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# The Influence of Encortone on Ultrastructural Changes in the Parotid Cells of Female White Rats of Wistar Race

Wpływ enkortonu na zmiany ultrastrukturalne komórek przyusznicy samic szczurów białych rasy Wistar

It is generally known that glucocorticoids exert an essential influence on digestive tract physiology (2). Many investigations carried out refer to the changes observed in the pancreas under different experimental conditions (4, 5, 6, 7, 8). The investigations of the influence of glucocorticoids on salivary glands and mainly on a parotid gland are very general. Salivary glands play an important role in the initial stages of taking up and assimilating food. Moreover, the produced growth factors and parotin play an important role in tissue development and maturation in young animals (12).

The purpose of this experiment was to find out whether physiological doses of encortone can cause the changes in ultrastructure of parotid serous cells.

## MATERIAL AND METHODS

The experiment was carried out on rats inbreeding Wistar race females weighing 210—220 g. The animals were divided into two experimental groups and two parallel control grups (including five animals each) regarding the age of animals and stress influence.

Control group I — the animals receiving distilled water by stomach-tube for 7 successive mornings.

Experimental group I — the animals receiving emulsion of encorton (of "Polfa" firm) in distilled water in the dose of 1 mg/kg of body mass by stomach-tube for 7 successive mornings before feeding.

Control group II — the animals receiving distilled water in the same way as the animals of control group I for 30 days.

Experimental group II — the animals receiving encortone in the same way as the animals of experimental group I for 30 successive days.

The administration of the drug in the case of both experimental groups began with the full dose, namely 1 mg/kg of body mass, then decreased to half and to $^1/_3$ at the end of the administration period. Each animal of experimental group I received together 0.94 mg of encortone and each animal

of experimental group II — 3.41 mg of encortone. The rats were fed with standard granulated fodder and water *ad libitum*. They were killed by the ether anaesthesia. The experimental animals showed the same increase of body mass as control animals: 30 g after a week and 50 g after a month. The parts of the parotid gland were fixed with glutaraldehyde and $OsO_4$ and then embedded in Epon 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate according to Reynolds. They were examined in Tesla BS-500 electron microscope.

## RESULTS

### Control groups I and II

There were no essential differences in ultrastructure of parotid serous cells in the animals of both control groups of the same acini. The acini showed mainly the same stage of secretion production but there were also acini with the cells of different stages of secretion production. Depending on the maturation stage and secretion condensation there were observed various morphological types of secretory granules: type I — the granules with very small electron density/bright present in the cells with a large number of rough membranes (Fig. 1); type II — the granules with a dense electron zonula and some of them also with medulla (Fig. 2); type III — the granules in which dense electron medulla is not formed in the condensation process (Fig. 3); type IV — the dense electron granules.

A number of rough membranes in the cells containing dense granules seems to be smaller compared with the cells containing the granules of smaller density. Free ribosomes are visible in the cytoplasm (Fig. 4).

### Experimental group I

Parotid serous cells of the animals from experimental group I showed differentiated metabolic activities. There were found in some animals (Fig. 5) well developed rough membranes and mitochondria in a low energetic stage with well developed cristae and dense matrix, in others (Fig. 6) considerably less active with a small number of ER membranes and mitochondria in the orthodoxal stage. Dense secretory granules were mainly found. The cells containing two cell nuclei each were less numerous compared with the control. At the same time single and small myelinin bodies were observed in many cells.

### Experimental group II

In the parotid serous cells of the animals from experimental group II also a strongly compact type of secretory granules was predominant. A number of granules was clearly smaller than in the control animals but a number of two-nucleic cells was larger. In many cells several myelinin bodies (Fig. 7)

