional conditions, depending on the interval after operation. It must be borne in mind, moreover, that after autotransplantation the organ is also denervated.

EXPERIMENTAL METHOD

White rats weighing 160-200 g, of both sexes, were used in the experiments. The operation of transplantation of the adrenals was carried out under light ether anesthesia. The rats were fixed in the prone position. The skin over the spine was incised (observing measures of asepsis and antisepsis), and the vertebral muscles exposed. Division of these muscles on both sides of the vertebral column in the region of the superior poles of the kidneys gave access to the adrenals. These glands were completely freed from their attachments and reburied in the peritoneal cavity near their natural location. The muscles and skin were sutured with silk.

Before and after the operation of transplantation of the adrenals, observations were made of the general condition and behavior of the animals, the healing of the operation wounds, the weight and the body temperature, which was measured rectally. In part of the experiments (10 rats) the reaction of the eosinophils of the peripheral blood was studied in response to the intramuscular injection of adrenocorticotropic hormone (0.1 unit per rat). Blood for eosinophil counting was taken from the tail, and diluted with Dunger's reagent.

At the end of the experiment the rats were sacrificed by decapitation, and the adrenals were removed for histological examination (fixation in formalin, staining with hematoxylin-eosin). Altogether, 75 experiments were performed. In 10 of these the indices chosen were studied in normal rats; in 10 experiments the operation was performed as described above, but the adrenals were not transplanted ("false" operation); in 15 experiments the adrenals were removed; and in other series of experiments (40 rats) the chosen indices were studied at various intervals of time after autotransplantation (7, 15, 60 and 80 days).

EXPERIMENTAL RESULTS

The animals withstood the operation of transplantation of the adrenals relatively well. After the "false" operation all the rats survived. After autotransplantation of the adrenals the mortality was 24%, and after removal of the adrenals, 60%. The animals died during the period from the fifth to the twentieth day after operation. In the first days after operation the rats were lethargic and sluggish. The comparatively high rate of survival among the rats after autotransplantation of the adrenals was evidently due to the presence of additional adrenal tissue in these animals. At the beginning of the second week the state of the animals undergoing operation improved appreciably.

In the rats with transplanted adrenals the operation wounds healed, as a rule, by first intention. In contrast to this, in most of the animals after the "false" operation suppuration took place in the wounds, which then healed by second intention. The difference observed in the wound healing may evidently have been due to a decrease or loss of the mineral corticoid activity of the

adrenal cortex after transplantation of the glands, since, according to reports in the literature [9], the violent course of the inflammatory reaction is due to an increase in the secretion of mineral corticoids. The growth of the hair around the operation wound in rats with transplanted adrenals was retarded by comparison with the rats subjected to the "false" operation; the hair fell out also in other parts of the skin, demonstrating the appearance of trophic disturbances.

The operation of transplantation of the adrenals had no appreciable effect on the temperature, and the changes that were observed were not of a regular pattern. By comparison with the animals undergoing the "false" operation, the rats with transplanted adrenals gained more in weight, especially in the series of experiments in which the animals periodically received ACTH. Two months after operation the weight of the animals with transplanted adrenals increased by 34%, but the weight of the same rats receiving ACTH had increased by 52% over the initial weight (the difference between the mean values is statistically significant, $R = 0.27$). If the definite influence of the adrenal cortex on growth and development of the animal is taken into consideration (which may be judged in particular by the increase in weight of the growing rats), it may be postulated that the administration of ACTH after autotransplantation stimulates the restoration of the function of the transplanted adrenals.

Histological study of the autotransplanted adrenals* showed the following (Fig. 1): One week after autotransplantation, a varying degree of progressive regeneration of the cortical substance was observed in the adrenals, as shown by the considerable hyperplasia of the deep layers of the capsule and subcapsular blastema, and by the formation of a zone of adrenal cortical cells. From its structural features, the latter zone had not yet formed typical cell complexes of the zona glomerulosa, which made it difficult to differentiate the regenerating tissue into typical zones of the cortex.

The regenerate area was very hyperemic. On section, the processes of regeneration were unevenly developed around the circumference of the organ. The central area of the transplant was dead. In individual rats of this group a considerable leucocytic infiltration of the outer layers of the necrotic parenchyma of the adrenal cortex was observed.

Two weeks after autotransplantation the almost complete regeneration of all the layers of the cortex was observed, and the zonal structure of the cortical layer was clearly apparent. The adrenal cortex was grossly hyperemic. In the deep layers of the cortex, intensive infiltration by leucocytes was observed. Two months after autotransplantation complete regeneration of the cortical substance had taken place, and its division into zones was pronounced. Medullary substance was absent (Fig. 3).

