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Ultrastructural Examination of the Regeneration of the Rat Adenohypophysis after Partial Hypophysectomy

W. Saeger[1] and H. Warnecke[2]

[1] Abteilung für Pathologie, Marienkrankenhaus Hamburg, Alfredstraße 9, D-2000 Hamburg 76, Federal Republic of Germany

[2] Institut für Pathologie, Universität Hamburg, D-2000 Hamburg 20, Federal Republic of Germany

Summary. Male Wistar rats were partially hypophysectomized and sacrificed at intervals of from 2 days to 1 year following the operation. The resected material was examined under the light microscope and the residual pituitary under the light and electron microscope. Neither regeneration nor an anatomical restoration of the adenohypophysis occurred even one year after partial hypophysectomy. An increased number of small chromophobe stem cells and juvenile chromophil cells were found in the residual pituitary. The amount of mitoses were not significantly increased.

The ACTH, FSH and prolactin producing cells demonstrated electron microscopically distinct changes which were interpreted as signs of intensified activity. This can be seen as a "functional regeneration" to maintain vital pituitary function for the organism.

Key words: Pituitary – Ultrastructure – Hypophysectomy – Regeneration.

Introduction

The regenerative power of the adenohypophysis following hypophysectomy or partial hypophysectomy has been occasionally examined in past decades without uniform findings. Koster and Geesink (1929) observed hypertrophy of the residual pituitary tissue located on the sphenoid bone after hypophysectomy. However, Smith (1930, 1932) could find no indication of regeneration after hypophysectomy in rats. He estimated that approximately 70% of the anterior lobe could be removed without the manifestation of endocrine deficiency. In contrast, Reiss et al. (1937) observed a striking hyperplasia of mainly acidophilic cells following partial hypophysectomy. Crooke (1938) reported hypertrophic sella portions after a subtotal hypophysectomy. Cameron (1952) assumed regenerative power if gland removal was not too extensive. Weinbren and Fitchen (1959)

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rejected the concept of regeneration of the pituitary and connected the post operative enlargement of the residual tissue with normal growth.

These differing results are due in part to the variable methods used. The parapharyngeal approach was used mainly in rats, however, standardization of partial hypophysectomy regarding a uniform operation technique and the removal of a definable amount of tissue is surrounded with anatomical and methodological difficulties.

Clinical examination of hypophysectomized patients occasionally demonstrates post operative recovery of pituitary function (van Buren and Bergental, 1960; Landolt, 1973) which has been attributed to regeneration. Morphological examination of human tissue with regard to this problem does not appear to have been carried out.

Modern microsurgery techniques, especially using the operating microscope, today allow better reproducibility in resection experiments and exact information regarding the ratio between the resected and the remaining portions of the pituitary. Advances in the fields of histochemistry, immunohistology and electron microscopy of the pituitary have given much information on hormone location and cell kinetics. The subject of the regenerative powers of the adenohypophysis has thus been studied with the aid of modern microsurgical and electron microscopic techniques.

In particular, we wanted to investigate whether eventual recovery of anterior lobe function is due to regeneration or an increased stimulation of the residual tissue. An indication of pituitary function could be derived from the extent of changes in size and weight and the structure of some other endocrine glands.

Materials and Methods

A partial hypophysectomy was performed on 50 male Wistar rats. Nine animals died during the operation from apnoea during tracheostomy or extensive haemorrhage after trepanation at the base of the skull. Six rats died spontaneously 1–12 days following the operation. The 35 animals which survived the surgery without substantial complications made up the group of partial hypophysectomized rats. To assess nonspecific stress reactions, a similar operation was carried out on 6 additional animals without removing a pituitary tissue. Furthermore, untreated rats served as control animals. All rats, divided into groups of 5 animals, were kept at constant conditions in standardized cages, given food and water ad libitum and were weighed each week.

The partial hypophysectomy was performed according to the method of Engelhard (1969) which is a modification of the Smith (1930) technique.