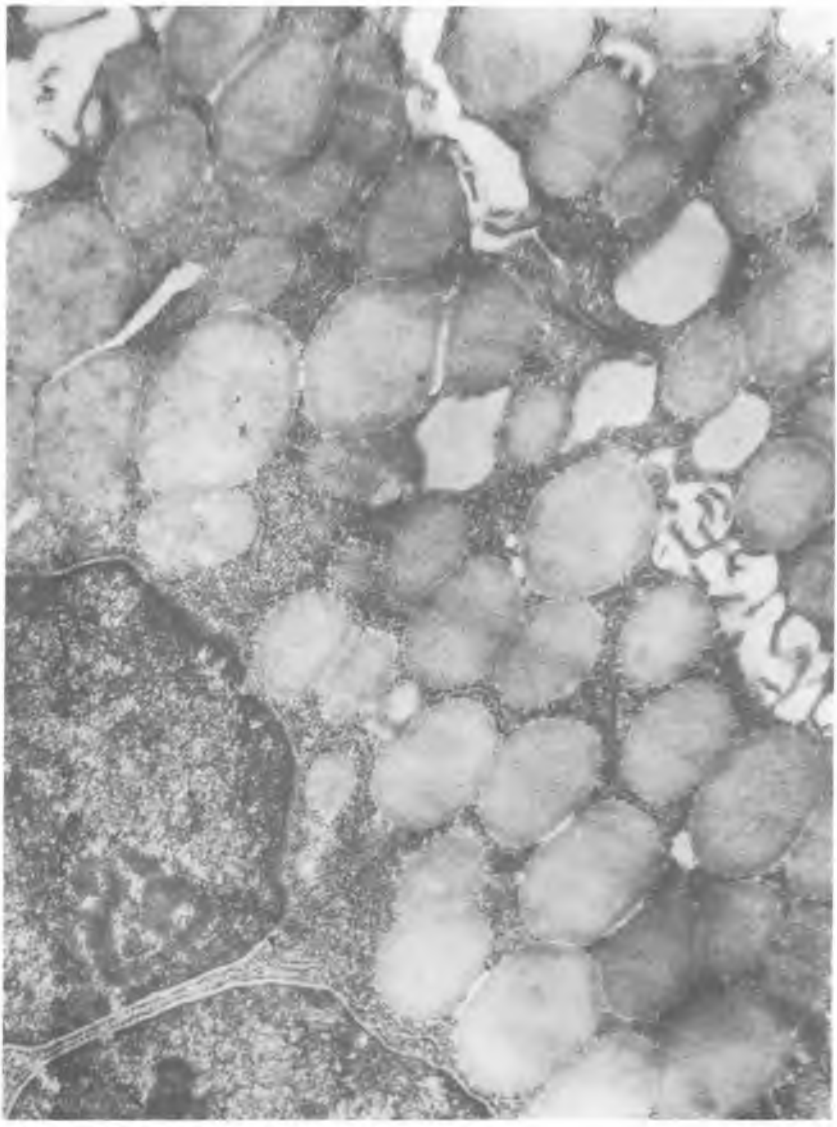

Fig. 1. First formation stage of secretory granules in the parotid cells from the control female rat. Secretory granules of low electron density are visible. Magn. 9000×

