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Correlated Morphometric and Autoradiographic Studies of the Effects of Corticosterone on Adrenocortical Cells of Intact and Hypophysectomized ACTH-Treated Rats

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Summary. The effects of corticosterone on adrenocortical cells of intact and hypophysectomized ACTH-treated rats were investigated by morphometric and autoradiographic methods. The data obtained in these experiments allow us to make the following conclusions:

1) The most important morphological parameter for assessing the activity of a steroid-secreting cell is the quantity of smooth endoplasmic reticulum.

2) The decrement in the smooth reticulum in adrenocortical cells of rats treated with corticosterone is due to an inhibition of the protein synthesis by the hormones themselves.

3) There is *in vivo* a direct negative feed-back control mechanism at the adrenal level, mediated by an inhibition of the RNA synthesis by the corticosteroid-hormones.

4) The trophism of the mitochondrial fraction of adrenocortical cells is controlled by ACTH. It is possible to hypothesize that ACTH intervenes in the regulation of the mitochondrial RNA and DNA synthesis.

Key-Words: Adrenal cortex — Corticosterone — Autoradiography — Morphometry.

Previously (Nussdorfer, 1970b) it was reported that the quantitative ultrastructural changes in adrenocortical cells of prednisolone-treated rats consist mainly of a significant decrease in the quantity of the smooth endoplasmic reticulum. This decrease is responsible for about 50% of the decrement in cell volume and of the adrenal hypotrophy, occurring after treatment of animals with prednisolone. It was suggested that the prednisolone inhibits the synthesis of enzymes and structural proteins in adrenocortical cells.

In order to verify this hypothesis and to find whether the action of the corticosteroids on adrenocortical cells is only indirect (through the inhibition of the hypothalamo-hypophysial axis) or also direct, morphometric and autoradiographic studies were carried out on the effects of corticosterone on the adrenocortical cells (zona fasciculata) of both normal and hypophysectomized ACTH-treated rats. The corticosterone is the most important hormone secreted by the rat adrenal cortex (Hofmann *et al.*, 1954; Lowy *et al.*, 1969).

Materials and Methods

Treatment of Animals. Forty male albino rats (Sprague-Dawley derived), weighing about 200 g, were divided into 4 stocks. One stock served as a control, the others received intraperitoneal injections of 20 mg/kg of corticosterone (Sigma) for 1, 2 and 4 consecutive days,

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respectively. Three hours before being sacrificed, each stock was subdivided into two subgroups, which were given intraperitoneal injections of 100 μc of 3H-orotate and 3H-leucine (New England Nuclear Corp.), respectively.

Forty male albino rats (Sprague-Dawley derived), weighing about 200g, were hypophysectomized by the parapharyngeal approach. Up to the day before the sacrifice the animals were treated with maintenance doses of ACTH (Sigma) (10 I.U./kg). At the 7th day after operation the animals were divided into 4 stocks. One stock served as a control, the others were given intraperitoneal injections of 20 mg/kg of corticosterone (Sigma) for 1, 2 and 4 consecutive days, respectively. Three hours before the sacrifice, each stock was subdivided into two subgroups, which received intraperitoneum 100 μc of 3H-orotate and 3H-leucine (New England Nuclear Corp.), respectively.

The animals were sacrificed by cervical dislocation, in such a way to induce the least possible stress.

Electron Microscopy. The adrenal fragments were fixed in 5% glutaraldehyde in cacodylate buffer (Sabatini *et al.*, 1963), post-fixed in 1% $Os0_4$ in phosphate buffer (Millonig, 1961) and embedded in D.E.R., according to Lockwood (1964). The zona fasciculata was examined on thick sections. At this level adjacent thin sections were cut with LKB I and III Ultrotomes. The thin sections were counterstained by Karnovsky's (1961) or Reynolds' (1963) method and observed in a Hitachi HU 11 electron microscope, at a direct magnification varying between × 800 and 60,000.

Morphometry. From each rat 3 tissue blocks, selected at random, were examined. The % cellular volume occupied by mitochondria, lipid droplets and smooth reticulum membranes (membrane space) was estimated on prints at a magnification of × 18,000 (80 micrographs/stock) with methods of differential point counting (Weibel, 1969; Weibel *et al.*, 1966, 1967). The membrane profile concentration (i.e. μ^2 of smooth reticulum membranes including Golgi apparatus/μ^3 of cell) was measured by the crossing method (Loud, 1962; Saltikoff, 1958) on prints at a magnification of × 40,000 (80 micrographs/stock). The absolute amount of organelles in the individual adrenocortical cell was calculated by determining the average cellular volume. This value was obtained with methods analogous to those described a previous by (Nussdorfer, 1970b). The average number of mitochondria per cell was estimated by the method of Weibel *et al.* (1969). The volume/surface ratio of mitochondria was measured by Chalkley's method (Chalkley *et al.*, 1949; Cornfield *et al.*, 1951).

Autoradiography. Five series of 0.5/1 μ thick sections, were made with LKB III Ultrotomes at the level of the zona fasciculata of all rats in each stock. The specimens were stained with 1% toluidine blue according to Trump *et al.* (1961). The thick sections were coated with carbon in order to prevent the background (Nussdorfer *et al.*, 1969). The specimens were then coated by dipping them into Ilford L4 liquid emulsion and were left to incubate in a desicator for 25 days at 4° C. The sections were developed in Microdol Kodak for 5 min. and fixed in 20% sodium thiosulphate for 5 min. The incorporation of the tracers was calculated by the method of the "mean grain count".

