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ACTH-induced ultrastructural changes in the zona fasciculata of the hamster adrenal cortex. Are intraadrenal thrombi regulators of corticosteroid secretion?

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Summary. Correlated stereological and functional studies were performed on the effect of massive ACTH doses on adrenal cortex of the female hamster.

ACTH resulted in a marked increase in adrenal gland weight at day 6 of treatment followed by a drop at day 9. Stereology showed significant enlargement of the zona fasciculata (ZF) cells with the highest value at day 6 and subsequent drop at day 9 of treatment. This hypertrophy was due to a notable increase in the volume of mitochondrial, SER, Golgi apparatus and lipid droplet compartments. Cortisol secretion by adrenal slices and homogenates was also highest at day 6 of ACTH administration and notably lower at day 9.

At day 6 of ACTH treatment in outer ZF thrombi were seen. In their vicinity the subendothelial space was dilated and endothelial cells dissociated from the basal lamina. Numerous erythrocytes were also visible among dissociated ZF cells. At day 9 of experiment in outer part of ZF numerous spaces devoid of parenchymal cells appeared.

The earlier authors considered the «empty spaces» or «holes» in hyperstimulated adrenal cortex as a sign of holocrine secretion of steroid hormones. The present findings enable us to introduce a new hypothesis on the development of these spaces. In our opinion in hyperstimulated adrenal cortex numerous thrombi may be formed leading thus to the degeneration of adrenocortical cells. Thus, the appearance of the «empty spaces» or «holes» in the gland is not connected with the holocrine secretion but with the regulation of the number of secretory cells in adrenal cortex by the thrombi-dependent mechanism. A similar mechanism may also be responsible for the remodelling of the gland after its enlargement due to ACTH administration.

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Introduction

Prolonged ACTH administration induces notable enlargement of the adrenal gland which is due to hypertrophy and hyperplasia of adrenocortical cells (for review see Nussdorfer et al., 1971; Belloni et al., 1978; Malendowicz, 1986; Nussdorfer, 1986; Müller et al., 1987; Malendowicz and Dembinska, 1990; Stachowiak et al., 1990). Such a treatment results in a marked increase in synthesis of structural and enzymic proteins, also including steroidogenic enzymes, and these changes are reflected by parallel changes in the surface area per cell of SER membranes and mitochondrial cristae in which these enzymes are contained (Nussdorfer and Mazzocchi, 1983; Nussdorfer, 1986). However, secretory activity of the adrenal cortex of animals treated chronically with massive doses of ACTH is the matter of discussion. Of interest are reports of some authors observing that prolonged corticotropin administration lowers corticosterone output by the gland, an effect which may depend on desensitization (or tachyphylaxis) of the cells to ACTH (Flack, 1970; Rani et al., 1983; Vreeburg et al., 1984; Malendowicz, 1986). However, the presence of other intraadrenal mechanisms regulating corticosteroid output by stimulated adrenal cortex must be taken into consideration. This includes not only the histological organization of the gland but also the innervation and vascular supply of the cortical cells (Hinson et al., 1990). In the present study we report data suggesting that intraadrenal thrombi may regulate the number of adrenocortical cells leading thus to lowering of the secretory activity of the gland.

Materials and methods

Animal treatment

Adult female hamsters *(Mesocricetus auratus* Waterhouse) were employed in this study. Animals were maintained under standardized conditions of light (14L:10D) and temperature (22 + 2° C) and fed with laboratory pellets with free access to tap water. Animals were administered subcutaneously with daily dose of 50 μg ACTH (Synacthen Depot, Ciba) for 3, 6 and 9 days except control group. Hamsters were sacrificed by decapitation, trunk blood collected (with 1 mg EDTA/ml), plasma separated and stored at -20° C until cortisol determination. Adrenals were promptly removed, freed of adherent adipose tissue and weighed.

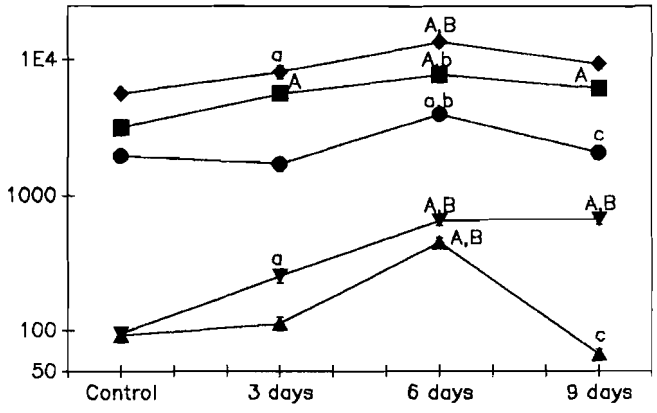

Fig. 1. The effect of ACTH treatment on the morphometric parameters of the zona fasciculata cells of the hamster adrenal cortex. ◆ Average volume of cell (μm³); average volume (μm³/cell) of the: • mitochondrial compartment; ▼ lipid droplet compartment: ▲ Golgi apparatus compartment; ■ smooth endoplasmic reticulum compartment. Other explanations as in Table 1.