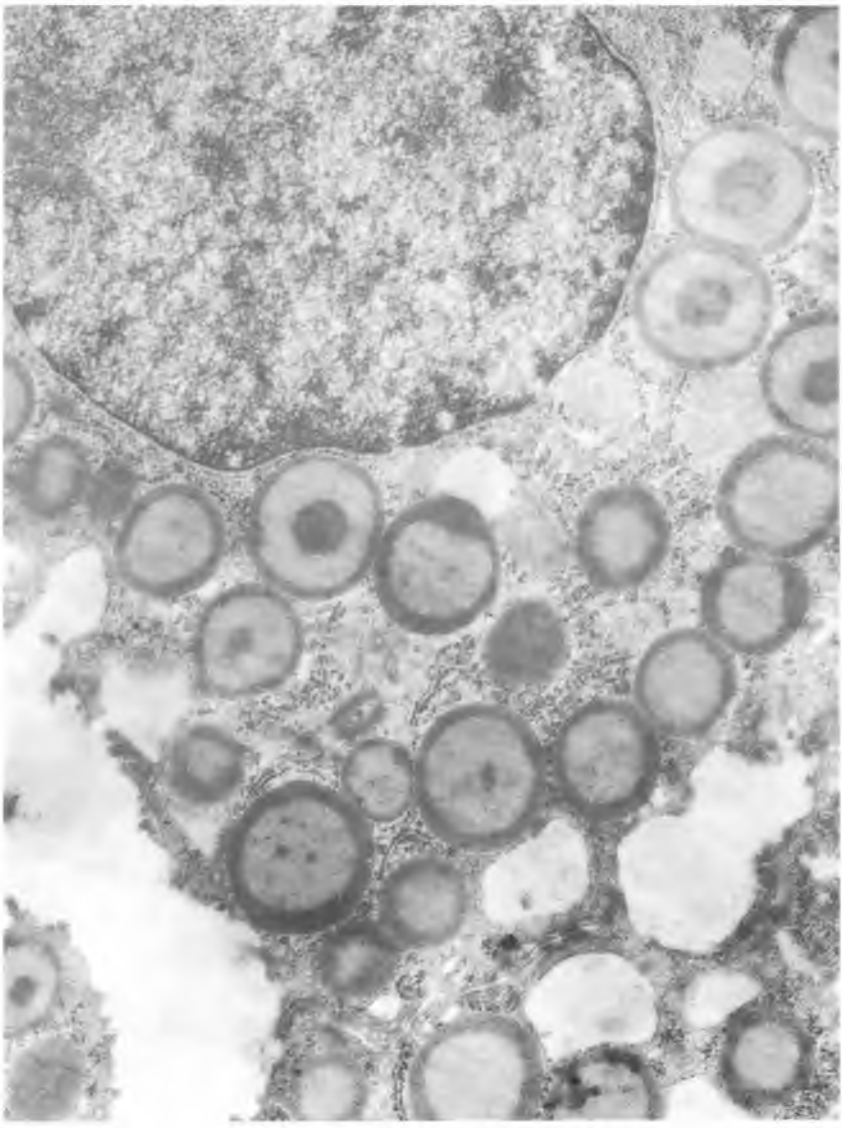

Fig. 2. Second formation stage of secretory granules in the parotid cells from the control female rat. Secretory granules with electron dense zonula and some of them also with dense electron medulla are visible. Magn. 9000 ×

