Michael's Hospital, Toronto, Canada,* V.A. Hospital, West Roxbury, MA, U.S.A.

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

HERRERA, J., LEON, C., MORAN, L., RAUDALES, L. and BERMUDEZ, J. A., Division of Biochemistry, Department of Scientific Research (IMSS), Mexico City, D.F., Mexico

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 sustrate to produce corticosterone selectively.

3C. Steroid biosynthesis: Ovary

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

KRAIEM, Z. and SAMUELS, L. T., Biochemistry Dept., University of Utah, Salt Lake City, Utah, U.S.A.

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 × 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, 20α-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

WU, D. H. and WIEST, W. G., Dept. of Ob. Gyn., Washington University Medical School, St. Louis, U.S.A.

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 progestin 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 $0{\cdot}511 \times 10^6$ viable cells per corpus lutem (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 20α-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-17β on progesterone biosynthesis in rhesus corpus luteum

ARCHER, D. F., Department of Obstetrics and Gynaecology, University Pittsburg School of Medicine, Pittsburgh, Penn., U.S.A.

There are no data on the utilization of sodium acetate as a substrate for progesterone biosynthesis in the rhesus corpus luteum. Estradiol-17β has been shown to decrease peripheral progesterone levels in this species. This study was designed to assess the effect of estradiol-17β 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,

divided into two fractions and incubated for 3 h in Krebs–Hensleit buffer pH 7·4 with ^{14}C-sodium acetate. Estradiol-17β, 300 ng/ml was added to one incubation. The rhesus corpus luteum was found to synthesize progesterone from sodium acetate. There was no significant difference in ^{14}C incorporation into progesterone between the control and experimental incubations. Progesterone content of the corpora lutea after incubation was 23·7 (n = 12) μg/g of wet tissue. No significant difference was found between control and estradiol-17β incubations. Thus estradiol-17β did not alter either parameter of progesterone biosynthesis in rhesus corpus luteum of menstruation.

71. Steroid metabolism and conjugation in the human ovary perfused *in vitro*

STURM, G., STÄHLER, E. and GROSCH, B., Dept. of Gynaecology and Obstetrics, University of Marburg/Lahn, Germany—BRD

Normal human ovaries with graafian follicles were extirpated from women attending the Gynaecology Department with different diagnosies. Immediately after extirpation the ovaries were perfused in a recycling perfusion system using a hemoglobin-free medium. The arterial pressure and parameters of energy metabolism such as oxygen and glucose consumption during perfusion were measured. In the experiments reported here 4^{14}C-androstenedione was used as a precursor. In the perfusion medium we were able to demonstrate the sulfates of testosterone, estradiol-17β and estrone. To confirm the results the following analytical methods were used: t.l.c. of the conjugates and of the free steroids after enzymatic hydrolysis and co-crystallisation to constant specific activity. Furthermore the ovaries being in a preovulatory phase were stimulated with LH. Under these conditions we were able to determine concentrations of the steroid conjugates.

72. Ovarian steroidogenesis in the aged female mouse

ALBRECHT, E. D., Biological Sciences Section, Purdue University, Fort Wayne, Indiana 46805, U.S.A.

Virgin C57Bl female mice 3 or 12–14 months of age were mated and killed 18 days post-coitum. A significant increase in resorption sites and decrease in litter size were exhibited by the aged females. Quantitive levels of free and esterified cholesterol and histochemical and biochemical levels of the steroidogenic enzyme Δ^5-3β-hydroxysteroid dehydrogenase (3β-OH-SDH) were determined. Levels of ovarian total cholesterol were significantly higher in the older mice. Substantial 3β-OH-SDH was present histochemically in the theca interna, granulosa, interstitium, and corpora lutea of the young females. Ovaries of those older animals possessing resorption sites only or within few living fetuses were comprised predominantly of corpora lutea which exhibited only a moderate amount of 3β-OH-SDH activity histochemically. Few or no follicles and a general lack of interstitial tissue were noted in the aged females each tissue with little or no enzyme activity histochemically. The reduction in 3β-OH-SDH activity histochemically was paralleled by a decline in concentration and total content of 3β-OH-SDH biochemically in the older animals. The build up of precursor cholesterol and lack of steroidogenic enzyme histochemically and biochemically would suggest that the inability to carry fetuses to term in the aged female may relate to a lack of ovarian steroid hormone synthesis, presumably estrogen and/or progesterone.

3D. Steroid biosynthesis: Testis

73. Steroid biosynthesis in scrotal structures in a family of true hermaphrodites

GUIZAR, E., CORTES-GALLEGOS, V. and GALLEGOS, A. J. División de Biología de la Reproducción, Departamento de Investigación Científica, IMSS.

The *in vitro* biosynthesis of androgens and estrogens in three members of the same family with a true hermaphrodite syndrome was studied. The patients' ages were 15, 13 and 11 years. The genotype was XX but every other single parameter was of the opposite sex. The ovotestes were removed from the scrotum and incubated during 5 days in Eagle's growth media with 3·8 μCi of DHEA-7-^{3}H at 37 °C. Final steroid identification was carried out after separation and recrystallization to constant specific activity. The production of testosterone; Δ_4-androstenedione; 5α-dihydrotestosterone, and the three classical estrogens was confirmed. Moreover, dehydroepiandrosterone sulphate and dehydroepiandrosterone glucuronides were identified. The major steroid products were estrone > estradiol > estriol, from ovarian and testicular structures. A positive correlation was found with plasma levels of estradiol and testosterone.

74. Biosynthesis of oestradiol-17β and testosterone in rat testicular tissues *in vitro*

DE JONG, F. H., HEY, A. H. and VAN DER MOLEN, H. J., Departments of Biochemistry (Chemical Endocrinology) and Internal Medicine III, Erasmus University of Rotterdam, Rotterdam, The Netherlands

In order to assess the capability of rat testicular interstitial tissue (INT) and seminiferous tubules (TUB) to produce estradiol (E_2) and testosterone (T), endogenous levels of E_2 and T were estimated by radioimmunoassay in 800 *g* pellets of INT and TUB and in supernatants of total and dissected testes before and after incubation. Furthermore, the conversion of ^{3}H-T (2×10^6 d.p.m.) to ^{3}H-E_2 by the same fractions was investigated. ^{3}H-E_2 was isolated after 3 chromatographic steps and identified after addition of ^{14}C-E_2 and measurement of constant ^{3}H/^{14}C ratios after chemical conversions and chromatographic procedures. The highest concentration of endogenous E_2 was found in the INT 800 *g* pellet [2·2 $\pm$ 1·3 (SD) pg/mg protein (P), n = 6] and in dissection medium [0·74 $\pm$ 0·35 (SD) pg/mg P, n = 4]. T was mainly localized in the INT supernatant [134 $\pm$ 75 (SD) ng/mg P, n = 6]. During incubation of INT or TUB supernatants levels of endogenous E_2 did not increase, although it was possible to show E_2 production during incubation of total testis tissue. T was produced during incubation of total testis, TUB and INT; the production per mg P in TUB was 1% of that in INT. The conversion of ^{3}H-T to E_2 was low (about 1%) but significant in TUB and total testis, it was suppressed after addition of T and enhanced when cyanoketone, an inhibitor of T production, was added. Although E_2 was isolated mainly from INT, the latter results suggest that E_2 is mainly produced in TUB. The biosynthesis of the larger part of T takes place in INT.