Ultrastructural and stereological studies

Fragments of the left adrenal gland were used for electron microscopy. They were sliced and immediately fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.4, and embedded in epoxy resin. Thin sections were counterstained with lead-hydroxide and observed in a JEM-100C electron microscope. Thick sections of the zona fasciculata were stained with methylene blue and on these sections stereological studies were performed. At a magnification of × 1050, using the eye-piece with a square lattice test system of type A81 (Weibel, 1979), the volume densities of nuclei and cytoplasm of parenchymal cells and stroma were estimated. Simultaneously the numerical density of parenchymal cell nuclei was counted. The number of nuclei per mm^3 of tissue was computed by the method of Weibel and Gómez (Weibel, 1979), as described in detail elsewhere (Nikicicz et al., 1984). For each adrenal

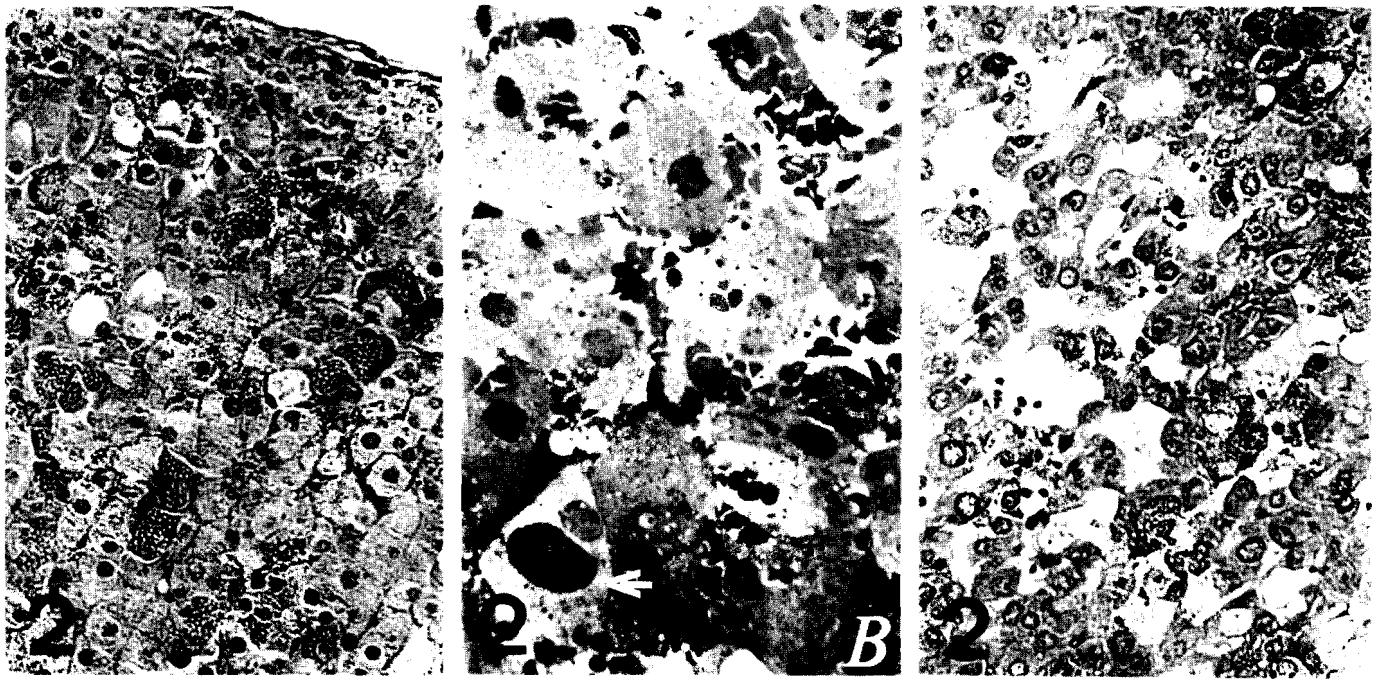

Fig. 2. Adrenal cortex of ACTH-treated hamster. **A.** At day 6 of experiment in the hypertrophied gland a notable dilation of the blood vessels and numerous thrombi are seen. H & E. Magnification approx. × 200. **B.** Semithin section of the outer zona fasciculata at day 6 of ACTH administration. Among dissociated adrenocortical cells numerous erythrocytes are seen. In cytoplasm of some adrenocortical cells methylene-blue-stained bodies (arrow) of various size are observed. Methylene blue. Magnification approx. × 1,200. **C.** On day 9 of ACTH treatment in the outer zona fasciculata numerous «empty spaces» or «holes» are visible. They contain fibrinaceous material in which polymorphonuclear granulocytes are embedded. H & E. Magnification approx. × 200