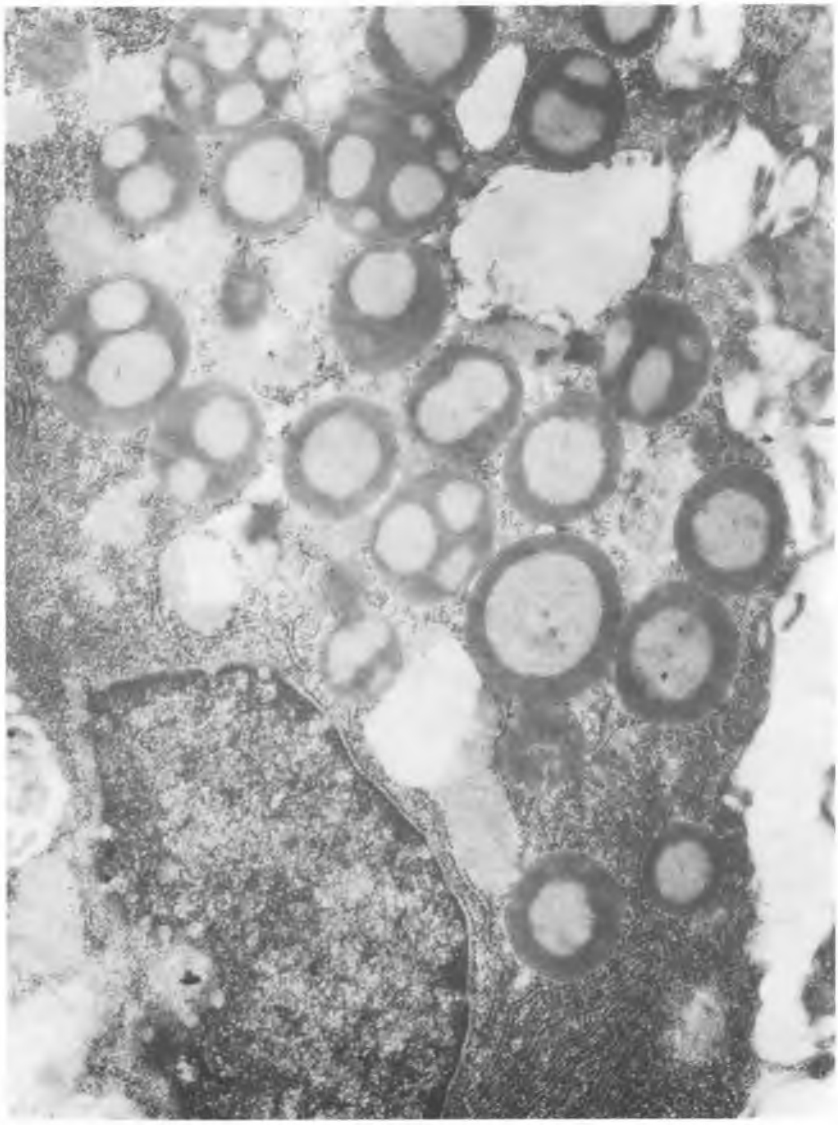

Fig. 3. Another modus of condensation of secretory granules in the parotid cells from control female rat is visible. Electron dense medulla in secretory granules is absent. Magn. 9000×