The 150–250 g rats were anaesthetized intraperitoneally with 0.8 µl Pentobarbital per gram body weight. Skin incision was performed in the midline from the mandibular region down to the sternum. A tracheotomy was necessary because the larynx was pressed aside with a spatula in the course of the operation. The skin and mandibular salivary gland were separated into equal portions and held apart to either side with clamps or spatulae. The sternohyoid muscle was exposed in the midline without cutting and split with the aid of fine forceps. The trachea was exposed in the region of the thyroid cartilage. A 4 cm long polyethylene cannula was introduced into the trachea above the first tracheal cartilage. The dissection was continued into the depth beneath the hyoid bone by laying aside the omohyoid muscle with the aid of an operating microscope (OpMi 1 from Zeiss). Dissection in the midline must be maintained to avoid lateral injury of the carotid. The demarcation points, oral and lateral, were the styloid process and the ear capsule, respectively. The styloid process should not be exposed as that would entail the risk of opening the nasopharynx. The deep neck musculature was carefully detached from its insertion and pressed

in the caudal, oral and lateral directions so that a 2 mm in diameter trepanation could be performed on spheno-occipital synchondrosis which lies in the lower one-third of the drill-hole. The trepine did not injure the dura which was then opened with a pointed hook. The pituitary lay directly in the drill-hole. After the body of the gland was cautiously mobilized. a portion of the adenohypophysis was aspirated by two glass pipettes connected to a suction pump. The resected material was recovered from the lower portion of the pipette for further study. Following the resection the drill-hole was occluded with a tampon of fibrin foam (Fibrospum®) (Saeger et al., 1980) and by layered suturing the tracheostomy was covered with musculature. The skin incision was closed with 4 button sutures.

The animals were sacrified in pairs together with a control animal of the same age after 2, 4, 6, 8 days, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks and after 9 and 12 months. Before autopsy perfusion fixation with 3% glutaraldehyde was carried out under ether narcosis. The site of the residual pituitary was photographed after removal of the brain. The rest of the hypophysis was dissected for electron microscopy. Brain, heart, lungs, liver, pancreas, spleen, kidneys, testes and adrenals were removed for histological study.

The length of the body and tail were measured both before the operation and before the autopsy.

The pituitary removed upon resection and also the residual found by the autopsy were weighed on a microgram balance. The total pituitary from the control animals was weighed in the same manner. By calculating a ratio between the weight of the control pituitary and the body weight a curve was established to obtain the approximate normal pituitary weight. By subtracting the weight of the residual pituitary found at autopsy from this curve, the amount of tissue remaining after the partial hypophysectomy was calculated. The calculated weights were compared to the actual weights obtained for the residual pituitary. The adrenals were weighed. The thickness of the complete cortex and the zona glomerulosa was measured on paraffin sections using a micrometer screw eyepiece (Leitz) to establish the degree of organ activation.

The organs were embedded in paraffin and stained with haematoxylin-eosin and PAS, the pituitaries additionally with performic acid-alcian blue-PAS-orange G, according to Adams and Swettenham (1958). The specimens for electron microscopy were fixed for an additional 2 h by osmium tetroxyde in 3% buffered glutaraldehyde and then buffered in 0.1 molar cacodylate, pH 7.2–7.4. After embedding in Epon 812, semithin sections were prepared and stained with toluidine blue for study with the light microscope. Ultrathin sections were prepared from representative random samples on the Reichert ultramicrotome. Uranyl acetate and lead citrate were used as a contrast medium. Electron microscopy and photography were carried out on the EM 9 S 2 from Zeiss. The numerous photographs were evaluated according to a scheme with consideration of cell type, cell size and cell number as well as the structural organelle properties. The Chi-Quadrat-Fourfield test was used to indicate significance in the incidence of certain cell types as compared with the controls.

Results

Operated Animals

The rats which underwent a sham partial hyophysectomy lost weight in the first weeks after the surgery. Hereafter, the sham operated animals developed like the control animals while those that were partially hypophysectomized did not reach the size or weight of the controls. The degree of impaired development correlated somewhat with the size of the resection material.

Morphology of the Resected Pituitary

The measurement of the resected pituitary tissue showed that between 4–100% of the pituitary had been removed. By examination with the light microscope

pure anterior lobe tissue was found in 24 rats. Intermediate lobe and neurohypophyseal tissue was also removed in 8 rats.