Statistical Treatment of Results. The quantitative data obtained from each experimental group were averaged and a calculation was made of the standard deviation from the mean (Standard Error = ± SE). In order to compare the mean values of different stocks, Student's test was used. The difference between two mean values was considered significant if the probability of error (P) was found to be less than 0.05.

Results

Effects of Corticosterone on Adrenocortical Cells of Intact Rats

The cell volume decreases significantly in relation to the duration of corticosterone treatment (Table 1). The nuclear diameter and volume also decrease significantly during the period of hormone administration (Table 1). Moreover, the nuclei of adrenocortical cells of the treated animals show a darker colouring and a more irregular form than those of the control rats. The ultrastructural

Table 1. *Synopsis of morphometric parameters of adrenocortical cells of intact rats after administration of corticosterone*

Duration of Treatment	Control	1 day	2 days	4 days
Volume of cells				
μ^3	2,000 ± 78.5	1,451 ± 58.7	1,180 ± 50	972 ± 40.2
$-\Delta v$ (%)		27.5	40.7	51.2
P		0.01	0.005	0.005
Volume of nuclei				
μ^3	187.3 ± 7.1	144.5 ± 5.2	120.1 ± 3.1	117 ± 1.8
$-\Delta v$ (%)		22.9	35.9	37.6
P		0.05	0.01	0.01
Diameter of nuclei				
μ	7.1 ± 0.284	6.5 ± 0.207	6.12 ± 0.124	6.07 ± 0.072
$-\Delta l$ (%)		8.5	13.8	14.5
P		0.05	0.01	0.01
Volume of mitochondrial fraction				
μ^3	623 ± 31.2	439 ± 24.1	390 ± 20.7	301 ± 18.8
$-\Delta v$ (%)		29.5	37.4	51.7
P		0.01	0.01	0.005
Volume of lipid fraction				
μ^3	220 ± 14.2	141 ± 11.5	91.7 ± 9.1	68.4 ± 5.3
$-\Delta v$ (%)		36	58.3	69
P		0.005	0.005	0.001
Membrane space				
μ^3	979.8 ± 39.1	726.8 ± 25.7	578 ± 22.2	485.6 ± 19
$-\Delta v$ (%)		25.8	41	52.5
P		0.05	0.01	0.01
Surface of smooth reticulum				
μ^2	10,481 ± 441	7,745 ± 329	6,382 ± 301	5,013 ± 237
$-\Delta s$ (%)		26.2	39.2	52.2
P		0.05	0.01	0.01

Table 2. *Synopsis of morphometric parameters of adrenocortical mitochondria of intact rats treated with corticosterone*

Duration of treatment	Control	1 day	2 days	4 days
Number per cell	668 ± 20.2	575 ± 15.8	530 ± 14.9	472 ± 13.8
P		0.05	0.05	0.01
Diameter (μ)	1.201 ± 0.018	1.140 ± 0.013	1.121 ± 0.011	1.054 ± 0.009
P		0.05	0.05	0.01
Volume (μ^3)	0.914 ± 0.062	0.768 ± 0.049	0.735 ± 0.041	0.622 ± 0.040
P		0.05	0.05	0.01
Surface to volume ratio	1.383 ± 0.040	1.487 ± 0.049	1.518 ± 0.051	1.601 ± 0.064
P		0.05	0.01	0.01

