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*

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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

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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

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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*

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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.