Morphology of the Testes and Adrenals

In only one animal, who survived for one year, did the testes show reduction of spermiogenesis resembling that seen in hypogonadotrophic hypogonadism. Other animals demonstrated lesser degree of decreased spermiogenesis without changes in the basal membrane or Leydig cells. Many rats demonstrated unchanged spermiogenesis.

The adrenals of 20 rats showed a moderate or distinct increase of lipids in the zona fasciculata, so the cortex could be compared in part to the normal cortex of a female rat. Intense dissociated atrophy of the cortex was observed in 1 animal who survived for 1 year. The zona fasciculata and zona reticularis were very much thinner but the zona glomerulosa remained unchanged.

Morphology of the Residual Pituitary

The weight of the resected and residual pituitary was not greater than the weight of the pituitary in control animals of the same age. Macroscopic regeneration could not be detected.

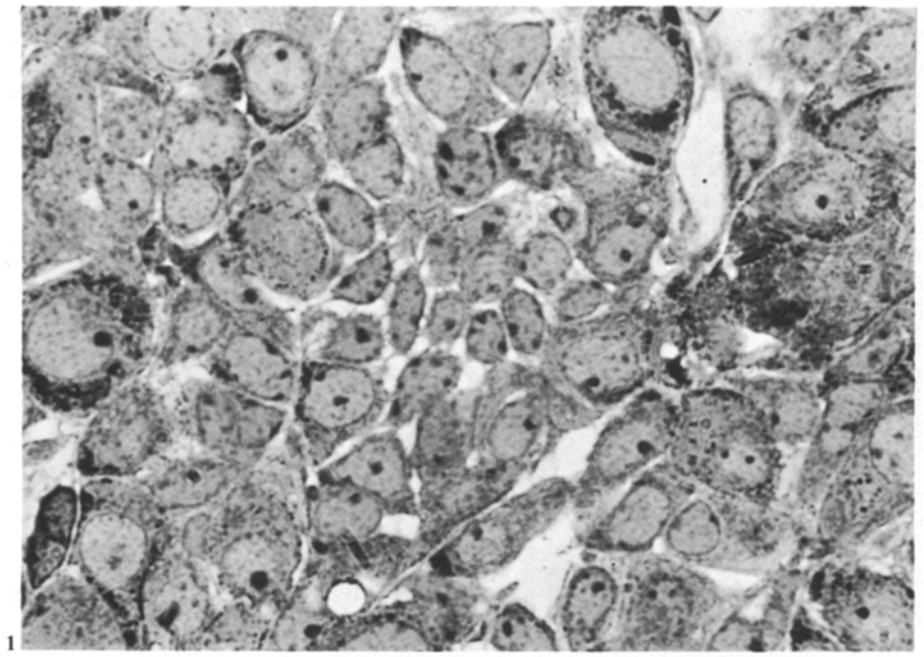
Anterior lobe, neurohypophyseal and intermediate lobe tissue was found throughout the residual pituitary by examination with the light microscope. Necrosis and fibrin exsudation was present in those animals sacrificed after a short time, while granulation tissue was observed in those killed after a longer period of time. Later scar tissue was also seen. Mitoses in the adjacent anterior lobe tissue were sporadic, however, they were not clearly increased after operation compared with the controls. Mature chromophil anterior lobe cells were inconspicuous. Small chromophobe stem cells were clearly increased compared with the controls. They formed cell nests in part (Fig. 1). Immature small chromophil cells were also plentiful.

Only the mature chromophil cells which could be identified without doubt were of interest for electron microscopic examination.

GH cells resembled both in relative number and in size GH cells in control animals. Golgi fields appeared to be smaller in the majority of the animals. The granular endoplasmic reticulum lay in parallel arrays around the nucleus while in the remaining cytoplasm it was fragmented.

Prolactin cells were identified by the characteristic structure of their secretory granules as well as by the typical arrangement of the rough endoplasmic reticulum. When an area of the same size were studied, they were found to be more plentiful in the residual pituitaries than in the controls. Their size remained unchanged (Fig. 2). The rough endoplasmic reticulum was more extensive and formed finger prints.

