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REGENERATION OF THE ADRENAL CORTEX

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There are numerous data dealing with the morphology of the adrenal gland, and among others, with the »dynamic morphology« of cortical cells. According to the latter theory, during life the cells of the cortex become exhausted, destroyed and replaced by new cells by means of regenerative processes in the cortex. *(Hoerr* [12] ; *Salmon* and *Zwemer* [18] ; and others.)

Authors accepting the possibility of cell migration and replacement uniformly agree that the site of cell destruction is the reticular zone. The facts supporting the assumption that destruction of reticular cells occurs are the general morphological pattern *(Zwemer, Wotton, Norkus)* [21] ; the presence of degeneration pigment *(Lewis)* [16] ; the reticular fibre pattern *(Bachmann)* [1] ; the diffuse discoloration of cells stained vitally with trypan blue *(Baxter)* [3], etc. Detailed information to this point may be found in *Bachmann*'s monograph (1941).

If the theory of cell destruction is adopted, the first question to arise is the origin of the cells required for reparation. In this respect opinions are by far not so unanimous as concerning the site of degeneration.

There are several theories as to the regeneration, or, more broadly speaking, replacement of cortical cells.

According to earlier views, some part of the cortex is responsible for this function. The role of a germinative layer had been attributed to the glomerulous zone, by others to the fasciculate zone, or to the cells at their border, or to the two zones together. (For detailed information see *Bachmann* [1].)

More recently, from 1937 on, the adrenal capsule has been considered by most authors as the germinative layer of cortical regeneration. In this respect two theories have been brought forward. One originates from Bachmann, who found a syncytium-like layer on the cortical aspect of the capsule, with large, oval or round nuclei containing small amounts of chromatin. *Bachmann* considered this layer as closely related to the mesenchymal tissue, attributed germinal capacity to it, and proposed the term »subcapsular blastema«. In his opinion, cortical cells originate from this layer. The other theory, supported first of all by *Zwemer* [21] and also by *Baker* and *Bailiff* [2], *Wotton* and *Norkus* [21], as well as *Salmon* [18], suggests that capsular cells through intermediary forms containing lipoid, would be converted into cortical cells. The fibroblast-like cells of the capsule are regarded as the primary ones.

Williams [20] is of the opinion that both the capsule and the glomerular zone are mutually responsible for the process of regeneration.

More recently, however, the possibility of adrenocortical cell migration has been subject to some scepticism, for instance on the basis of *Giraud, Martinet* and *Bellon*'s [10] studies about the action of ascorbic acid on the adrenal glands of the horse, or of the recent reports by *Tonuti* [19], *Baxter* [3], *van Dorp* [5] and others.

The primary aim of the present investigations was to determine the site of origin of cellular replacement.

Materials and Methods

A total of 79 white rats, both male and female ranging in weight from 150 g to 250 g were used. Environmental conditions and diet were the same both before and during the experiment.

The cortex was injured in ether anaesthesia, under aseptic conditions. An incision was made on the shaven skin of the left renal area, the back muscle was cut, the adrenal gland carefully extracted from the peritoneal fatty tissue and a section of the adrenal cortex was cut off with iris scissors. After closing the wound by layers, primary healing ensued in all cases. There were no deaths.

Subsequently, the animals were sacrificed by bleeding after various intervals and the injured adrenal glands to be studied were divided into three groups.

Group A (48 rats). Injured adrenal glands of animals killed on each of the first 12 post-operative days, and those of animals killed after 20, 30, 40 and 50 days, respectively, made up the test material. For each test, 3 animals were used in both A and B groups. The material was fixed according to Susa, Zenker, or Maximov, and embedded in paraffin. Sections 5 micron thick were cut and stained with haematoxylin-eosin, or iron haematoxylin, or according to Mallory and Giemsa.

Group B (21 rats). The material consisted of the lesioned adrenals of animals killed 4, 8, 12, 20, 30, 40 and 50 days after the operation. After fixation in formaldehyde, the preparations were frozen and treated with fat stains (Scarlet R., Nile blue).

