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Electron microscopy has revealed the presence of microtubules in the adrenocortical cells of rats. They are randomly distributed in the cytoplasm and fail to accumulate around the mitochondria or adjacent to the plasmalemma, nucleus or cytoplasmic organelles. Vinblastine (5 mg/100 g, i.v.) in 450 g Sprague-Dawley male rats, caused disruption of microtubules, and paracrystalline inclusions were found within two hours in the cytoplasm. No marked alterations were seen in other cellular constituents. The paracrystalline inclusions, which were evident in the cells of the zona glomerulosa and fasciculata, consisted of closely packed tubular structures in longitudinal sections and resembled a honeycomb pattern in cross sections. The functional significance of microtubules in the adrenal cortex is not clear. Temple and Wolff (1973) showed that vinblastine stimulates steroid production by mouse adrenal tumour cells *in vitro* and proposed that in normal adrenal cortex, microtubules restrict the access of cholesterol to the mitochondria. Antimicrotubular agents, such as vinblastine, by removing this physiologic inhibition, enhance the rate of steroid production above the normal level. The fine structural changes detected during the present study may represent the morphologic manifestations of this functional abnormality.

**67. Effect of ACTH on the steroid metabolic pools in the rat adrenal gland**

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To study *in vivo* the mechanism of ACTH action on steroid biosynthesis in the rat adrenal gland, we determined in the subcellular fractions by radioimmunoassay the metabolic pool changes of pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone and their corresponding sulfates, as well as progesterone, 17-hydroxyprogesterone, corticosterone and cortisol, establishing a correlation with the serum concentrations. To achieve this purpose we utilized 44 Sprague-Dawley strain male rats: 26 received 0.1 I.U./g weight of ACTH I.P. and the control group (18) received isotonic saline solution; both groups were decapitated at 0, 5, 10 and 20 min after injection. Our results showed at zero time the highest serum concentrations in corticosterone, pregnenolone-sulfate and dehydroepiandrosterone-sulfate, while in the mitochondria pregnenolone and also corticosterone were in greater quantity and progesterone was the highest in the microsomal fraction. The ACTH activity depleted the pools of pregnenolone and progesterone, increasing the one of corticosterone with minor modifications in the others; its maximal effect was obtained at 10 min. In contrast with previous reports, we found 17-hydroxylated compounds such as cortisol, revealing 17-hydroxylase enzyme activity. We conclude that the ACTH not only stimulates pregnenolone synthesis but also increases the enzymatic activity of other systems which utilized this compound as a substrate to produce corticosterone selectively.

**3C. Steroid biosynthesis: Ovary**

**68. Influence of hFSH and hFSH + hLH on steroidogenic enzymes in immature mouse ovaries**

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Most evidence indicates that FSH alone does not stimulate estrogen biosynthesis in immature ovaries in spite of increased follicular growth, but the influence of "biologically pure" FSH on enzymes of steroid biosynthesis has not been determined. We have studied this problem. Female mice 21-22 days old were injected for 3 days with either saline preparations having both FSH + LH activities, or the same preparations in which the LH activity had been neutralized with 2 x the neutralizing dose of anti-hCG. In one series endogenous mouse LH was neutralized by administration of anti-rLH. Each major step between cholesterol and estrogens was studied by incubating appropriate substrates with aliquots of ovarian homogenates. The major effect of FSH was to increase the enzyme activities in proportion to the general increase in protein, a growth effect. FSH + LH caused marked differential increases in cholesterol side chain splitting, 20 $\alpha$ -hydroxysteroid dehydrogenase, and aromatizing activities per mg protein. These enzyme activities per mg protein were slightly increased in the FSH groups, even with twice the dose of anti-hCG needed to inactivate the LH contamination. This would seem to indicate a slight intrinsic LH effect of FSH analogous to the MSH activity of pure ACTH. (Supported by USPHS Grant CA-10935 and a grant from Mr. S. Lunenfeld).

**69. Steroidogenesis in dispersed, superfused corpora luteal cells**

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Enzymic digestion has been used to disperse pregnant rat corpora luteal cells. The quality of dispersed cell preparations has been monitored by electron microscopy (EM), dye exclusion, and measurement of progesterin secretion during superfusion. Prolonged incubation (> 1 h) with trypsin and collagenase reduced the yield of viable cells. Reduction of exposure time to 30 min with 0.12% trypsin, 0.25% collagenase and 0.2% hyaluronidase followed by 30 min exposure to 0.2% lima bean trypsin inhibitor yielded  $0.511 \times 10^6$  viable cells per corpus luteum (25%). EM examination demonstrated intact microvilli. Mitochondria appeared normal, and smooth endoplasmic reticulum predominated over rough. Such characteristics appear compatible with active steroid secretion. Superfusion of dispersed cells, using Dulbecco's modified Eagle medium, provided evidence of *de novo* steroidogenesis. Progesterone (P) was secreted at an average rate of 26 ng/h/ $10^6$  cells, representing a 14-fold higher secretion rate than that obtained with intact corpora lutea. P exceeded 20 $\alpha$ -dihydro-P in the effluent medium by 1.8-fold. Replenishment time of the P content of the average luteal cell was estimated at about 1 h.

**70. Effect of estradiol-17 $\beta$  on progesterone biosynthesis in rhesus corpus luteum**

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There are no data on the utilization of sodium acetate as a substrate for progesterone biosynthesis in the rhesus corpus luteum. Estradiol-17 $\beta$  has been shown to decrease peripheral progesterone levels in this species. This study was designed to assess the effect of estradiol-17 $\beta$  on two indices of progesterone biosynthesis: (a) The *de novo* incorporation of sodium acetate and (b) total progesterone content, in rhesus corpora lutea of menstruation. Corpora lutea were excised,