ACTH cells were recognized by cytoplasmic branches and a characteristic size and distribution of secretory granules. The ACTH cells in the operated



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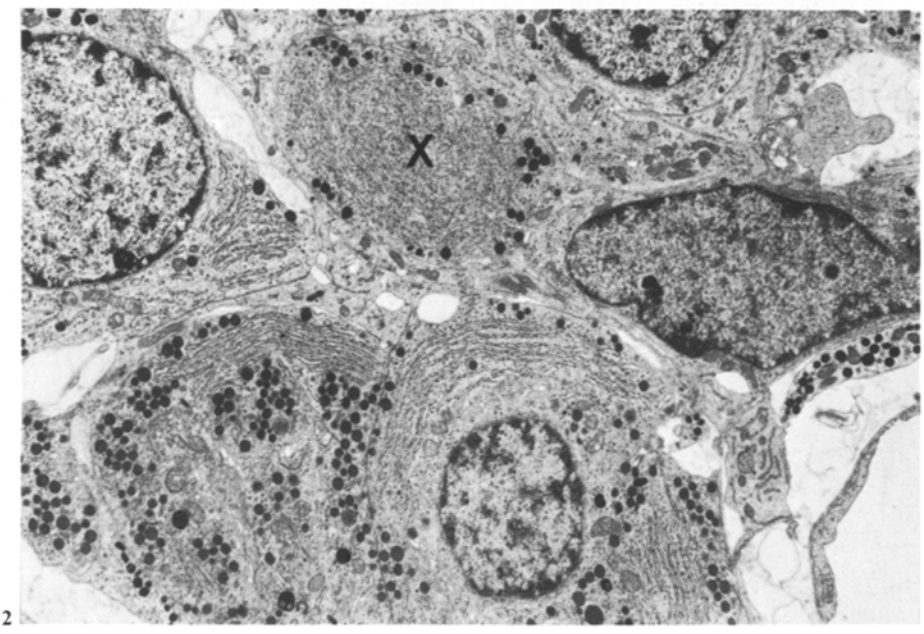

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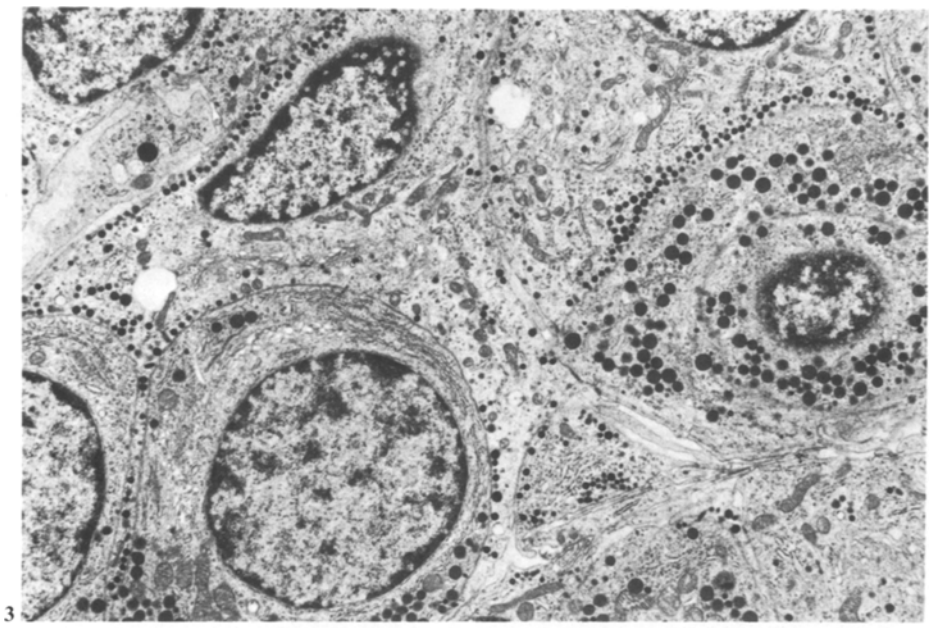
Fig. 1. Residual pituitary (14 days postoperative): in the middle is a nest of small chromophobe cells. Epon semithin section, toluidine blue stain, magnification 1,150 ×

Fig. 2. Prolactin cells (57 days postoperative): increased rough endoplasmic reticulum, formation of a fingerprint (X), regular secretory granules. Epon ultrathin section, uranyl acetate-lead citrate, magnification 5,700 ×