Group C consisted of 10 control rats.

Observations

The state after operation was studied on the adrenal cortex of animals killed next day. On the sections two parts were discernible, typical adrenal tissue, and the haemorrhage at the surface of the wound. This mass is surrounded by fatty tissue that, adhering to the surroundings, encapsulates the clot. On the surface of the wound fine formations similar to connective tissue fibres and probably consisting of fibrin or precollagen can be seen.

They stain reddish-blue with Mallory's triple dye in contrast with the steel-blue colour of definite collagenous fibres of the capsule. A degeneration zone is present under the wound surface. Comparing this zone with intact cortical areas, its cells appear pale with indefinite outlines and the nuclei show signs of chromatolysis and pyknosis. The sinuses are markedly distended and contain a large number of leucocytes, many of them outside the vessels. Sections cut at different heights prove that cells in the immediate neighbourhood of the marrow show normal structure. From these two observations it may be concluded that the blood vessels supplying the described area had been severed when inflicting the lesion. As to the normal marginal cells, it is assumed that these are supplied with blood vessels from the marrow. Another observation appears to confirm this assumption. On examining a circumscribed area of degeneration in the cortex, the capsule overlying this area was found to be distorted, broken and a small subcapsular blood vessel was also ruptured. The vascular lesion occurred probably during operation, when the adrenal gland was extracted.

On both sides of the wound the trancised capsular margin with the lesioned adjacent cortical tissue and numerous extravasal leucocytes can be clearly seen.

The observations of the following days reflect mainly the completion of degeneration. Up to the fifth day there was no sign of regeneration. The fibres at the surface of the wound stain differently than on the first day and appear to be intermediary forms between the former and the definite capsular fibres. At the border between the proliferating haemorrhagic tissue and the fat layer, large cells containing hemosiderin can be found. In the central part of the cortex and in the marrow hyperaemia is present.

From the fifth day on, a change occurs in the histological picture, signs of regeneration making their first appearance. Preparations from this period present the following aspect. The section still contains two parts, characteristic adrenal tissue and proliferative tissue at the surface of the lesion. The trancised capsular margins are markedly thickened at the points corresponding to the wound surface (Fig. 6). Here cells abound the capsule, with numerous amitotic forms among them. The two margins of the capsule are connected with a new capsule made up of fresh collagenous fibres which cover the surface of the proliferative tissue as well. In the proliferative tissue elongated cellular forms are present. In the thickened parts of the capsule, the transitory cellular forms described by *Zwemer* et al. can also be found (Figs. 1 and 2), appearing as rounded cells of a considerable size, containing a central nucleus rich in chromatin surrounded by a granulated, resp. vacuolized cytoplasm staining reddish-violet. The intermediary cellular forms just described appear from the fifth day on.

Besides these cellular forms at some sites of capsular thickening, areas resembling syncytium appear (Figs. 3 and 4). The cells show no definite outlines, the nuclei are large, elongated in shape, and contain chromatin in fine, dust-like distribution. It is thought that these areas correspond to *Bachmann*'s »subcapsular blastema«. This blastema was considerably less frequently encountered than the intermediary forms.