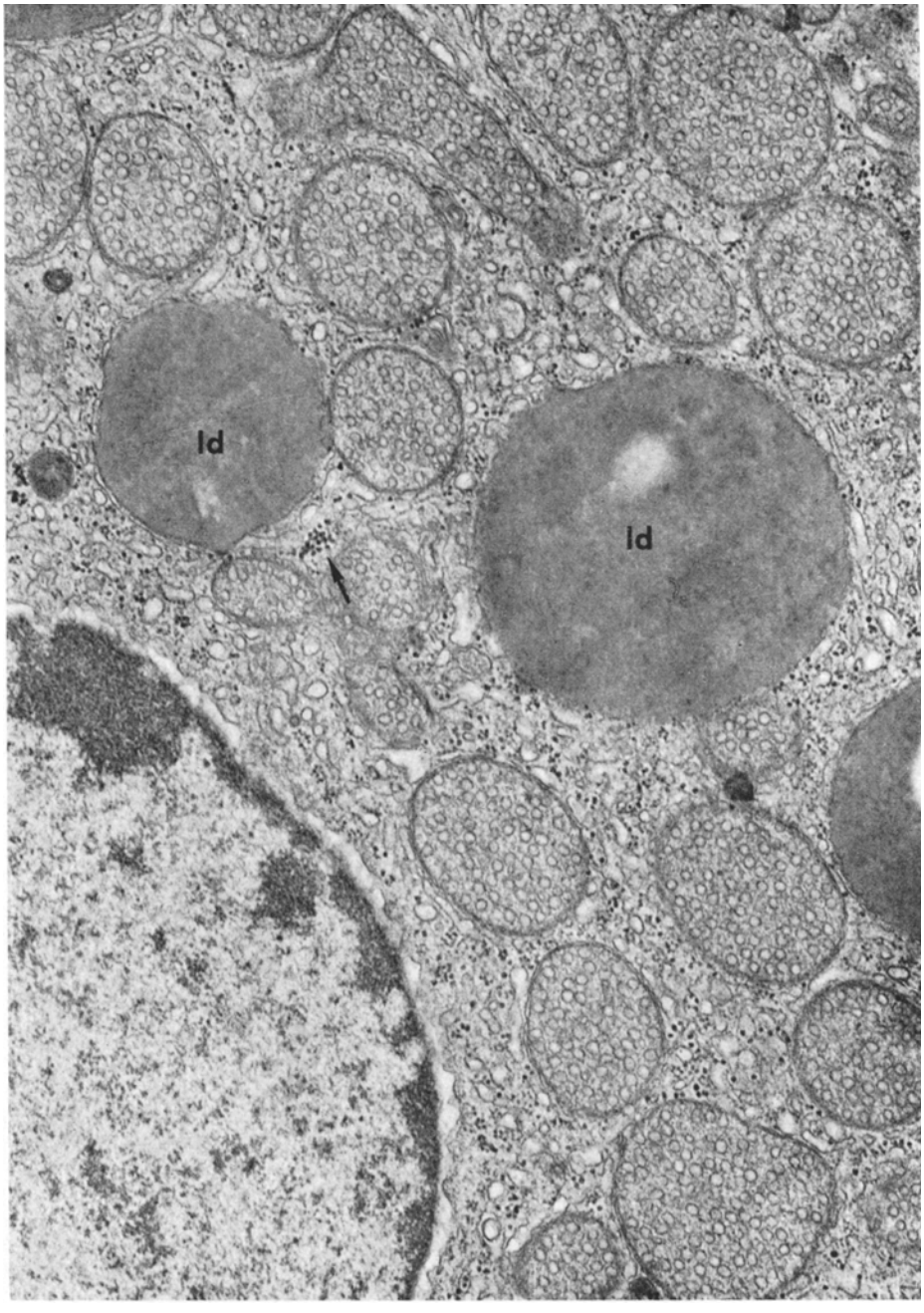


Fig. 1. Adrenal cortex of intact rat. Cell of the zona fasciculata. The smooth endoplasmic reticulum and the lipid droplets (*ld*) are very abundant. Very scarce is the intracellular storage of glycogen (arrow). × 20,000

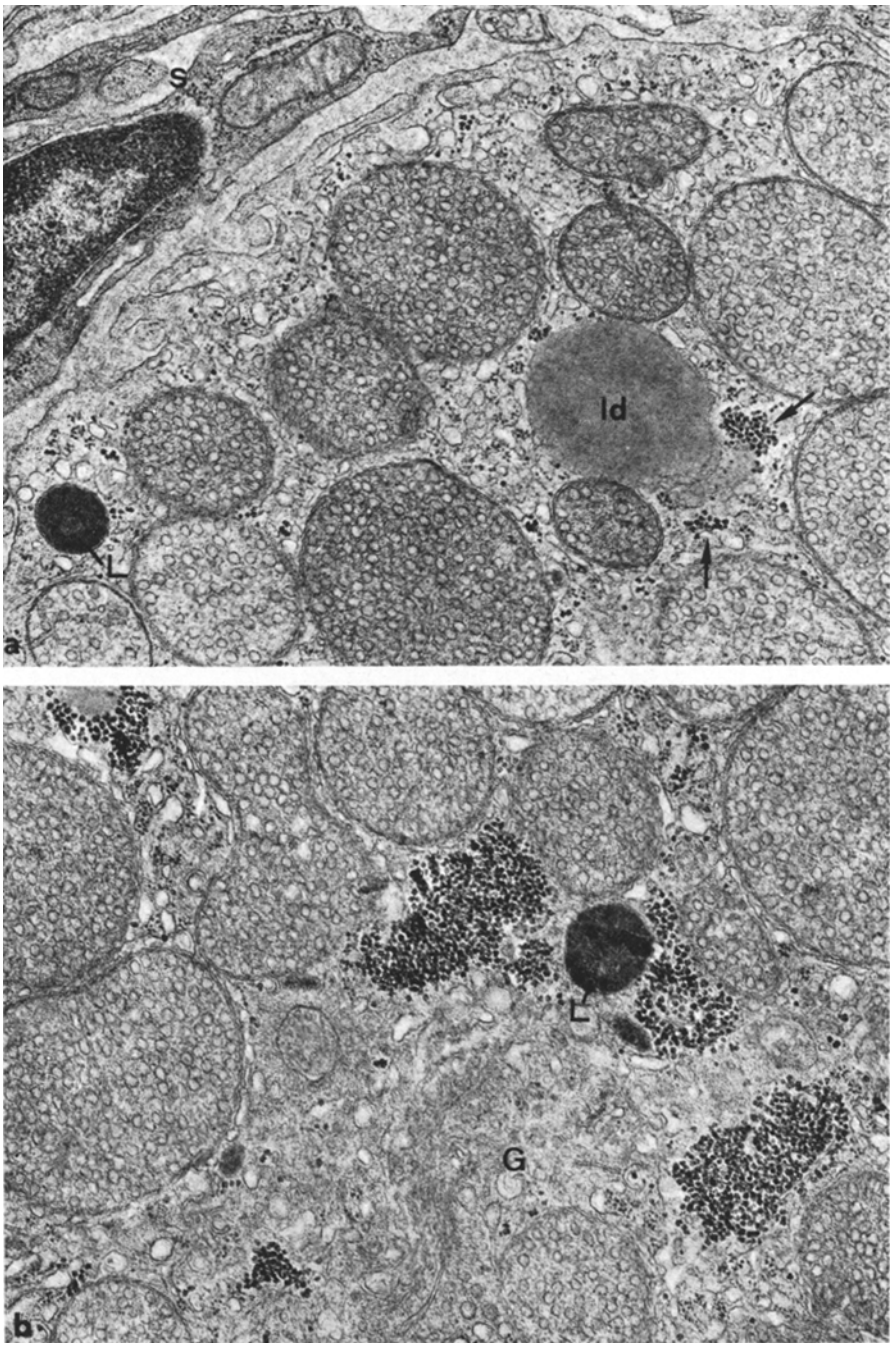
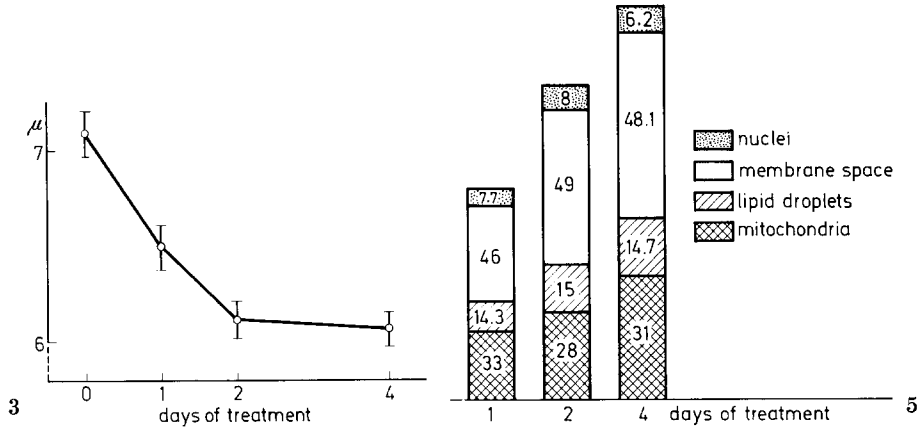