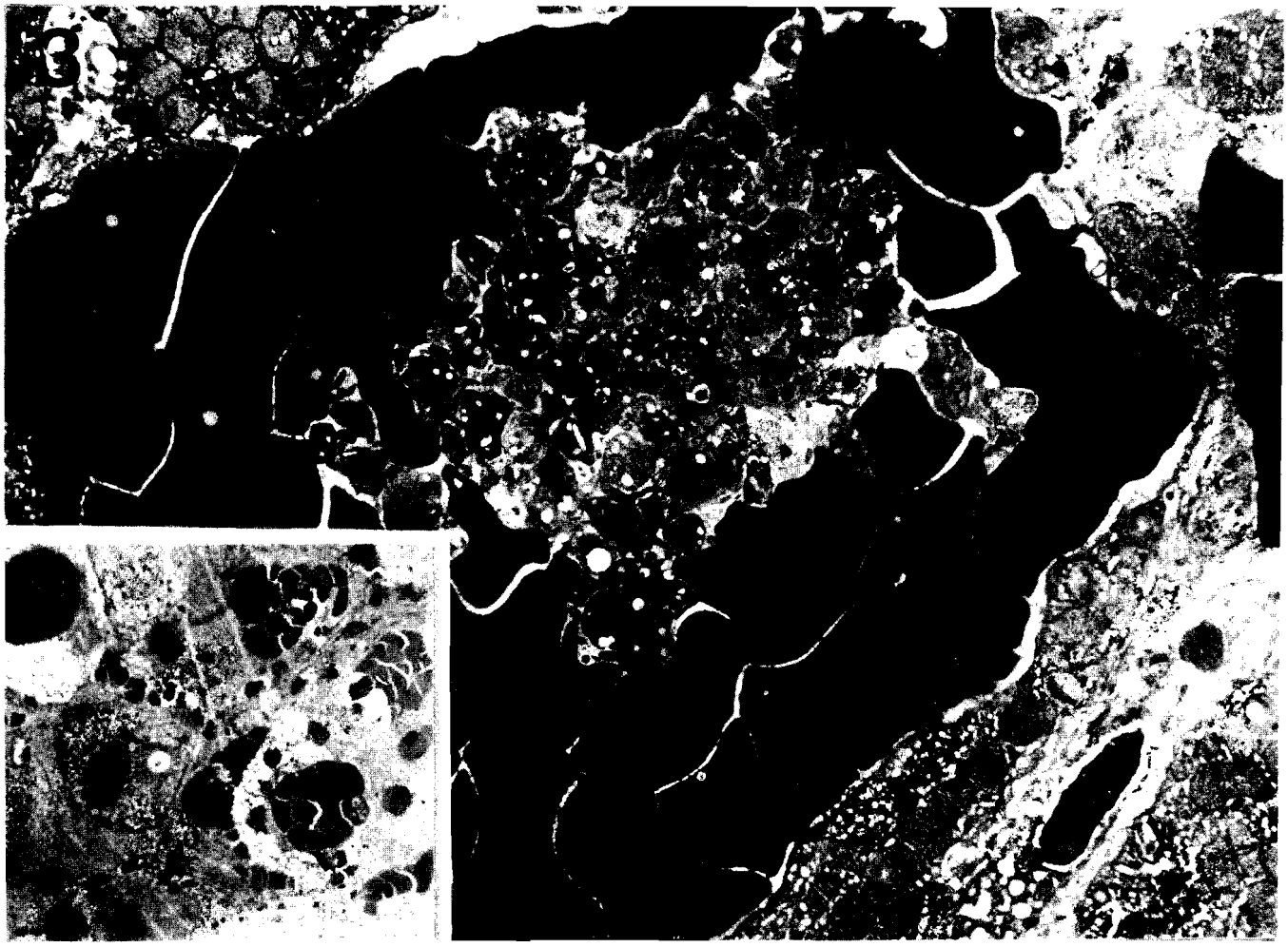

Fig. 3. Adrenal cortex of ACTH-treated hamster. On day 6 of experiment in the outer part of the zona fasciculata thrombi are formed. Their core is composed of thrombocytes containing typical granules. Magnification approx. × 6,500. Insert: semithin section of the outer zona fasciculata with thrombi and erythrocytes localized among dissociated adrenocortical cells. Methylene blue. Magnification approx. × 1,200

Table 1. The effect of long-term ACTH administration on adrenal gland weight, adrenal cortisol content and serum cortisol level in female hamster. Results expressed as means ± SE.

	Control (a)	3 days (b)	6 days (c)	9 days (d)
Adrenal weight [mg]	20.1 ± 1.9	22.6 ± 2.6	40.1 ± 4.9^{A,B}	29.2 ± 1.8^{C}
Cortisol content [ng/adrenals]	85.9 ± 15.5	121.3 ± 44.4	138.8 ± 25.0	106.7 ± 12.6
Plasma cortisol [nmol/l]	19.5 ± 4.9	16.9 ± 2.1	25.5 ± 3.8	17.5 ± 2.0

Number of animals in each group n = 6. Statistical evaluation of the results by ANOVA followed by the multiple range test of Duncan (groups marked in parentheses as a,b,c, and d) - small letters $p < 0.05$; capital letters $p < 0.01$.

zona fasciculata 4-8 randomly chosen electron micrographs at a final magnification of × 20,000 were selected. On these electron micrographs the V_v of mitochondria, membrane space (SER), Golgi apparatus and lipid droplets were estimated by differential point counting, using a test system A100 (Weibel, 1979).

Adrenal homogenate and slice assays

The remaining part of the left adrenal gland was sliced into small pieces, preincubated for 15 min at 37° C in Krebs-Ringer bicarbonate buffer with 0.2% glucose (KRBG). The medium was discarded and slices incubated for 60 min in KRBG with 0.4% bovine serum albumin (fraction V, Sigma). The right adrenal gland was homogenized in 0.154 M KCl, spun at 2,000g for 10 min and the supernate was incubated as described previously (Malendowicz, 1976). All incubations were performed at 37° C with continuous shaking at 50 rpm. Cortisol content was determined in

