

CHANGES IN  $3\beta$ -OH-STEROID DEHYDROGENASE ACTIVITY IN THE ADRENALS  
OF RAT FETUSES DECAPITATED *in utero*

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Activity of  $3\beta$ -OH-steroid dehydrogenase in the adrenal cortex of 21-day rat fetuses in the control and 3 days after decapitation *in utero* was estimated quantitatively by microspectrophotometry. Activity of the enzyme, calculated per conventional cell in the zona fasciculata was considerably higher than in the developing zona glomerulosa. After hypophysectomy the  $3\beta$ -OH-steroid dehydrogenase activity was reduced in both zones of the adrenal.

KEY WORDS: Rat fetuses; hypophysectomy; adrenal cortex;  $3\beta$ -OH-steroid dehydrogenase.

Among indices of the state of adrenocortical function the activity of  $3\beta$ -OH-steroid dehydrogenase, the key enzyme of steroid synthesis, is of considerable interest. In the rat fetal adrenals this enzyme can be detected histochemically from the twelfth day and its activity reaches a maximum in 19-day-old fetuses [11]; biochemical observations show that the activity of this enzyme also increases to the 19th day [5]. Intensive stimulation of the adrenal by ACTH has been demonstrated in rat fetuses aged 18-20 days [3, 6, 7, 14]. Correlation between increased  $3\beta$ -OH-steroid dehydrogenase activity in the adrenal cortex and increased pituitary adrenocorticotrophic function also exists in the rabbit fetus [12, 14] and chick embryo [10, 17]. Hypophysectomy in adult rats leads to a decrease in the activity of this enzyme in the adrenal cortex [8, 15]; the same effect is observed after decapitation of chick embryos [10].

Changes in activity of  $3\beta$ -OH-steroid dehydrogenase in the adrenal cortex of rat fetuses in the presence of ACTH deficiency, caused by decapitation of the fetuses, were investigated by means of a quantitative histochemical reaction for the enzyme [2].

#### EXPERIMENTAL METHOD

Fetuses of seven Wistar rats were investigated. At the age of 18 days some of the fetuses of each female were decapitated *in utero*; the rest served as the control. Three days later the adrenals were removed from the fetuses and the glands from the experimental and control fetuses of each litter were mounted in the same block, thus ensuring uniform thickness and simultaneous treatment of the sections. Activity of  $3\beta$ -OH-steroid dehydrogenase was detected in cryostat sections, 7  $\mu$  thick, by the use of dehydroepiandrosterone as the substrate. The optical density of the formazan deposits was measured by photographic densitometry [1]. The specimens were photographed in a narrow range of the visible spectrum (590 nm), using a  $\times 100$  objective (planachromatic). All the photographed material was developed at the same time. Photometry of the negatives was carried out on an IFO-451 dual-beam recording microdensitometer, equipped with integrator with discriminating device for density thresholds and with an intensimeter, by means of which the background could be adjusted to a constant level and the accuracy of measurement accordingly increased. Because of the irregular arrangement of the formazan granules and the impossibility of detecting the cell boundaries in the histochemical preparations, the activity of the enzyme was determined