Fig. 2. a Adrenal cortex of intact rat after 2 days of treatment with corticosterone. Cell of the zona fasciculata. The cell shows no ultrastructural qualitative differences in comparison with the analogous elements of the control animals, except for a slight accumulation of glycogen (arrows). *S* sinusoid; *ld* lipid droplet; *L* lysosome. × 20,000. b The intracellular storage of glycogen is much more conspicuous in adrenocortical cells of intact rats after 4 days of corticosterone treatment. *G* Golgi apparatus; *L* lysosome. × 20,000



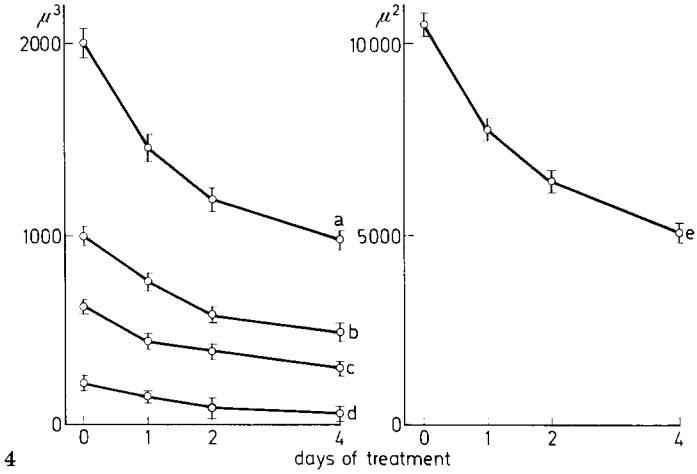


Fig. 3. Changes in diameter of nuclei of adrenocortical cells of intact rats induced by corticosterone treatment. Standard errors are indicated

Fig. 4. Changes in morphological parameters of adrenocortical cells of intact rats as function of the number of days of corticosterone treatment. Standard errors are indicated. *a* Volume of cells; *b* membrane space; *c* volume of mitochondrial fraction; *d* volume of liposomes; *e* surface of smooth endoplasmic reticulum

Fig. 5. Histogram showing the total decrements in volume of adrenocortical cells of intact rats after 1, 2 and 4 days of corticosterone treatment. The percentages of the total decrement due to the decrease in mitochondria, liposomes, membrane space and nuclei are indicated

features of the adrenocortical cells reveal no qualitative differences, except for a slight storage of glycogen in the adrenocortical cells of the treated animals (Figs. 1, 2a–b).

The volume of the mitochondrial fraction decreases slightly, but significantly, in relation to the duration of treatment (Table 1). Also the mitochondrial diameter, volume, and number of mitochondria per cell decrease with the number of days

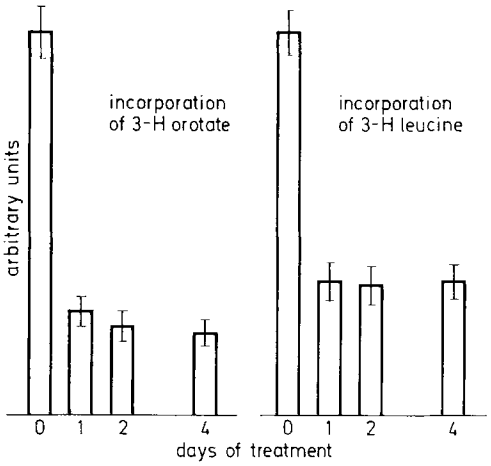


Fig. 6. Histogram showing the extent of inhibition of 3H-orotate and 3H-leucine incorporation into adrenocortical cells of intact rats, induced by corticosterone treatment

of treatment (Table 2). These data show that the decrease in volume of the mitochondrial fraction is about 50–55% due to the decrease in number of mitochondria per cell, and about 45–50% due to the decrease in volume of the organelles themselves. On the other hand, the surface/volume ratio of mitochondria increases significantly in relation to the duration of the corticosterone treatment (Table 2).