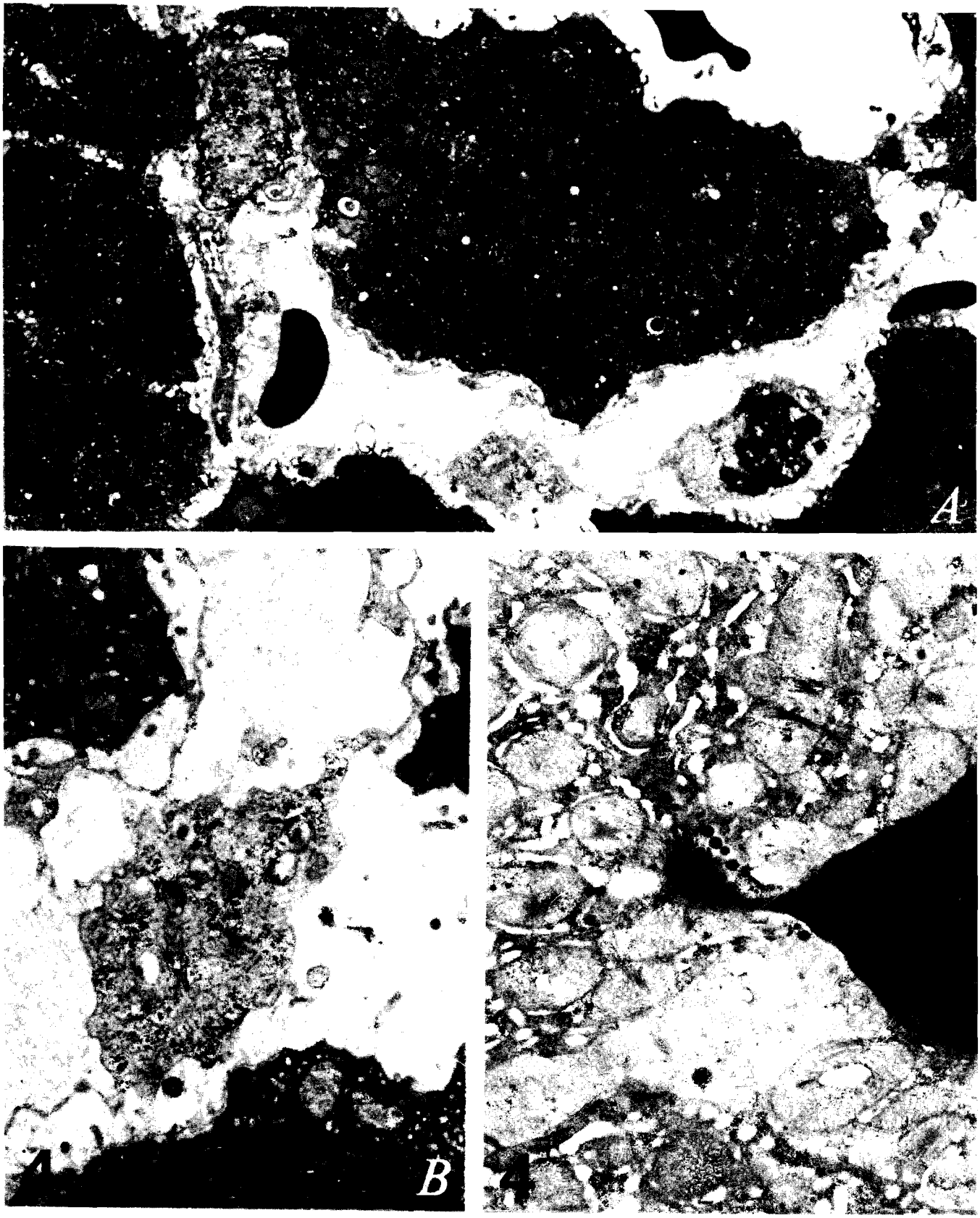

Fig. 4. Zona fasciculata of the hamster adrenal cortex at day 6 of ACTH administration. **A.** The dilated subendothelial space containing fibrinaceous material is seen. Some of endothelial cells dissociated from the basal lamina and among adrenocortical cells an erythrocyte is seen (the upper right corner). Magnification approx. × 5,000. **B.** Higher magnification showing endothelial cell dissociated from the basal lamina. Magnification approx. × 12,500. **C.** Zona fasciculata cell containing small, round bodies and a fragment of invaginated erythrocyte. A marked dilation of SER cisternae. Magnification approx. × 15,000

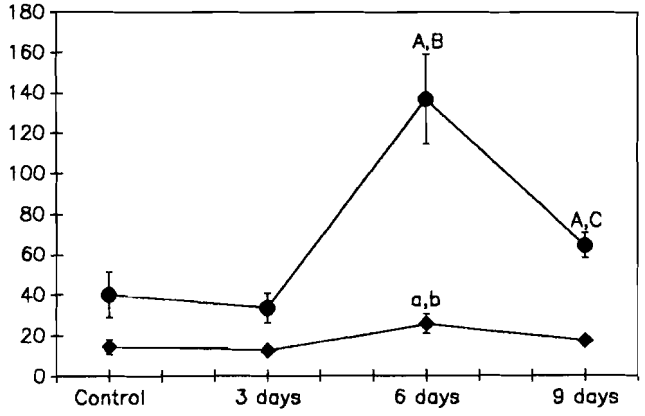

Fig. 5. The effect of ACTH treatment on cortisol secretion by adrenal slices ◆ or homogenate • (μg/adrenals/60 min). Other explanations as in Table 1.

unincubated supernate and cortisol output by adrenal homogenate was expressed as difference in cortisol concentration between incubated and nonincubated samples.

Cortisol determination

Cortisol in plasma and incubation medium was quantified, without prior extraction, by RIA with RIA cortisol (125I) kit of Farmos Diagnostica (Finland). With the kit, cross-reactions with progesterone and corticosterone were < 0.01% and < 2% respectively and interassay variation 4%.

Statistical treatment of the results

The data obtained from each animal were averaged per experimental group and the standard error was calculated. Comparisons among the experimental groups were performed by ANOVA and the multiple range test of Duncan.

Results

ACTH administration resulted in a marked increase in adrenal gland weight at day 6 of treatment followed by a drop at day 9 of experiment (Table 1). Stereology showed that ACTH-induced adrenal growth was associated with significant enlargement of the zona fasciculata cells with the highest value at day 6 and subsequent drop at day 9 of treatment (Fig. 1). This hypertrophy was due to a notable increase in the volume of mitochondrial, SER, Golgi apparatus and lipid droplet compartments.