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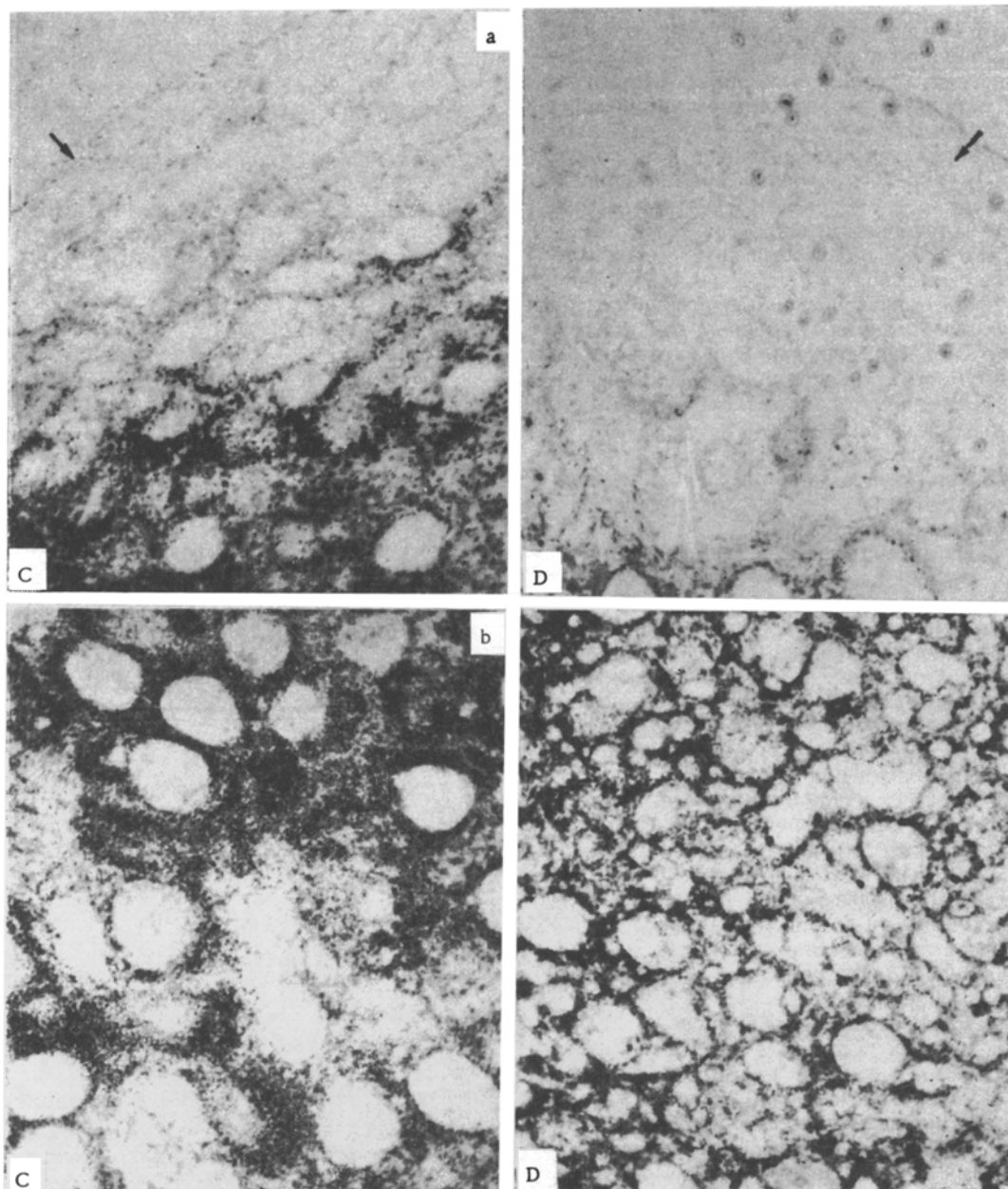


Fig. 1. Deposition of formazan granules in reaction for  $3\beta$ -OH-steroid dehydrogenase in adrenal cortex of 21-day-old rat fetuses in control (C) and after decapitation at the age of 18 days (D): a) developed zona glomerulosa (arrow marks edge of adrenal capsule); b) zona fasciculata; 1000 $\times$ .

quantitatively in an assigned area (600  $\mu^2$ ), after which it was expressed relative to a conventional cell, the relative area of which was determined in a morphological section by means of an ocular grid.

#### EXPERIMENTAL RESULTS AND DISCUSSION

In the adrenal cortex of 21-day-old fetuses cells of the zona glomerulosa and the middle part of the zona fasciculata could be distinguished sufficiently clearly. On the third day after hypophysectomy of the fetuses the most marked morphological changes were found in the cells of the zona fasciculata: a reduction in the volume of the cytoplasm and the cell nuclei, and vacuolation of the cytoplasm [4].

$3\beta$ -OH-steroid dehydrogenase was discovered throughout the cortex of the adrenals of the control fetuses, including in the bands of cells of cortical origin in the medullary part of the gland; the intensity of the reaction was greatest in the zona fasciculata. On visual