A considerable decrement of the lipid fraction and of the smooth reticulum, both in volume (membrane space) and in surface was also found (Table 1).

If the data given in Table 1 are plotted on a graph as function of the number of days of corticosterone treatment, it may be noted that the reduction in the cellular, nuclear, mitochondrial and lipid volume and the decrease in volume and surface of the smooth reticulum are not related linearly with the duration of treatment (Figs. 3, 4). In fact, 50–55% and 80–85% of the total decrement in volume of cells and of other subcellular organelles occur as early as the 1st and 2nd days of treatment, respectively.

The above-mentioned data show that the decrease in cell volume is 48% due to the reduction in the smooth reticulum (membrane space), while the decrease in volume of nuclei, mitochondrial fraction and of lipid fraction are responsible for 8, 30 and 14% of the decrease in cellular volume, respectively (Fig. 5).

The incorporation of 3H-orotate is reduced by about 70% for all the times of the experiment. On the other hand, the incorporation of 3H-leucine is reduced by about 55%, independently of the duration of the hormonal treatment (Fig. 6). It therefore seems that with the high doses of corticosterone used, the maximum effect has already been obtained by the 1st day of corticosterone administration. The incorporation of the tracers in the capsular fibroblasts was not found to be significantly reduced (4–5%) ($P < 0.2$).

Table 3. *Synopsis of morphometric parameters of adrenocortical cells of hypophysectomized ACTH-treated rats injected with corticosterone*

Duration of treatment	Control	1 day	2 days	4 days
Volume of cells				
μ^3	1,877.6 ± 62	1,608 ± 49	1,455 ± 39	1,275 ± 36
$-\Delta v$ (%)		14.4	22.6	32.1
P		0.05	0.01	0.01
Volume of nuclei				
μ^3	165.4 ± 3.7	158.1 ± 3.2	148.3 ± 3	135.3 ± 2.8
$-\Delta v$ (%)		4.5	10.3	18.2
P		0.1	0.05	0.05
Diameter of nuclei				
μ	6.81 ± 0.148	6.71 ± 0.128	6.57 ± 0.12	6.37 ± 0.112
$-\Delta l$ (%)		1.5	6.5	9.3
P		0.1	0.05	0.05
Volume of mitochondrial fraction				
μ^3	497.4 ± 22	452 ± 22	455 ± 26	478.4 ± 23.8
$-\Delta v$ (%)		not significant	not significant	not significant
P				
Volume of lipid fraction				
μ^3	248.5 ± 16.4	180 ± 12.3	151.1 ± 11.2	127.5 ± 9.8
$-\Delta v$ (%)		27.7	38.8	54
P		0.05	0.01	0.01
Membrane space				
μ^3	966.3 ± 44.1	817.9 ± 30.9	700 ± 28.2	533.8 ± 22.9
$-\Delta v$ (%)		15.4	26.6	44.8
P		0.05	0.05	0.01
Surface of smooth reticulum				
μ^2	10,320 ± 321	9,017 ± 281	7,752 ± 211	6,155 ± 189
$-\Delta s$ (%)		12.7	24.9	40.4
P		0.05	0.05	0.01

Effects of Corticosterone on Adrenocortical Cells of Hypophysectomized ACTH-Treated Rats

The cell volume decreases significantly in relation to the duration of treatment (Table 3). There is also a significant decrease in the nuclear diameter and volume (Table 3). The nuclei show structural modifications similar to those described in the experiment with intact animals. No appreciable storage of glycogen was found in the adrenocortical cells chemically inhibited with corticosterone.

The mitochondrial fraction does not reveal significant variations in volume (Table 3). On the other hand, there is a highly significant decrease in the lipid fraction and the smooth reticulum (both in volume and in surface) (Table 3).

If the data given in Table 3 are plotted on a graph, it may be noted that in this experiment, as also in that performed on intact rats, the decreases in the various cellular morphological parameters are not linearly related to the number