At day 6 of ACTH treatment, in the outer part of the adrenal cortex a notable widening of the blood vessels was observed and in some regions of the cortex thrombi were seen (Fig. 2). The central part of the thrombus contained clusters of thrombocytes surrounded by changed erythrocytes (Fig. 3). In other regions of the gland subendothelial space was dilated and endothelial cells dissociated from the basal lamina (Fig. 4). In some areas the continuity of endothelium was disrupted and erythrocytes were seen among dissociated adrenocortical cells. Some of them were invaginated into the cytoplasm of adrenocortical cells giving an impression of the appearance of acidophilic bodies in the cytoplasm of parenchymal cells (Fig. 2). In the cytoplasm of some adrenocortical cells small, round bodies appeared and their electron density was comparable to that of erythrocytes. Part of the adrenocortical cells contained dilated cisterns of SER (Fig. 4).

In the outer part of the zona fasciculata at day 9 of ACTH treatment numerous spaces devoid of parenchymal cells appeared. In these spaces, embedded in fibrinaceous material, polymorphonuclear granulocytes were seen (Fig. 2).

ACTH administration had no effect on plasma cortisol concentration and adrenal cortisol content (Table 1). On the other hand, ACTH resulted in a marked increase in cortisol secretion by adrenal slices and homogenates, with the highest values at day 6 and subsequent drop at day 9 of experiment (Fig. 5).

Discussion

Results of the present study confirm the earlier findings on the thropic effect of ACTH on the adrenal cortex of various animal species. This effect depends mainly on enlargement of parenchymal cells which is principally due to the hypertrophy of mitochondrial and SER compartments and parallelled by notable increase in the surface area of mitochondrial cristae and SER membranes (Nussdorfer et al., 1971; Canick and Purvis, 1972; Nussdorfer and Mazzocchi, 1983; Miskowiak et al., 1986; Nussdorfer, 1986; Müller et al., 1987; Malendowicz and Dembinska, 1990). As revealed in the present study, the highest hypertrophy of the zona fasciculata cells was found at day 6 of ACTH administration; however, with prolonged injections of massive doses of ACTH on day 9 of experiment a notable lowering of the studied stereological parameters was found.

The above mentioned changes were parallelled by changes in cortisol output by adrenal slices and homogenates with the peak at day 6 and a subsequent drop at day 9. Similar decline in corticosterone output by adrenals after prolonged corticotropin administration was also reported by other authors (Flack, 1970; Rani et al., 1983; Vreeburg et al., 1984; Malendowicz, 1986) and this effect may depend on desensitization of adrenocortical cells to ACTH. However, in the present study in adrenal cortex of ACTH-treated hamsters numerous thrombi and extravasations of erythrocytes were seen at day 6 of treatment and in the same regions of the gland the spaces devoid of parenchymal cells were visible at day 9 of treatment. Thus the thrombi appeared before regressive changes took place in the adrenal cortex. Also, Pudney et al. (1984), in adrenal cortex of

ACTH-administered rat, described numerous erythrocyte extravasations. At light microscope level, in stimulated adrenal cortex, the earlier authors frequently described the «empty spaces» or «holes» devoid of parenchymal cells, which were mainly visible in the outer part of the cortex. It was generally agreed that these spaces resulted from the holocrine secretion of steroid hormones (for review see Bachmann, 1954; Piroth, 1961; Mietkiewski et al., 1966). This hypothesis was readvanced by ultrastructural findings of (Nussdorfer, 1986), although now it has only historical value. The present findings enable us to introduce a new hypothesis on the development of the «empty spaces» or «holes» in the cortex of stimulated adrenal gland as well as on their physiological significance. In our opinion in hyperstimulated adrenal cortex numerous thrombi may be formed, and the formation of thrombi leads to the degeneration of adrenocortical cells. Thus, the appearance of the «empty spaces» or «holes» in the gland may not be connected with holocrine secretion, but with the regulation of the number of secretory cells in the cortex by the thrombi-dependent mechanism. The mechanism in which thrombi are involved may also be responsible for remodelling of the gland after its enlargement due to prolonged ACTH administration.

A similar mechanism of thrombi formation may be involved in the luteolysis in superstimulated ovaries of immature hamsters in which they may contribute to the onset of luteolysis by a temporary ischemia (Spanel-Borowski and Heiss, 1986).

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