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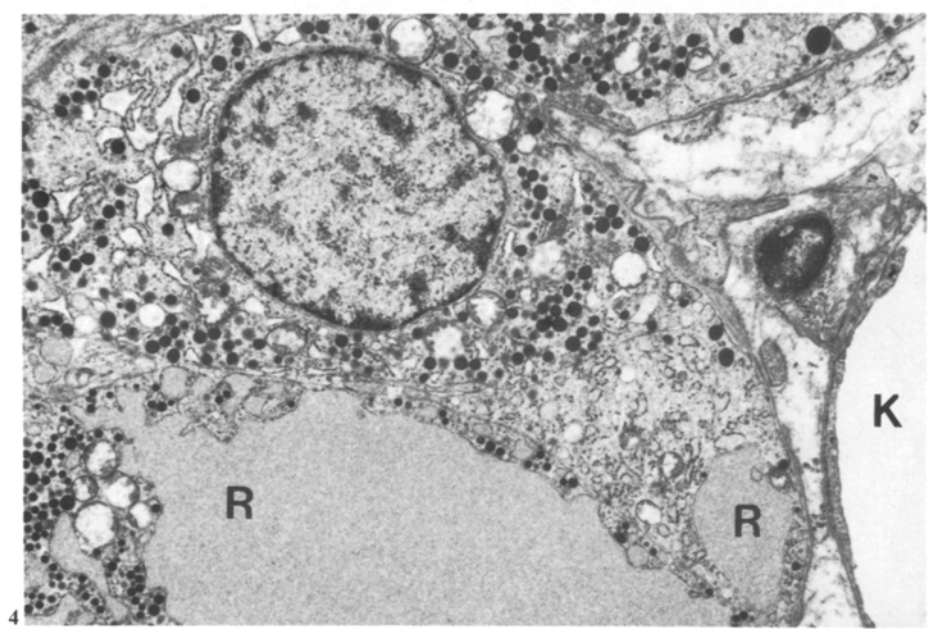

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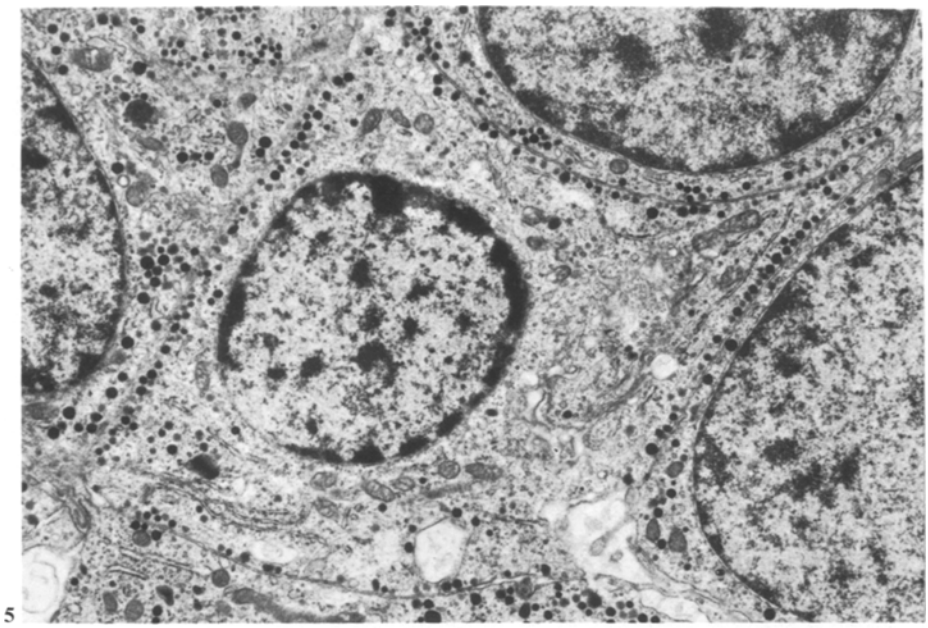
Fig. 3. ACTH cells (57 days postoperative): enlarged cytoplasm with long grasping processes, large Golgi fields with immature secretory granules, peripherally arranged small secretory granules, localized numerous microtubuli and cytofilaments. Epon ultrathin section, uranyl acetate-lead citrate, magnification 6,250 ×