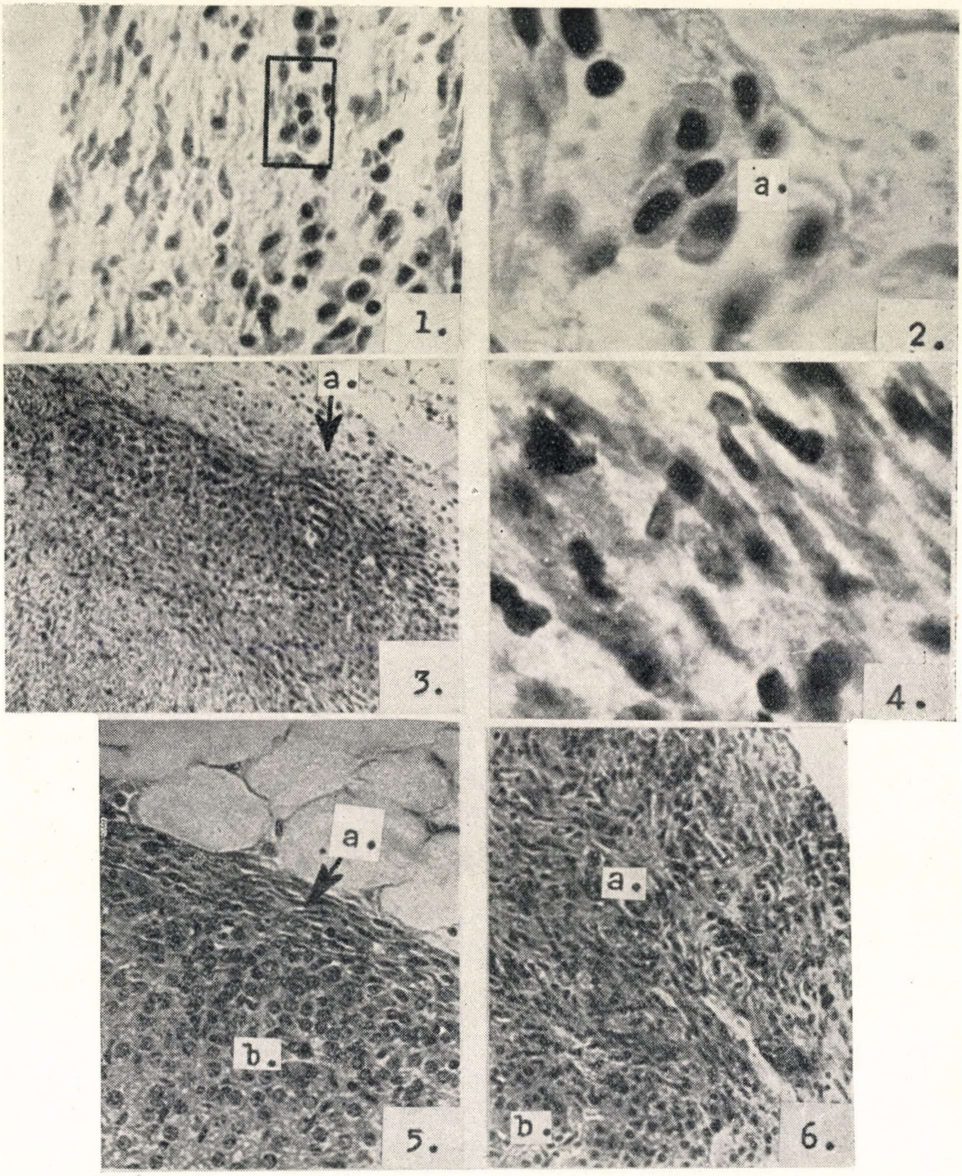
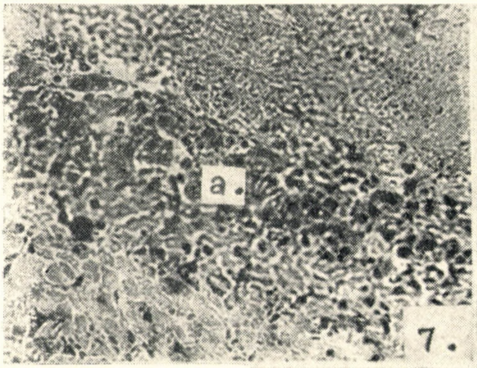
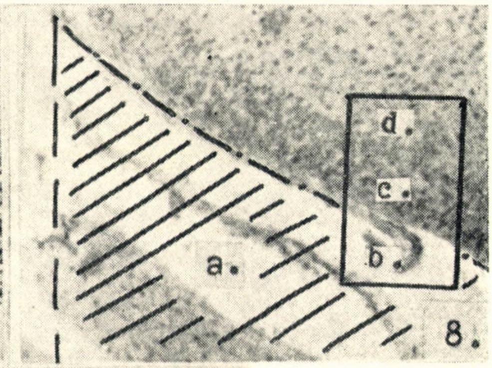