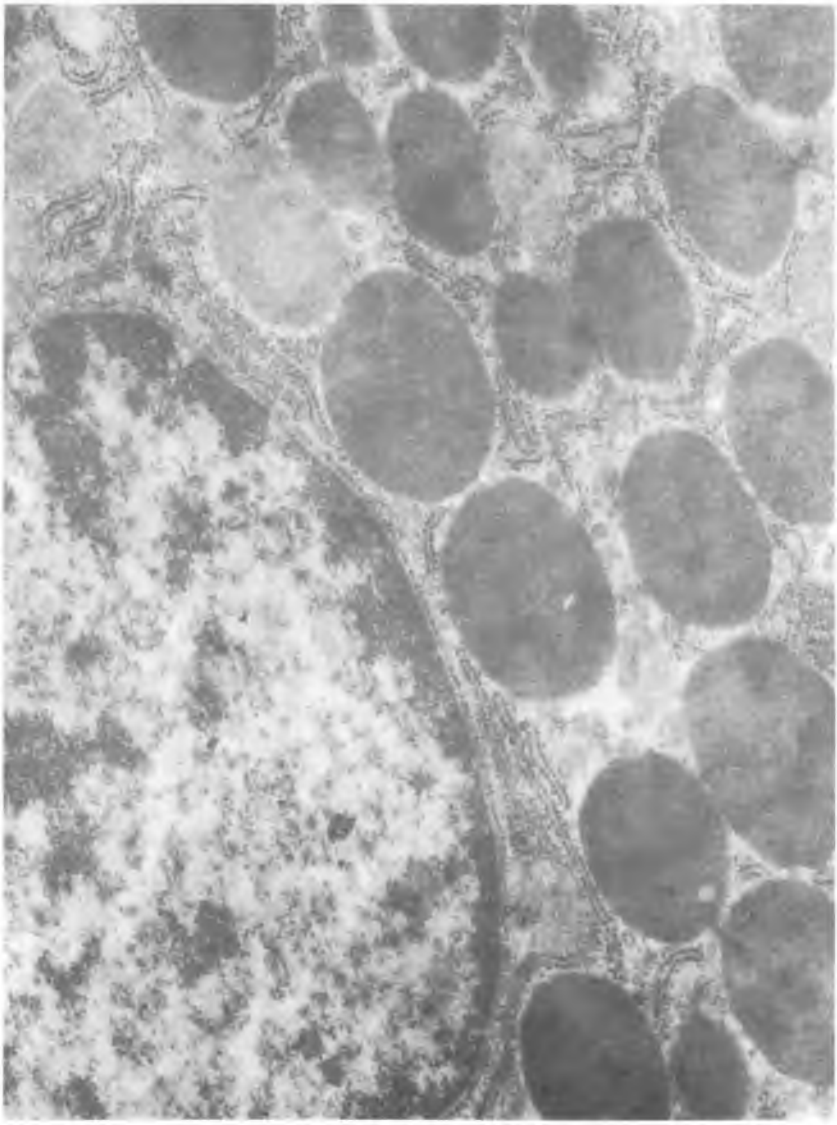

Fig. 4. Third formation stage of secretory granules in the parotid cells from the control female rat. Dense secretory granules are visible. Magn. 12000 ×

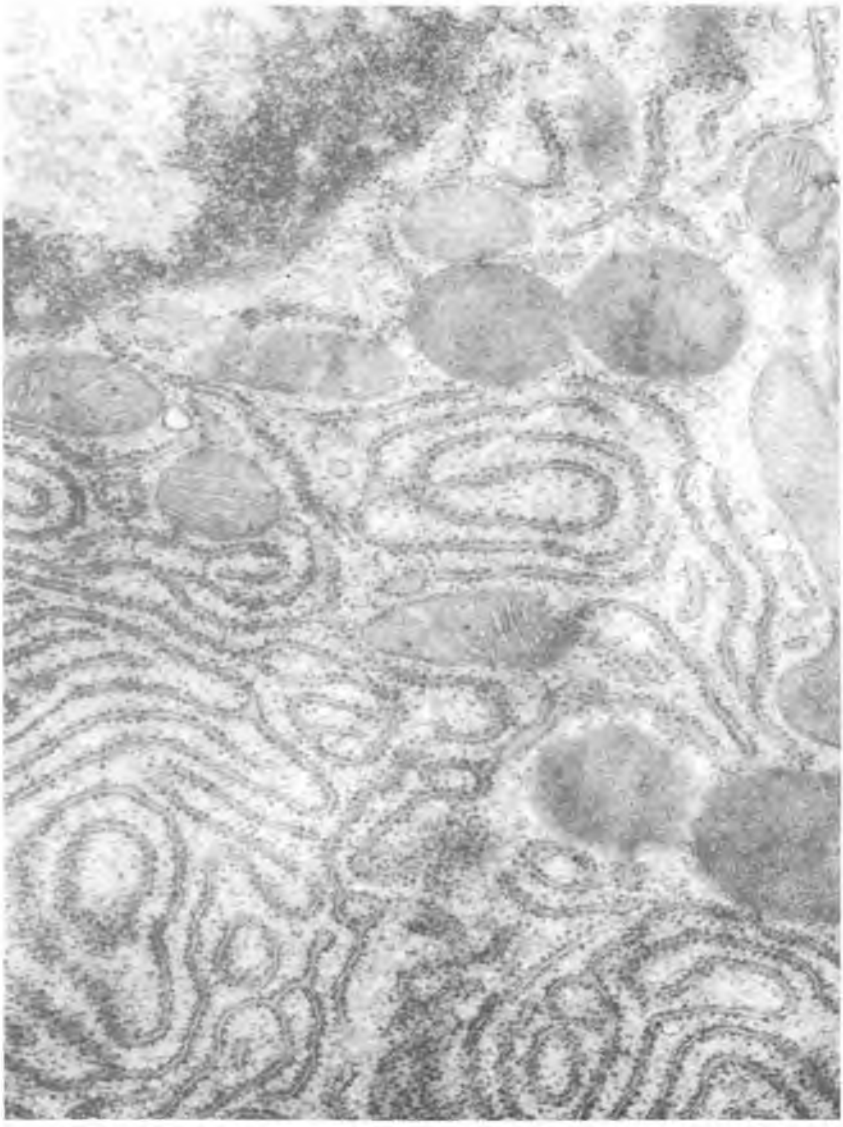

Fig. 5. The parotid cell from female rat of experimental group I. Well developed rough membranes and mitochondria in low-energetic stage are visible. Magn. 16000×