Fig. 4. FSH cells (57 days postoperative): dilated rough endoplasmic reticulum, irregular electron dense secretory granules. An FSH cell is transformed into a castration cell through a high degree of dilation of the ergastoplasm (*R*). Cross-section of a capillary (*K*). Epon ultrathin section, uranyl acetate-lead citrate, magnification 7,360 ×



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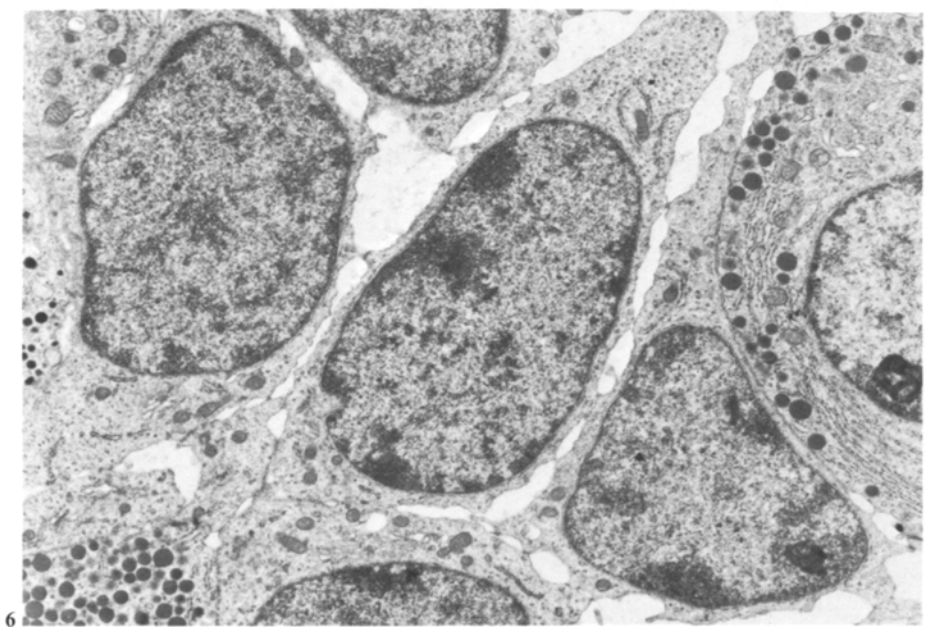

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Fig. 5. ICSH cells (57 days postoperative): enlarged cytoplasm, medium to large Golgi fields, peripherally arranged small to medium large secretory granules. Epon ultrathin section, uranyl acetate-lead citrate, magnification 9,350 ×

Fig. 6. A nest of small chromophobe cells (4 days postoperative): narrow cytoplasm without granules, very scanty rough endoplasmic reticulum, groups with polyribosomes. Epon ultrathin section, uranyl acetate-lead citrate, magnification 7,050 ×