Fig. 1. Eyepiece, Leitz Periplan 10x. Lens, Zeiss 4 mm. In the framed area Zwemer's transitory cellular forms can be seen. *Fig. 2.* Eyepiece, Leitz Periplan 10x. Lens, Leitz H. I. 100x. High-power view of the framed area of Fig. 1. »a« : transitory cellular forms. *Fig. 3.* Eyepiece, Periplan 10x. Lens, Zeiss 16 mm. The arrow labelled with »a« points to a thickening of capsule following trancision. *Fig. 4.* Eyepiece, Leitz Periplan 10x. Lens Leitz H. I. 100x. High-power view of Fig. 3. Area resembling syncytium. *Fig. 5.* Eyepiece, Leitz Periplan 10x. Lens, Zeiss 16 mm. »a«, normal adrenal capsule; »b«, normal cortical tissue. *Fig. 6.* Eyepiece, Leitz Periplan 10x. Lens, Zeiss 16 mm. Margin of capsule showing signs of regeneration. »a«, thickening ; »b«, cortical tissue.

During the following period regeneration progresses. In addition to the aforementioned regenerative activity of the capsule, the glomerular zone too exhibits signs of cellular reparation. On the 20th day, a group of cells resembling cortical cells appears within the proliferation tissue (Fig. 7). These cannot be





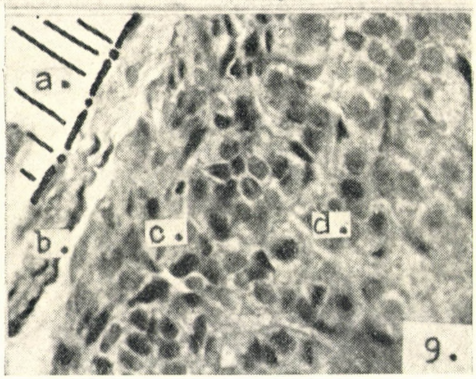


Fig .7. Eyepiece, Leitz Periplan 10x. Lens, Zeiss 16 mm. Group of cells in granulation tissue resembling in shape cortical cells (»a«).

Fig. 8. Eyepiece, Leitz Periplan 10x. Lens, Zeiss 16 mm. On the 50th day following operation. The broken line indicates the original site of cortical margin ; the shadowed area labelled with »a« represents the place of the cortical tissue removed at the operation ; the result line indicates the regenerated cortical margin. »b«, capsule; »c«, newly formed glomerulous zone; »d« fasciculate zone.

Fig. 9. Eyepiece, Leitz Periplan 10x. Lens, Leitz H. I. High power view of the framed area of Fig. *8.* Signs as above.

considered to be remnants of the destroyed cortex. We think it likely that these cells have developed from granulation tissue cells on some appropriate chemical effect. The hyperaemia of the central part of the cortex and of the marrow has subsided by this time. Newly formed cells can be found in large numbers. The fibres of the new capsule are not much different in staining from the definite fibres. The pace of regeneration is reduced.

When studying late phases, in one instance where the lesion involved only a very small section of the cortex, complete restitution was seen. Recovery of more extensive lesions, on the other hand, was not complete even by the 50th day (Figs. 8 and 9). In one of the cases the fibres of the adrenal capsule already enclosed the cortex as definite structures. At the site of the lesion an impression was present on the surface and here the cortex was at least 20 to 30 rows of cells thinner than at other sites. The reticular zone retained its normal width. The tissue defect is located in the glomerular zone, follows the upper margin of the fasciculate. Here a newly formed layer can be found (Fig. 9) all morphological features of which, except the somewhat different shape of the nuclei, indicate that it represents a glomerular layer. All these facts make it obvious that in the course of 50 days a restitution with the formation of a new glomerulous zone has resulted from the regenerative action of both the capsule and the glomerulosa cells. No evidence indicating an increase in, or segmentation of, the fasciculate zone could be detected in the sections.