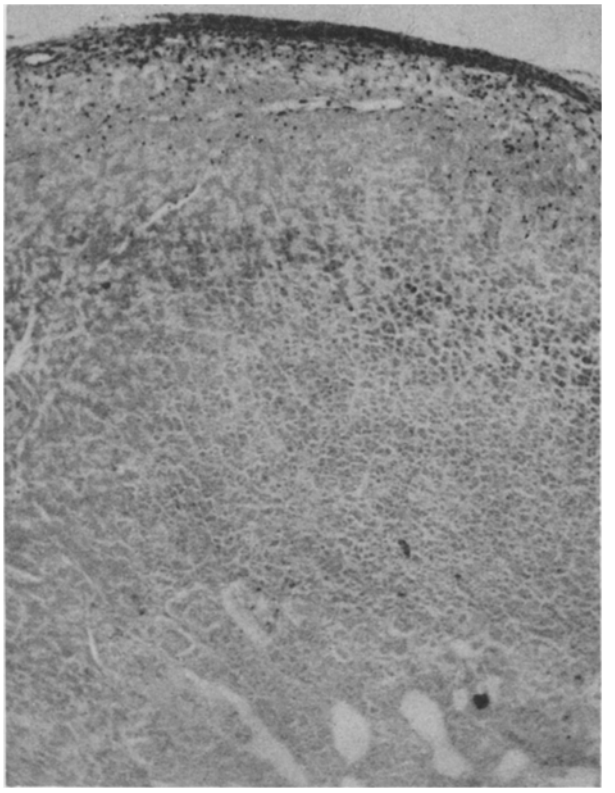

Fig. 1. Adrenal gland one week after autotransplantation. Hyperplasia of the deep layers of the capsule and of the subcapsular blastema. Stained with hematoxylin-eosin. Magnification 30 ×.

In order to evaluate the functional state of the adrenal cortex after autotransplantation, changes in the number of eosinophils in the peripheral blood in response to the injection of ACTH were analyzed. With the dose of the hormone selected (0.1 unit/rat), on the fourth day after injection of ACTH into healthy animals the eosinophils had completely disappeared from the peripheral blood. It must be pointed out that injection of the hormone at first caused, as a rule, an increase in the number of eosinophils, presumably as the result of a redistributive leucocytosis, and then a gradual fall to their complete disappearance after 3-4 hours. In contrast to this, in the animals with transplanted adrenals, changes of an unusual character in the eosinophil count were observed in response to the injection of ACTH. During the first 24 hours after operation, injection of ACTH led to an increase in the eosinophil count, which lasted until the third hour, after which it returned to its initial level, or to a higher level than before operation. The phase of eosinopenia was thus absent at this period. On the third day after operation, immediately after a slight fall, a progressive increase was observed in the eosino-

* This investigation was carried out in consultation with Prof. E. A. Moiseev.

phil count. On the sixth or seventh day the curve showing the changes in the eosinophil count was almost normal in character, although at a much higher level. On the 14th day the initial number of eosinophils was lower than in previous investigations, but the injection of ACTH caused a distorted reaction — an increase in the number of eosinophils. On the 20th and 27th days after operation the reaction of the adrenal cortex to injection of exogenous ACTH was also abnormal. Only on the 56th day did the reaction of the eosinophils in response to injection of the hormone become normalized at a lower level of the eosinophil count in the peripheral blood.

The results of these investigations confirmed the description in the literature of the course of the morphological changes in the adrenals after autotransplantation. During the first few days after operation degenerative changes occurred in both cortical and medullary substance. Soon, however, the cortical substance began to regenerate. Source of regeneration was the cells of the subcapsular blastema. Whereas one week after operation regeneration of the cortical substance was confined to the subcapsular layer, after 2 weeks the almost complete restoration of the adrenal cortex was observed, and the characteristic zonal structure of the cortex gradually became apparent. Two months after autotransplantation regeneration of the cortical substance was finally complete.

Fig. 2. Autotransplanted adrenal gland 2 weeks after operation. Well-advanced regeneration of the cortex can be seen. Stained with hematoxylin-eosin. Magnification 30×.