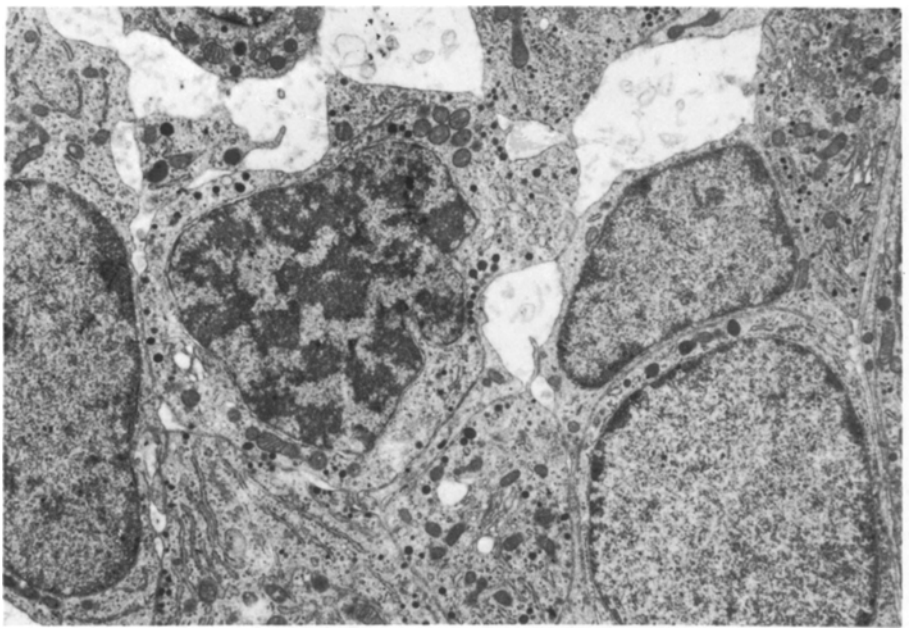

Fig. 7. Mitosis of an immature chromophil cell (84 days postoperative): chromatin condensation and beginning membrane disintegration of the nucleus, few secretory granules, on 2 cell poles distributed mitochondria. Epon ultrathin section, uranyl acetate-lead citrate, magnification 7,100×

animals appeared to be larger (Fig. 3) with longer cytoplasmic branches which might completely surround adjacent cells. The rough endoplasmic reticulum was not as a fragmented as in controls but had longer membranes. The Golgi fields appeared to be larger and the number of immature secretory granules increased.

TSH cells were rarely identifiable. Their ultrastructure was unchanged compared to the control animals. Their thin cytoplasm contained little rough endoplasmic reticulum and very small secretory granules.

FSH cells occurred in part as castration cells (Fig. 4) which can be identified by the lacuniform, dilated rough endoplasmic reticulum. They were also present in the control pituitaries with the same size and structure. FSH cells not fully transformed into castration cells were larger in the residual pituitaries than in the controls.

ICSH cells were observed only in small numbers. They occasionally demonstrated an enlarged cytoplasm (Fig. 5). Their organella structures did not differ from ICSH cells in the control animals.

The increase in *small chromophobe cells* in the residual pituitary was statistically significant ($p < 0.05$) when compared with the controls. They were often present in nests (Fig. 6). The individual cells were not different in structure from those of controls.

An increased number of *immature chromophil cells* was observed in the residual pituitaries (Fig. 7) with cytoplasm that was approximately twice as wide

as in the small chromophobe cells. They contained a relatively large number of free ribosomes, small Golgi fields and a varying number of secretory granules. Classification was not possible.

Near to the intermediate lobe numerous *follicle cells* were observed which contained microvilli, cilia and some desmosomes. The nuclear matrix was irregular but otherwise they were very similar to the small chromophobe cells.

Discussion

Effect on the Animal

A clear correlation between the amount of tissue removed and the growth of the animal has already been established in rats where 40% of the adenohypophysis has been removed. However, Smith (1932) found an impaired growth only when more than 70% of the anterior lobe tissue had been resected. We were unable to demonstrate ultrastructural alterations of the remaining intact GH cells.

Some of the operated animals showed an increase of lipids in the zona fasciculata of the adrenal cortex which indicates reduced ACTH stimulation (Mitschke and Saeger, 1975).

Spermatogenesis was reduced in those animals where more than 80% of the pituitary was removed. This result corresponds to the findings of Smith (1932).

Regeneration

Obvious regeneration of the anterior hypophysis did not occur. The total weight of the residual pituitary and the removed pituitary was not larger in any animal than the pituitary weight in the controls of the same size although additional scar tissue in the operation area was included. At no stage after the operation was an enlargement of the gland observed which could not be attributed to total body growth. This is in agreement with the results of Weinbren and Fitschen (1959) and in contrast to the findings of Reiss et al. (1937) and Crooke (1938).

An increase of small chromophobe cells, so called stem cells, was observed by examination with the light and electron microscope. However, since they did not lead to an anatomical restoration of the gland even in those animals which remained alive for a long period of time, this change can at best be referred to as a regeneration attempt.

Degree of Stimulation of the Residual Pituitary

The relative number of prolactin cells was increased. ACTH cells demonstrated an increase in the rough endoplasmic reticulum and enlarged Golgi areas. FSH

cells showed dilatation of the rough endoplasmic reticulum up to the extent found in castration cells.

These results suggest an increased stimulation or a decreased suppression of the glandular tissue remaining after the operation. We regard this as an attempt of the pituitary function to adapt to the requirements of the organism following partial hypophysectomy. Consequently, this hyperactivity should be designated as a "functional regeneration".

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