Discussion

The above specified technique of injuring the adrenal cortex and the resulting tissue conditions have rendered it possible to study the course of regenerative activities. The primary aim of this work was to follow the process of regeneration and it was not intended to study the migration of cells. Thus, no contributions of major significance have been made regarding the latter point.

The absence of histochemical evidence confirming the results is undoubtedly a shortcoming of the present investigations. According to *Feldman* [9], and other authors, no undisputably reliable test or test combination is known that would render the ketosteroids visible. In spite of this fact, we are planning to complete our investigations with that procedure.

First of all it must be pointed out that with the technique described only a slight regenerative tendency was found in the cortex. Namely, when only small areas of the cortex had been injured, tissue reparation was complete, but more extensive lesions involving the whole width of the cortex were not replaced by newly formed tissue even in 50 days. *Greep* and *Deane* [11] reported a much higher regenerative activity, but they performed bilateral enucleation and retained the capsule only. The difference between the methods applied explains the difference in results. Considering *Ingle*'s findings [13] it may be stated that regeneration of the adrenal gland stand under the influence of the regulative action of the adrenocorticotropic hormones of the cortex and the anterior lobe of the pituitary gland, and, as put by *D. I. Ingles* »it runs parallel with physiological need«. When one adrenal gland is extirpated and the other enucleated, regeneration is rapid. Leaving one adrenal gland intact and enucleating the other one, the pace of regeneration is much slower.

As indicated by transplantation experiments (*Williams*, [20,] et al.) and studies made after enucleation (*Greep* and *Deane* [11]), the adrenal capsule with a small account of underlying cortical tissue is capable of producing fresh cortical tissue. Thus, the site of cellular reparation must be sought also in the capsule. On the cortical aspect of the capsule intermediary cellular forms, also described by *Zwemer* et al., could be observed. The rounded cells containing fine vacuoles and representing a transitory form between capsular and cortical cells could also be detected. Considering that these structures were present in most of our cases, it is thought that even this mode of cellular reparation may have played a part in our series. The question might arise whether these cells located at the border of the granulation tissue were not identical with macrophages containing debris. This possibility may be excluded since these cells were demonstrable even at sites where no cell destruction occurred in the surrounding tissues.

This mode of regenerative action of the capsule, was, however, not the only one that could be detected. At certain parts, particulary where the trancised capsular margin was markedly thickened, areas resembling the »subcapsular blastema« of *Bachmann* were also found. In these areas, as already mentioned, syncytium-like tissue could be seen.

Beside the regenerative activity of the capsule, signs of cellular reparation were present in the cortical tissue too. According to our results only the cells of the granulous zone play a role in this respect. This is in agreement with the data reported by *Greep* and *Deane* [11], *Williams* [20], and others. The fasciculate zone is not thought to be capable of taking an appreciable part in the regeneration process. We were unable to confirm *Rotter*'s assumption that the reticular and glomerulous zones would be products due to the segmentation of the peaks of the fasciculate columns. On the other hand, *Rotter*'s observations were made on human material and this may be responsible for the difference in the findings.