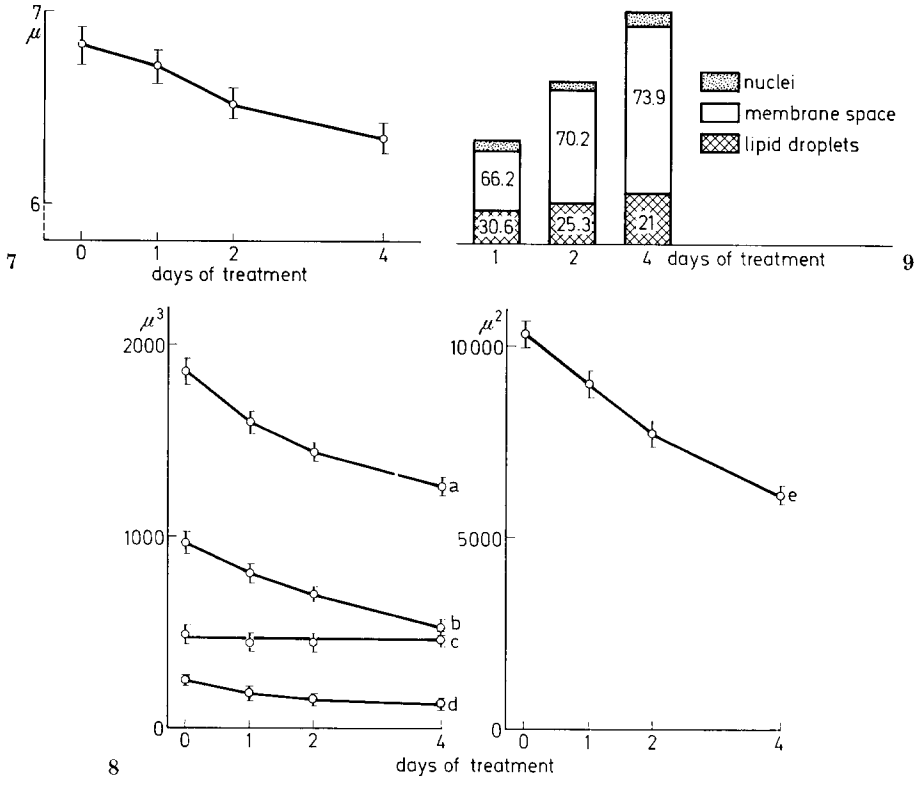


Fig. 7. Changes in diameter of nuclei of adrenocortical cells of hypophysectomized ACTH-treated rats induced by corticosterone treatment. Standard errors are indicated

Fig. 8. Changes in morphological parameters of adrenocortical cells of hypophysectomized ACTH-treated rats as function of the number of days of corticosterone treatment. Standard errors are indicated. *a* volume of cells; *b* membrane space; *c* volume of mitochondrial fraction; *d* volume of liposomes; *e* surface of smooth reticulum membranes

Fig. 9. Histogram showing the total decrements in volume of adrenocortical cells of hypophysectomized ACTH-treated rats after 1, 2 and 4 days of corticosterone treatment. The percentages of the total decrement due to decrease in liposomes, membrane space and nuclei are indicated

of days of corticosterone treatment (Figs. 7, 8). In fact, 45–50% and 70–75% of the total decrement in these parameters are observed as early as the 1st and 2nd day of treatment, respectively.

From Fig. 9 it may be seen that the reduction in the volume of adrenocortical cells of hypophysectomized ACTH-treated rats as a result of corticosteroid treatment is 70% due to the reduction in the smooth reticulum. The decrease in nuclear and lipid volume are responsible for 4 and 25% respectively of the decrement in cellular volume.

The quantitative autoradiographic study shows that the incorporation of ^{3}H-orotate in adrenocortical cells of treated rats is reduced by about 55–60% for all the times of the experiment, while the incorporation of ^{3}H-leucine is

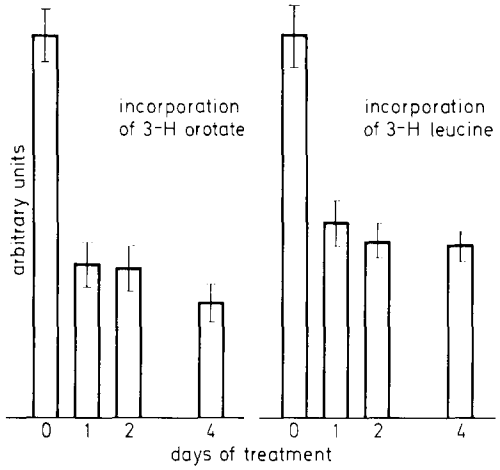


Fig. 10. Histogram showing the extent of inhibition of 3H-orotate and 3H-leucine incorporation into adrenocortical cells of hypophysectomized ACTH-treated rats, induced by corticosterone treatment

reduced by 40–45% (Fig. 10). It may therefore be noted that, also in this experiment, the high doses of corticosterone used produced a maximum effect as early as the 1st day of treatment. The reduction in the incorporation of the tracers in the capsular fibroblasts was found not significant (less than 3–4%).

Discussion

Examination of the morphometric data obtained in the present study indicates that the most conspicuous effect of corticosterone on the fine structure of adrenocortical cells of intact rats consists of a considerable reduction in the smooth reticulum. These data, which are largely comparable to those obtained by treating intact rats with prednisolone (Nussdorfer, 1970b), confirm the hypothesis (Nussdorfer, 1970a) that the quantity of smooth reticulum is the most important morphological parameter for assessing the functional activity of a steroid-secreting cell. To a lesser extent, the quantity of the mitochondrial fraction is also related to the activity of adrenocortical cells.

These data can be easily explained by taking into account the fact that (1) in the smooth reticulum and at the level of the mitochondrial cristae the enzymes that take part in the synthesis of cholesterol from acetate (smooth reticulum) and of adrenocortical hormones from cholesterol (smooth reticulum and mitochondria) are localized (Harding *et al.*, 1968; Inano *et al.*, 1969; Péron *et al.*, 1968; Samuels *et al.*, 1967; Satre *et al.*, 1969; Siekevitz, 1963; Simpson *et al.*, 1968; Srere *et al.*, 1948), and that (2) quantitative variations in these subcellular fractions correspond to mutual variations in the enzymatic activity of these fractions (Stäubli *et al.*, 1969). The reduced synthesis of cholesterol induced by the corticosterone treatment in adrenocortical cells, may explain the significant reduction in the lipid droplets in adrenocortical cells of treated animals.