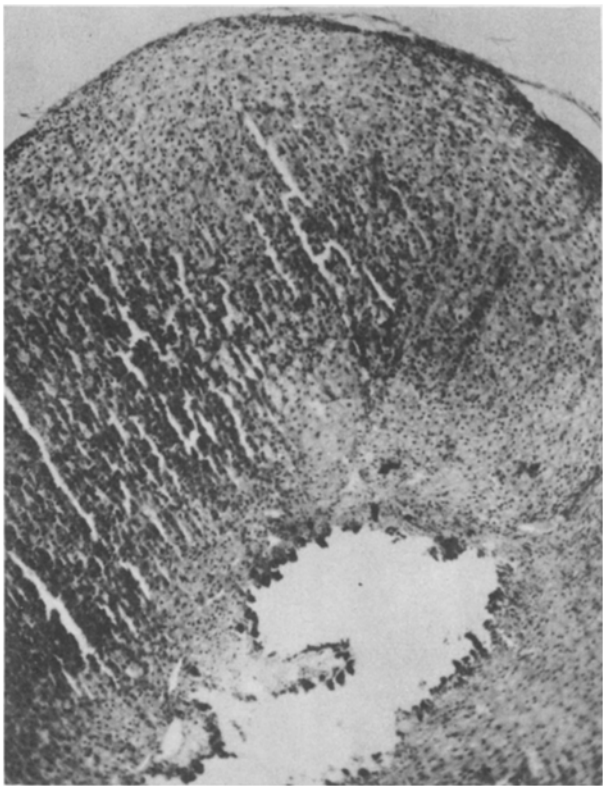

Fig. 3. Autotransplanted adrenal gland 2 months after operation. Complete regeneration of the cortical substance with marked zonal pattern. Medullary substance absent. Stained with hematoxylin-eosin. Magnification 30 ×.

The autotransplanted adrenals responded for a considerable time by an abnormal reaction (judging by changes in the number of eosinophils in the peripheral blood) to injection of exogenous ACTH, which was associated either with the functional imperfection of the cortical cells or with the denervation of the adrenals. It is most probable that both factors are important [2]. On the basis of this investigation and of information in the literature, the sequence of changes in the function of the autotransplanted adrenals may be represented schematically as follows: 1) the period of acute adrenal cortical insufficiency — 1-2 weeks after operation; 2) the period of relative adrenal insufficiency — 2-4 weeks after operation; 3) the period of normalization of the adrenal function.

The second and (in particular) the third periods are characterized by the complete, or almost complete, restoration of the structure of the organ. However, the presence of a higher eosinophil count than initially, together with the abnormal reaction of the adrenal cortex

to ACTH, is evidence of the functional impairment of the adrenal glands.

The possibility cannot be entirely ruled out that hyperfunction of the adrenals may develop at a later period after autotransplantation, as occurs after ligation of the vessels supplying the adrenal gland. In these conditions, in association with morphological changes in the regenerating cortical substance analogous to those described above, hyperfunction develops, as may be judged by the increased excretion of 17-ketosteroids in the urine, the more energetic reaction of the adrenals to the administration of ACTH and the hypertrophy of the anterior lobe of the pituitary [8].

SUMMARY

Experiments were performed on white rats at various periods following autotransplantation of the adrenal glands. The authors studied the morphological changes occurring in the adrenals, the weight and temperature of the body, the wound healing and the reaction of the transplanted adrenal glands to ACTH administration.

Proof was obtained that autotransplantation is followed by complete degeneration of the medullary, and partial degeneration of the cortical, substance. After a week, however, the cortical substance begins to proliferate, with the cells of the subcapsular blastema serving as a source of regeneration. At the end of the second week regeneration of the cortical substance is almost complete, and typical zones of adrenal cortex appear. Restoration of the function of the adrenal cortex takes place later, since during the first month there is a relatively large number of eosinophils in the peripheral blood and an inadequate eosinophilic reaction in response to ACTH administration. Periodic ACTH administration has a favorable effect upon the growth and development of rats with transplanted adrenal glands.

LITERATURE CITED

1. E. I. Tarakanov, Problemy Éndokrinol. i Gormonoterap. 4, 114 (1958).
2. I. M. Shapiro, The Nervous System in the Process of Healing and Restoration of the Function of the Thyroid and Adrenal Glands after Autotransplantation. Author's abstract of doctoral dissertation [in Russian] (Moscow, 1954).
3. H. Christiani and A. Christiani, J. de physiol. et path. gén. 4, 982 (1902).
4. W. J. Dempster and I. Doniach, Arch. Internat. Pharmacodyn. 101, 398 (1955).
5. R. O. Greep and H. W. Deane, Endocrinology 45, 42 (1949).
6. D. J. Ingle and G. M. Higgins, Endocrinology 22, 458 (1938).
7. H. Poll, Arch. mikr. Anat. 54, 44 (1899).
8. G. Poumeau-Delille, E. Fels, and M. Kijewska, Compt. rend. Soc. biol. 148, 1658 (1954).
9. H. Selye, Annual Report on Stress (Montreal, 1951-1956).
10. L. C. Wymen and C. Tum-Suden, Am. J. Physiol. 101, 662 (1932).