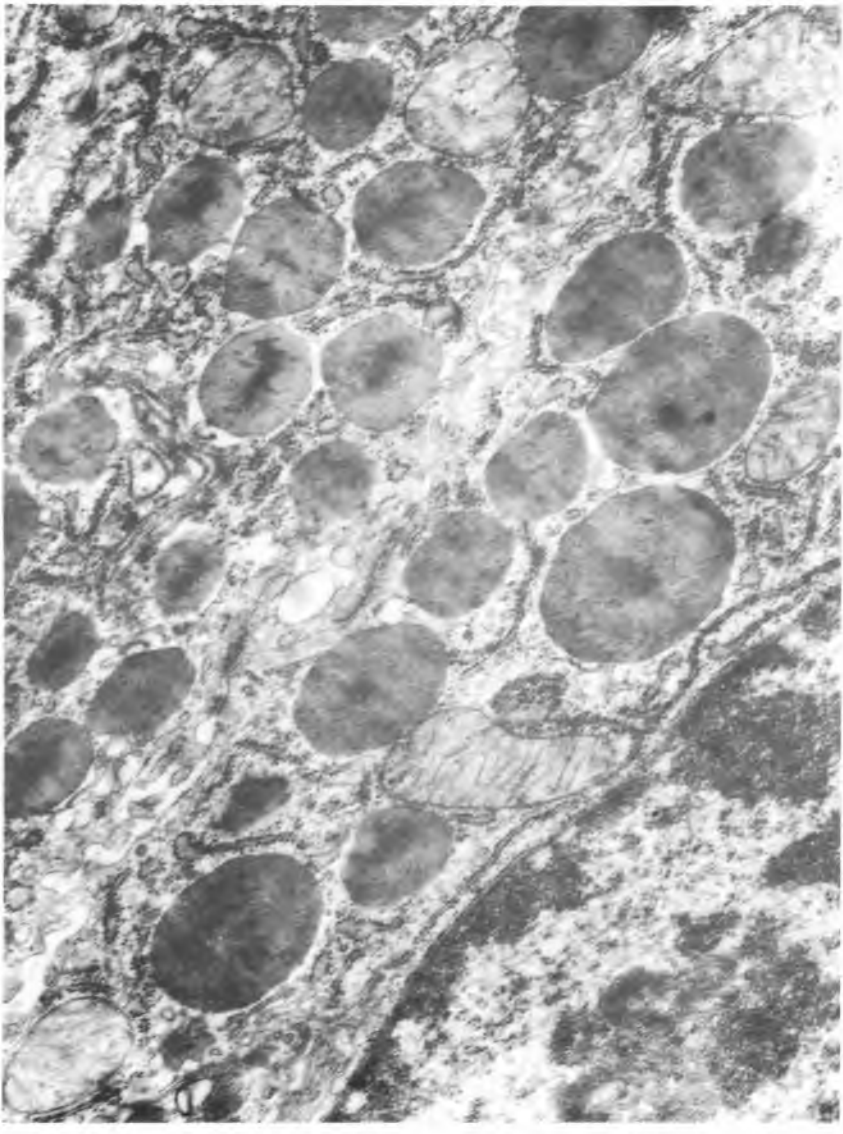

Fig. 6. The parotid cell from female rat of experimental group I. The small number of ER membranes and mitochondria in orthodoxal stage are visible. Magn. 12000×