Another mode of cellular replacement should also be considered. The cells of the indifferent granulation tissue formed at the site of the haemorrhage are known for their pluripotency. *Krompecher*'s studies made on joints have shown that under different environmental conditions these cells might differentiate into a variety of types. In the former experiment a »morphodifferentiation« in a mechanocyte, physical direction occurred, due to physical action. Data indicating the possibility of a chemically determined differentiation of these pluripotent cells can also be found in the literature. Supporting this view are the histiocyte experiments of *Chevremont* and *Chevremont-Comhaire* [4], as well as the investigations into bone formation carried out by *Levander* [15], *Engström* and *Orell* [7]. We feel that these possibilities could be equally applied to our special case. It is assumed that under the influence of the cortical hormone, as an adequate stimulus, the cells of the granulation tissue may be converted into cortical cells. Certain signs supporting this point were observed in the course of the experiment , but really convincing results cannot be obtained except with histochemical methods. It should, however, be pointed out, that in this area abounding with macrophages containing cortical cell debris, it would be difficult to evaluate the results, since it is likely that the debris of the cortex would react similarly to the dye. As to the question of cellular division in the adrenal cortex, our observations support *Bachmann*'s view, in contrast with numerous other data. Similary to *Bachmann*, we have found only a very limited number of mitotic forms in the adrenal cortex, in spite of the fact that the process of regeneration was rather extensive. Only a few typical mitotic forms could be detected in the whole experimental series. Studying the postnatal development of the adrenal cortex of the rat, *Engström* [6] counted the number of mitoses detectable in each of the layers and found the highest number of mitotic forms in the period between the 10th to 40th days. Later on, the number of indirect mitoses decreases. This may account for the absence of mitotic forms in our material : in advanced age the rate of mitoses is presumably reduced. The colchicine method appears to present serviceable means for the solution of this problem. Still, it must be applied with some criticism, as according to the data of Selye the drug excites the adrenal cortex, and thus, when arresting mitosis, it would simultaneously stimulate the fasciculate zone *(Greep* and *Deane*, 11).

In contrast with the rather low number of mitotic forms, sandglass-like nuclei in the cells of the adrenal capsule were frequently observed. This nuclear form is not a decisive sign of amitosis, or, at least, as suggested by *Bachmann*, not every nucleus bearing a constriction can be considered to be a direct mitotic form [1]. Still, considering the scarcity of indirect mitoses and also the fact that the findings were made on adult animals, these might be taken for forms of mitosis.

Summary

Experiments were carried out on 79 adult rats, male and female. One of the adrenal glands was injured and the morphology of the ensuing regeneration process was studied with the usual histological technique. The following conclusions were drawn :

1. Under the conditions specified, the adrenal cortex showed a relatively low regenerative activity. After an extensive cortical injury restitution was not completed even by the 50th post-operative day.

2. The capsule and the glomerular zone are held responsible for the regeneration. It is pointed out that both signs indicating a regenerative tendency of the capsule, i. e. the »transitory cells« of *Zwemer* and the »subcapsular blastema« of *Bachmann*, could be demonstrated in the material examined.

3. In contrast with other data, no regenerative activity of appreciable degree is attributed to the fasciculate zone.

4. It is suggested that the pluripotent cellular elements of the granulation tissue may also participate in the process of cellular replacement. These cells would be converted into cortical cells under the influence of the cortical hormone.

REFERENCES

1. *Bachmann, R.* : (1941) Nebennierenstudien. Ergeb. der Anat. und Entwicklungsgesch. **33,** 31—134.

2. *Baker, D. D.* and *R. N. Bailiff* : (1938) The role of the capsule in suprarenal regeneration. Anat. Rec. Suppl. 3.

3. *Baxter, J. S.* : (1946) The growth cycle of cells of the adrenal cortex in the adult rat. Journ. Anat. **80,** 139.

4. *Chévremont, M.—S. Chévremont—Comhaire* : (1945) Recherches sur le determinisme de la transformation histiocytaire. Acta Anat. **1,** 95—144.

5. *van Dorp, A. W. V.—H. W. Deane* : (1950) A morphological and cytochemical study of the postnatal development of the rat's adrenal cortex. Anat. Rec. **107,** 265—282.

6. *Engström, H.* : (1936) Studien über die postnatale Entwicklung der Nebennierenrinde der weissen Ratten. Anat. Anz. **83,** 1—19.