Analogous effects are produced by corticosterone on the fine structure of adrenocortical cells of hypophysectomized ACTH-treated rats. Also in this case the most conspicuous effect consists of reduction in the smooth reticulum membranes and, secondarily, in the lipid droplets. On the other hand, it should be noted that the volume of the mitochondrial fraction does not vary.

The results of the quantitative autoradiographic studies indicate an inhibition of the protein synthesis by corticosterone at the level of adrenocortical cells, both in intact and in hypophysectomized ACTH-treated rats. The specificity of this effect is confirmed by the lack of inhibition of the incorporation of tracers in the fibroblasts of the adrenal capsule. The incorporation of 3H-orotate and 3H-leucine is considerably reduced in both experiments, and it is well known that the incorporation of these precursors may be considered a valid parameter for an assessment of the incorporation of aminoacids in polypeptide chains and of RNA synthesis, respectively.

Our autoradiographic data indicate that corticosterone inhibits, primarily, the RNA synthesis. Secondarily, this leads (for exposures to corticosterone greater than 24 h) to a reduction in the incorporation of labelled aminoacids in polypeptide chains. This is also confirmed by studies of the effects of a short exposure to corticosterone upon the incorporation of orotate-3H and leucine-3H in adrenocortical cells of hypophysectomized ACTH-treated rats (Nussdorfer *et al.*, 1970). In fact, if the tracers are administered 1 hour after exposure of animals and the incorporation of labels is estimated for 2 hours, a significant reduction is found in the incorporation of 3H-orotate and only a slight reduction in the incorporation of 3H-leucine. This finding may be easily explained by taking into account the fact that the RNA present in the cells at the moment of corticosterone administration has continued to exercise its function in protein synthesis during the 3 hours before the sacrifice of animals.

Karyometric assessments showing a decrease in the nuclear volume as a result of corticosteroid treatment, and the nuclear morphological characteristics are in agreement with a reduced nuclear synthesis of RNA (Mitro *et al.*, 1970).

Also the accumulation of glycogen, found in adrenocortical cells of intact rats treated with corticosteroids may be explained by a lesser requirement of energy due to the reduced synthesis of proteins and/or corticosteroids. Since an obvious accumulation of glycogen has not been found as a result of corticosteroid treatment in the adrenocortical cells of hypophysectomized ACTH-treated rats, it may be thought that the accumulation of glycogen is due to inhibition of the hypothalamo-hypophysial axis by corticosterone. In fact, it is shown that the stresses and the ACTH cause depletion of glycogen in adrenocortical cells (Cohen, 1961; Cohen *et al.*, 1962a, 1962b). This is in agreement with the hypothesis of Haynes-Berthet (1957) that the ACTH stimulates, at the level of adrenocortical cells, the production of $NADPH_2$ by activation (via 3′,5′-AMP) of phosphorylase, which increases the breakdown of glycogen into glucose-1-phosphate, and of the hexose-monophosphate shunt.

Hence, our data support the hypothesis that the reduction in the various cellular fractions and especially in the membranes of the smooth reticulum are the expression of reduced protein synthesis. Since, for short exposures to corticosterone, no quantitative variations are observed in the ultrastructural character-

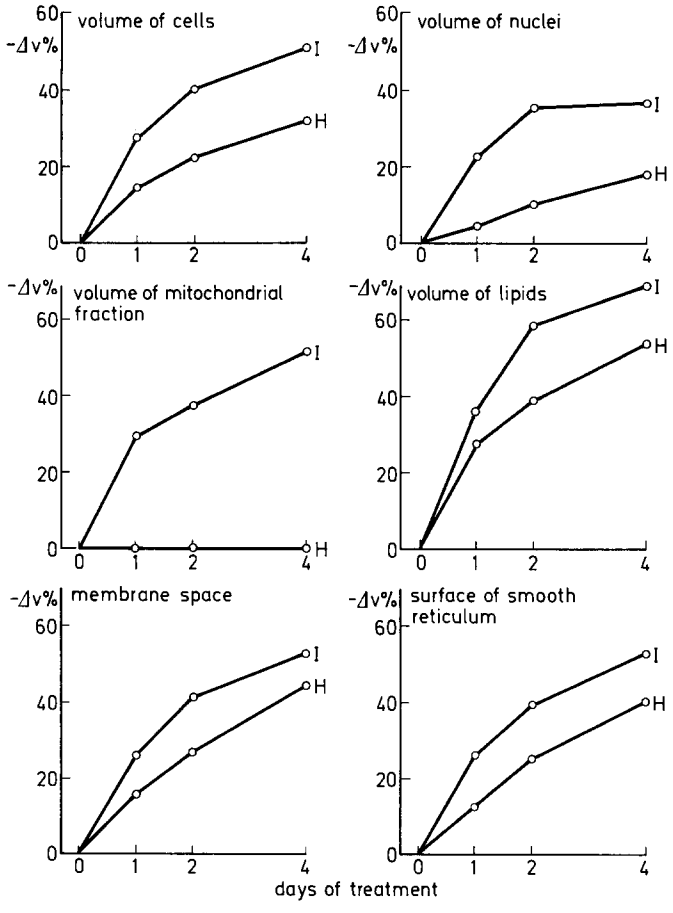


Fig. 11. Graphs showing the % decrement ($-\Delta v$) in various morphological parameters of adrenocortical cells of intact (*I*) and hypophysectomized ACTH-treated rats (*H*) as function of the number of days of corticosterone treatment