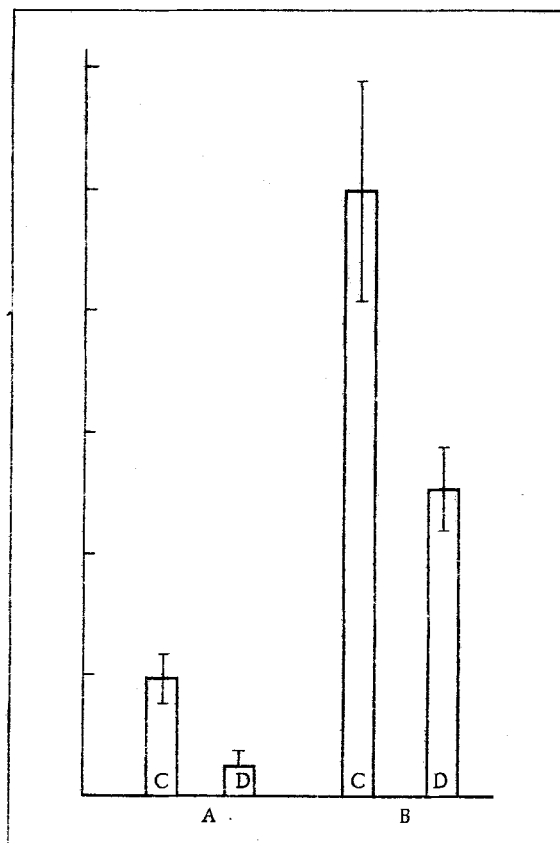


Fig. 2. Changes in  $3\beta$ -OH-steroid dehydrogenase activity in adrenal cortex of rat fetuses after decapitation. Abscissa, zones of adrenal cortex (A — glomerulosa, B — fasciculata); ordinate, enzyme activity (in instrumental units per conventional cell). C) Control 21-day-old fetuses; D) experimental 21-day-old fetuses decapitated at 18 days.

comparison the adrenals of the decapitated fetuses were distinguished by the sharp irregularity of the reaction in the zona fasciculata and the reduced enzyme activity in the zona glomerulosa (Fig. 1). The results of quantitative estimation of the histochemical reaction are given in Fig. 2. Activity of the enzyme in the zona fasciculata clearly was much higher than in the zona glomerulosa ( $P < 0.001$ ). After decapitation the activity of the enzyme per cell fell considerably ( $P < 0.01$ ) in both zones. The function of the zona fasciculata of the adrenal cortex is under the control of ACTH. This suggests that ACTH deficiency due to decapitation of the fetuses was responsible for the lowering of  $3\beta$ -OH-steroid dehydrogenase activity in the zona fasciculata of the adrenals. The results are in agreement with the observed fall in the corticosterone level in the adrenals of rat fetuses after decapitation [3].

Morphological changes in the zona glomerulosa of the adrenal cortex after hypophysectomy were much less marked than in the zona fasciculata. This agrees with observations of other workers [14]. Meanwhile the zona glomerulosa in rat fetuses at the end of uterine development evidently responds to absence of the fetal pituitary. The question of pituitary regulation of the zona glomerulosa of the adrenals has not been finally settled, but judging from data in the literature [9, 13, 16], ACTH may have a definite role in this process.

On the whole the results indicate that the steroidogenic function of the adrenal cortex in rat fetuses is under the control of the fetal pituitary and that the reduction in steroid synthesis after hypophysectomy may be connected with reduced activity of  $3\beta$ -OH-steroid dehydrogenase.

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## NITROGEN-SPARING ACTION OF LIPID EMULSIONS FOR PARENTERAL FEEDING OF ALBINO RATS WITH THYROTOXICOSIS

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The nitrogen-sparing action of lipid emulsions (intralipid and lipidin) during parenteral administration of casein hydrolysate was studied in 65 albino rats with thyrotoxicosis. If casein hydrolysate was given together with lipid emulsions and physiological regulators of metabolism the negative nitrogen balance was found to be quickly changed to positive, the free amino acid nitrogen level in the blood and tissues (skeletal muscle, heart, liver) fell, and the body weight of the animals increased.

KEY WORDS: *Parenteral feeding; lipid emulsions; nitrogenous substances; nitrogen-sparing action; thyrotoxicosis.*

Rational parenteral feeding must include not only nitrogenous substances but also sources of energy. Otherwise the nitrogenous preparations administered would be partially utilized to meet the energy requirements of the organism and this would naturally reduce the effectiveness of the parenteral feeding [5-7].

In this investigation the nitrogen-sparing action of two lipid emulsion preparations, intralipid (Sweden) and lipidin (L'vov Institute of Hematology and Blood Transfusion; B. V.

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