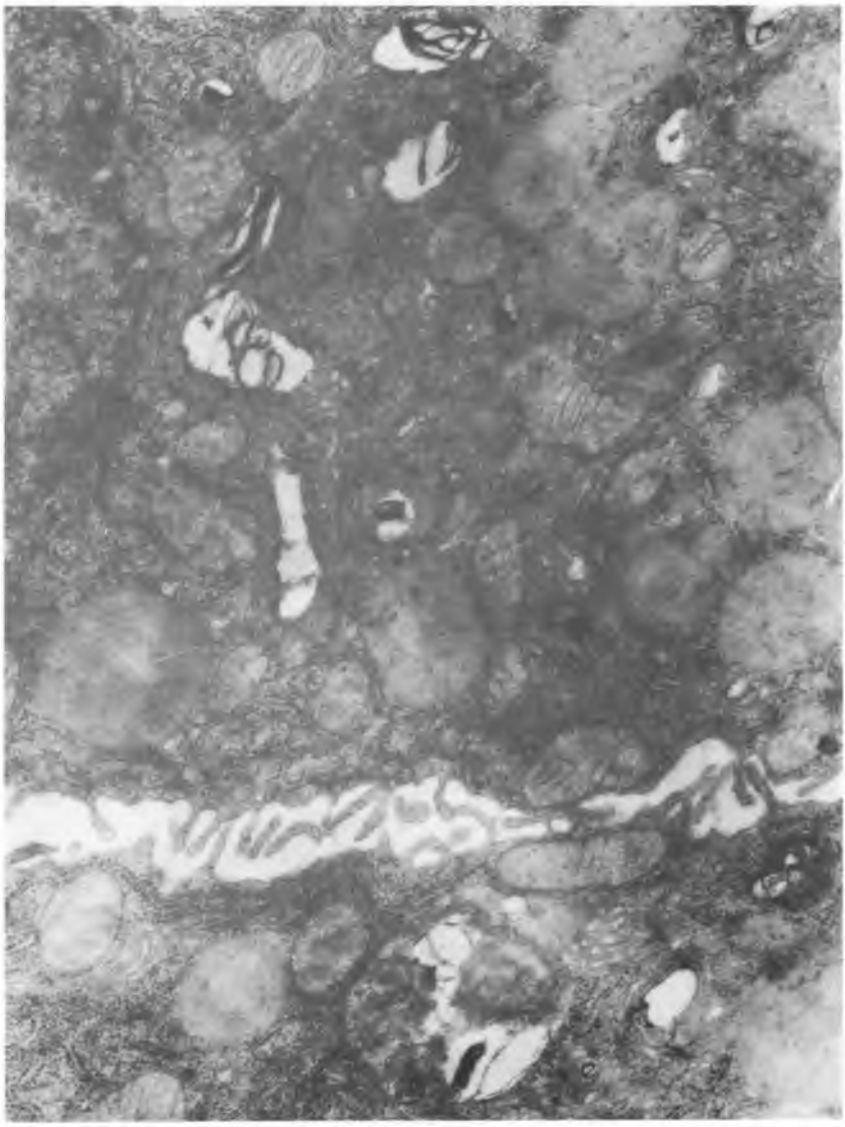

Fig. 7. The parotid cell from female rat of experimental group II. Several myelinic bodies in the same cell are visible. Magn. 12000 ×

Fig. 8. The parotid cell from female rat of experimental group II. The small number of ER membranes and pathological mitochondrium are visible. Magn. 12000×

sometimes reaching large sizes were often present. These bodies were situated also in the intercellular spaces and in the cells stuffing the intercalated ducts. A number of ER membranes often of irregular arrangement was evidently smaller than in the control and mitochondria showed a highly energetic stage (Fig. 8).

## DISCUSSION

Encortone (prednisone) is the drug administered quite often in extrasubstitutive treatment because of its strong antiphlogistic action, diminished ability of $Na^+$ ions holding and not very long period of reversible repression of corticotropin (2). At the same time it is known that glucocorticoids exert different influence on all organs and tissues of the organism, because there are receptors for these hormones almost in all of them (8). Already after a short 7-day period of treatment with a physiological dose of encortone distinct changes in ultrastructure of serous cells were observed. A small number of strongly condensed secretory granules seems to point to diminished production of secretion or increased rate of secretion. The increase of ER membrane number along with the presence of mitochondria in a low-energetic stage was observed in some cells while the decrease of membrane number and mitochondria in an orthodoxal stage were found in others. In the first case the cells showed probably mobilization for the increased activity and perhaps quicker rate of secretion, in the second stage this activity was distinctly smaller. Morphometric investigations of parotid in the same animals (16) showed the increase of acini section area, serous cells and a number of cells in acini and the decrease of cell nuclei section area. These data are in agreement with the observations by means of electron microscope and suggest that energetic mobilization of many cells was dependent on the hypertrophia and hyperplasia processes. Similar processes in the acinar cells of pancreas after small doses of hydrocortisone were observed by Morisset et al. (4, 5) and Otsuki et al. (7) also showed that hydrocortisone treatment in the dose of 1.25 mg/kg of body mass exerted an influence on concentration decrease of the enzymes in the exocrine part of rat pancreas, which may result from the decreased synthesis or the increased secretion. However, Polakova (8) and Morisset et al. (4) report the increased production of zymogene and amylase activity increase following the administration of small doses of hydrocortisone for several days.