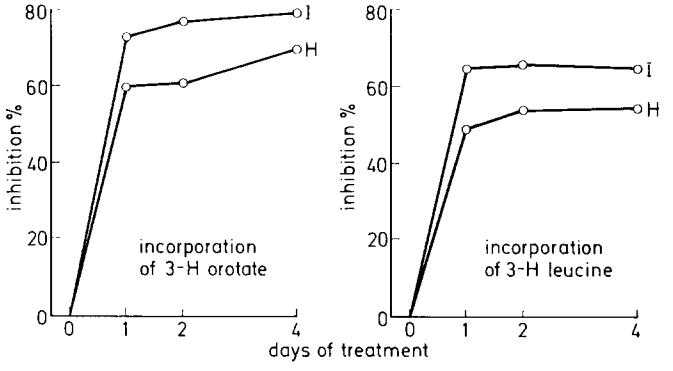


Fig. 12. Graphs showing the % inhibition of the incorporation of 3H-orotate and 3H-leucine in adrenocortical cells of intact (*I*) and hypophysectomized ACTH-treated rats (*H*) as function of the number of days of corticosterone treatment

istics of adrenocortical cells, in relation with the reduced protein synthesis (Nussdorfer *et al.*, 1970), it may be thought that either (1) the corticosteroids initially inhibit the enzyme synthesis and only secondarily inhibit the synthesis of structural proteins, or (2) the morphological effect of the inhibited synthesis of structural proteins requires at least 20–24 hours to be detectable. This second hypothesis appears the more probable and is also supported by studies carried out by Orrenius *et al.*, (1968) on enzyme induction in hepatocytes by prednisolone: in fact, the effects on the microsomic fraction were appreciable only 24 hours after prednisolone administration.

On the basis of these considerations, it may be thought that the decrement in the smooth endoplasmic reticulum is the expression of the physiological intracellular katabolism of membranes, in the presence of a deficit in the synthesis of new membranes (Nussdorfer, 1970b).

Comparing the quantitative data obtained in the two above-described experiments, it may be stated that corticosterone inhibits RNA synthesis in adrenocortical cells by a double mechanism. In fact, it acts not only at the level of the hypothalamo-hypophysial axis (for references, see Mess *et al.*, 1968), but also directly on the adrenocortical cells. In fact from Figs 11 and 12 it results that the % decrement in the various morphological and autoradiographic parameters, is far less pronounced in the adrenocortical cells of hypophysectomized ACTH-treated rats than in those of intact animals. This indicates that corticosterone has less effect on adrenocortical cells of hypophysectomized ACTH-treated animals. These results may be easily explained by the fact that corticosterone in intact rats inhibits the adrenal cortex either indirectly (by blocking the hypophysial secretion of ACTH) or directly, whereas in hypophysectomized ACTH-treated rats the inhibition is only direct. Since ACTH stimulates the protein synthesis in the target cells (Brandsome *et al.*, 1964; Farese, 1968, 1969; McKerns, 1968), it follows that the reduction in the incorporation of orotate-3H and leucine-3H in adrenocortical cells of hypophysectomized ACTH-treated rats will be less than in those of intact animals. These data confirm, *in vivo*, the hypothesis—based on the results of *in vitro* studies (Burrow *et al.*, 1966, 1968; Ferguson *et al.*, 1967; Morrow *et al.*, 1967)—of a direct action of corticosterone on adrenocortical cells.

The absence of any decrement in the mitochondrial fraction of adrenocortical cells as a result of corticosterone treatment of hypophysectomized ACTH-treated rats deserves some discussion. In fact, it indicates that the trophism of the mitochondrial fraction of adrenocortical cells is controlled by ACTH. As shown in the results, the volumetric reduction in the mitochondrial fraction of adrenocortical cells of intact rats treated with corticosterone, is 50% due to a reduction in the number of the organelles and 50% due to a decrease in their volume. Considering that the mitochondria are able to synthesize DNA and RNA (for references, see Roodyn *et al.*, 1968), the reduction in the number of these organelles suggests that ACTH intervenes in the regulation of the synthesis of mitochondrial DNA or/and RNA. The reduction in the volume of the mitochondria in correlation with an increase in the surface/volume ratio may also indicate that ACTH produces a swelling of the adrenocortical mitochondria. This is also supported by personal morphometric data (Nussdorfer, results unpublished). Moreover, these morphological data may be correlated to the biochemical data of Koritz (1968), which indi-

cate that ACTH (via 3′,5′-AMP) increases the permeability of the mitochondrial membrane to pregnenolone. The swelling of mitochondria could therefore be the morphological expression of this increased permeability. Moreover, others chemicals producing mitochondrial swelling, increase the rate of corticosteroid synthesis in adrenocortical cells (Hirshfield *et al.*, 1964).

In conclusion, it seems possible to state that the most important morphological parameter for assessing the activity of a steroid-secreting cell is the quantity of smooth reticulum. Moreover, the combined quantitative morphometric and autoradiographic results of the present study indicate that the decrement in the smooth reticulum membranes of adrenocortical cells of rats treated with corticosterone is due to inhibition of the protein synthesis. Furthermore, it seems to be confirmed that, also *in vivo*, there is a direct negative feed-back control mechanism at the rat adrenal level, mediated by the inhibition of the RNA (RNAm ?) synthesis by corticosteroid hormones.

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