7. *Engström, H., Orell, S.* : (1943) Über Regeneration Rings um subcutane Knochen-¡mplantate. Zeitschr. mikr. Anat. Forsch. **53,** 285—318.

8. *Everett, N. B.* : (1949) Autoplastic and homoioplastic transplants of the rat adrenal cortex and medulla to the kidney. Anat. Rec. **103,** 335—348.

9. *Feldman, J. D.* : (1950) Histochemical reactions of adrenal cortical cells. Anat. Rec. **107,** 347—359.

10. *Giroud, A., Martinet, M.* and *Bellon, M. T.* : (1940) Répartition de l'hormone corticale dans le cortex surrenal, valeur de la zone glomerulée. C. R. Soc. Biol. **134,** 441—443.

11. *Greep, R. O.* and *Deane, H. W.* : (1949) Histological, cytochemical and physiological observations on the regenerations of the rat's adrenal gland following enucleation. Endocrinol. **45,** 42—56.

12. *Hoerr, N. L.* : (1931) The cells of the suprarenal cortex in the guinea pig. Their reaction to injury and their replacement. Amer. J. Anat. **48,** 193—197.

13. *Ingle, D. J.* : (xxx) Control of regeneration of the adrenal cortex in the rat. Pituitary-Adrenal Function.

14. *Krompecher, I.* : (1943) Izületképzés. Erdélyi Múzeum-Egyesület Orvostudományi Szakosztálya, Kolozsvár. (Hung.)

15. *Levander, G.* : (1949) On tissue induction. Acta Path. et Microbiol. Scand. XXVI 113—142.

16. *Lewis, M. R.* : (1948) Nile blue staining of adrenal glands of living mice. Anat. Rec. **102,** 37—44.

17. *Rotter,* : (1949) Das Wachstum der foetalen und kindlichen Nebennierenrinde. Zeitschr. f. Zellforsch. u. mikr. Anat. **34,** H. 5.

18. *Salmon, Th. N.* and *Zwemer, R. L.* : (1941) A study of the life history of corticoadrenal gland cells of the rat by means of trypan blue injections. Anat. Rec. **80,** 421—430.

19. *Tonutti, E.* : (1942) Zur Histophysiologie der Nebennierenrinde : Bau und Histochemie bei der Atrophie des Organs nach Hypophysectomie. Zeitschr. mikr. Anat. Forsch. **51,** 346—392.

20. *Williams, R. G.* : (1947) Studies of adrenal cortex : Regeneration of the transplanted gland and the vital quality of autogenous grafts. Amer. J. Anat. **81,** 199—231.

21. *Zwemer, R. L., Wotton, R. M.* and *Norkus, M. G.* : (1938) A study of corticoadrenal cells. Anat. Rec. **72,** 249—263.

РЕГЕНЕРАЦИЯ КОРЫ НАДПОЧЕЧНИКА

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Резюме

Авторы проводили экспериментальные исследования над 79 взрослыми крысами-самцами и крысами-самками. Один из надпочечников был нарушен оперативно, а затем была исследована наступающая регенерация с морфологической точки зрения. Применяя обычную гистологическую методику исследования, они установили, что кора надпочечника при условии упомянутого выше способа повреждения обладает относительно небольшой способностью к регенерации. В случае более значительного повреждения, даже спустя 50 дней не наблюдался полный возврат к норме. В связи с этим, авторы указывают на гормональные и нервные факторы, оказывающие влияние на кору надпочечника и играющие несомненно значительную роль в регенерации. По их мнению регенерация происходит за счет капсулы гломерулярной зоны надпочечника. Фасцикулярная зона, — по мнению авторов — не участвует в процессе регенерации. Они считают возможным, что в замещении погибших клеток участвуют и плюрипотентные клеточные элементы грануляционной ткани, возникшей на месте повреждения, а именно таким образом, что под влиянием гормона коры надпочечника, они превращаются в корковые клетки.