In the parotid cells of male rats which were given encortone in the same way well marked increase of secretion production as well as secretion increase were observed (14). However, the results of morphometric investigations did not show cells hypertrophy and their number increase in the acini (15). Different reaction of male and female parotid cells to the administered hormone may be related to

the presence of different receptors (a different number of receptors) for adrenal cortex and sexual hormones. Probably encortone acts directly on the parotid cells, but also its indirect action through adrenal cortex cannot be excluded (3, 8, 9, 10, 11). In the case of androgen deficiency in males glucocorticoids bind with the androgen receptors (3) and also modulate capacity and concentration of the receptors for EGF (1). It can be supposed, that there is a number of receptors for androgens (13) in parotid of females. It is also possible that glucocorticoids bind with the estrogen receptors in the females.

After 30-day physiological dose encortone treatment the parotid cells showed a small metabolic activity, which is shown by a small number of greatly condensed secretory granules, a small number of ER membranes, mitochondria in a highly energetic stage. In comparison with the control, a greater number of the two-nucleic cells can show that in these cells only the first stage of mitosis caryokinesis takes place but there is no cell division — cytokinesis. Morphometric investigations of parotid in the same animals showed the decrease of section area of the cells, acini and a number of cells in acini (16). Ricciardi et al. (11) report that the dexamethasone administered in the dose of 2 mg/kg of body mass for 14 days caused atrophy of acini, changes of cell shape and nuclei pycnosis in the rat submandibular gland. Such a pycnosis was not observed in our experiment. At the same time it is quite probable that cell divisions are more intensive than in the control. The 30-day hormone treatment may stimulate new cells formation or hyperplasia manifesting itself mainly in new acini formation. Secretory granules synthesis decreased significantly in the division period. This stage may precede atrophic changes developing little by little and may be the evidence of glandule mobilization for the increased secretion in the later period.

## Conclusions

1. 7-day administration of encortone in the dose 1 mg/kg of body mass caused in some parotid cells decrease of activity (decrease of ER membrane number, mitochondria in a highly energetic stage) in others — increase of activity (increase of a number of ER membranes, mitochondria in a low energetic stage).

2. 30-day treatment of encortone caused a significant decrease of the processes of secretion (the decrease of ER membrane number, mitochondria in a highly energetic stage) and an increase of 2-nucleic cell number, which can indicate hyperplasia results.

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## STRESZCZENIE

Dojrzałe samice szczurów otrzymywały enkorton w dawce 1 mg/kg masy ciała przez 7 dni i 30 dni. Ultracienkie skrawki przyusznicy były barwione według metody Reynoldsa i badane w mikroskopie elektronowym BS-500 firmy Tesla. Zaobserwowano następujące zmiany w komórkach surowiczych przyusznicy zwierząt doświadczalnych w porównaniu z kontrolą: a) po 7 dniach podawania enkortonu w niektórych komórkach przyusznicy nastąpiło obniżenie aktywności (zmniejszenie liczby błon ER, mitochondria w stanie wysokoenergetycznym), w innych stwierdzono wzrost aktywności (wzrost liczby błon ER, mitochondria w stanie niskoenergetycznym); b) po 30 dniach podawania enkortonu nastąpiło wyraźne obniżenie procesu wydzielania (zmniejszenie liczby błon ER, mitochondria w stanie wysokoenergetycznym) i stwierdzono wzrost liczby komórek 2-jądrowych, co może być wyrazem hiperplazji.