75. Steroidogenesis in quail embryonic gonads cultured *in vitro*

GUICHARD, A., HAFFEN, K. and SCHEIB, D., Maternité de Port-Royal (E.R. CNRS No. 123) Paris XIV and Collège de France, Paris V, France

Gonads from quail embryos of 10 and 15 days incubation were explanted for 24 h on a synthetic medium (Eagle) containing either pregnenolone-16-^{3}H and progesterone-4-^{14}C in equimolar quantities or DHA-4-^{14}C. The study of the conversion of pregnenolone-16-^{3}H into tritiated progesterone indicates that the enzyme system Δ_{5-4} isomerase-3β HSDH is more active in female than in male gonads but increases with developmental age. The biochemical data confirm earlier histochemical findings. DHA is formed from tritiated pregnenolone by male and female gonads. Embryonic testis produces mainly testosterone and labelled progesterone is a better precursor than is DHA or pregnenolone. Labelled oestradiol-17β and oestrone have been identified in the media of gonads of both sexes but are more abundant with ovaries. Oestrogen formation is higher with DHA than with pregnenolone or progesterone and in the male gonads, the oestrogens are synthesized with a higher yield than is testosterone, which is significant only in the 15 day testis. It seems likely that for the synthesis of sex hormones, the quail embryonic gonads use preferentially the Δ5-3β-hydroxysteroid pathway and the lower Δ5-3β-HSDH activity in male gonads effects only testosterone production. It is interesting to compare these results with structural abnormalities in the quail testis.

76. New pathway of testosterone synthesis

DE LA TORRE, B. and DICZFALUSY, E., Reproductive Endocrinology Research Unit, Swedish Medical Research Council, Karolinska sjukhuset, Stockholm, Sweden

Decapsulated testes of adult mice secrete microgram quantities of testosterone (*T*) when incubated in the presence of human chorionic gonadotrophin (VanDamme *et al.* Acta endocrinologica 74 (1973) 642). Serial radioimmunoassays revealed that the gradually increasing quantities of *T* in the incubation medium are associated with little, if any progesterone (*P*), 17α-hydroxyprogesterone (*17α-OH-P*), pregnenolone (*Δ^5-P*), pregnenolone sulphate (*Δ^5-PS*), 17α-hydroxypregnenolone (*17α-OH-Δ^5-P*) or dehydroepiandrosterone (*DHA*). Only androstenedione (*A*) showed a rise corresponding to that of *T* but at a much lower level. When incubations were repeated in the presence of carbon-14 labelled sodium acetate, large amounts of radiochemically homogenous *T* and smaller amounts of *A* were isolated. No carbon-14 labelled *P*, *17α-OH-P*, *Δ^5-P*, *17α-OH-Δ^5-P*, *DHA*, androstenediol (*Δ^5-A*), *Δ^5-PS*, dehydroepiandrosterone sulphate (*DHAS*) or androstenediol sulphate (*Δ^5-AS*) could be detected in the medium or in the tissue. The same pattern of labelling was found in the presence of non labelled *P*, *Δ^5-P*, *17α-OH-P* and *17α-OH-Δ^5-P*, used as "trapping agents". It is concluded that decapsulated testes of mice synthesize testosterone from sodium acetate by a pathway which does not involve *P*, *Δ^5-P*, *Δ^5-PS*, *DHA* or *DHAS* as intermediates. The role of *Δ^4*-sterols as possible precursors is considered.

77. *In vitro* steroid metabolic studies of testes with a 17β-reduction defect

STANCZYK, F. Z., HALL, T. D., PAUL, W. L. and GOEBELSMANN, U., University of Southern California School of Medicine, Department of Obstetrics and Gynaecology, Los Angeles, U.S.A.

Steroid metabolism and kinetics were studied in minces and homogenates of testes from a male pseudo-hermaphrodite with gynecomastia and virilization in order to investigate the testosterone (T) biosynthetic defect attributed to a testicular 17β-reduction defect (17RD) as based on *in vivo* findings. Incubations with ^{14}C-labelled precursors were carried out with and without cofactors. The metabolites were quantitated and identified by paper chromatography and reverse isotope dilution. The 17RD testes produced considerably more androstenedione (A) from pregnenolone, 17-hydroxyprogesterone and dehydroepiandrosterone (DHA) than control testes. In the 17RD testes the velocity of the conversion of A to T and of estrone (E_1) to estradiol (E_2) was about 18 and 8 times less, respectively, than in the control. Oxidation of T to A by the 17RD testes, however, was not impaired. Results of 17β-reduction rate studies of A, E_1 and DHA with varying concentrations of NADH or NADPH as well as findings in our other kinetic studies were consistent with the assumption of two 17β-hydroxysteroid dehydrogenases, one requiring NADPH and the other, NADH. The T biosynthetic block in the 17RD patient thus may be caused by a deficiency of the NADPH-requiring 17β-hydroxysteroid dehydrogenase or by a decreased affinity of this enzyme for NADPH.

78. Kinetic studies on the 3β-hydroxysteroid dehydrogenase (HSD) from rat leydig cells

WIEBE, J. P., Regulatory Mechanisms Laboratory, Zoology Department, University of Western Ontario, London, Canada

Testes from 24 rats at age 29 days were processed in a manner that separated interstitial cells from tubular and peritubular cells. The 12,000 *g* supernatant from homogenized interstitial cells was employed, without further purification, in kinetic studies of the NAD-linked HSD. Relative reaction velocities were obtained with 16 steroid substrates: 5β and 5α isomers of 3β-OH-androstan-17-one (100; 60); 5α and 5β isomers of 5α-OH-androstan-17-one (7·5; 1·5); isomers of 17β-OH-androstan-3-one and androstan-3,17-dione (0·0); Δ^5-3β-OH-androsten-17-one (DHA; 8·0); Δ^5-3β-OH-pregnen-20-one (2·1). DHA, 5β-androstan-3β-ol-17-one (A) and 5α-androstan-3β-ol-17-one (B) were employed in further substrate studies; the pH optimum, with phosphate buffer, was between 7·8 and 8·2 and the temperature optimum lay between 30° and 35°. The Q_{10} for A decreased from 3·3 (5°–15°) to 1·7 (20°–30°) and inactivation was rapid at 40°. The V_{max} values for A and B were 20 to 50 times greater than for DHA but affinity for DHA ($K_m = 1{\cdot}4 \times 10^{-5}$ M) and B ($K_m = 1{\cdot}5 \times 10^{-5}$ M) was somewhat greater than for A ($K_m = 4{\cdot}7 \times 10^{-5}$ M). Varying the [A] or [B] in the presence of constant [DHA] indicated that DHA may act as a competitive inhibitor (V_{max} unaltered, K_m increased). It is suggested that 5-H isomers of androstan-3β-ol-17-one may function as intermediates of steroid hormone biosynthesis in rat Leydig cells.