92. Conversion of testosterone-4-^{14}C into 5α-dihydrotestosterone in the human placenta *in vitro*

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Testosterone-4-^{14}C (T) was incubated with sections of the placenta and amnion and its conversion into 5α-dihydrotestosterone (DHT) was investigated. Added T-^{3}H and DHT-^{3}H following incubation served for the estimation of recovery, and the ratio $^{14}C/^{3}H$ was a comparative measure of quantitative estimation. Steroids were purified as follows: (1) thin layer chromatography (t.l.c.) on silica gel HF_{254} in the system of chloroform:methanol (99:1). (2) t.l.c. on silica gel in the system ethyl ether:benzene (70:30). (3) acetylation and t.l.c. on aluminium oxide in the system benzene:ethyl ether (80:20). (4) hydrolysis of acetates and crystallization. It has been found that amnion, in the presence of NADPH, converts T into DHT with a yield of about 0·7%, whereas placenta does not possess such properties. Furthermore it has been estimated that the quantity of unconverted T in amnion incubates is very high compared to low quantities in placental incubates. Further investigations of the problem are under way.

93. An unusual inherited form of male pseudohermaphroditism. A model of 5α-reductase deficiency in man

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We have investigated 12 families with 22,46 XY pseudohermaphrodites born with ambiguous genitalia. They masculinize at puberty without breast enlargement, have normal testes histologically, no Mullerian structures, complete Wolffian differentiation, small phallus, bifid scrotum, urogenital sinus with perineal hypospadias and blind vaginal pouch. Plasma testosterone (T) is normal; however, 5α dihydrotestosterone (DHT) is less than 2% of plasma T (normal 10%). During constant infusion of radioactive T, less than 1% is converted to DHT (normal 4–7%). The urinary 5β/5α androstane 17-ketosteroid (etiocholanolone/androsterone) ratio is markedly elevated. After glucuronidase hydrolysis, 7–14/1 (normal 0·4–2·0/1) – after glucuronidase and hot acid hydrolysis, 4·5–8·0/1 (normal 0·4–1·3/1). Urinary 5β/5α androstane (3α/3β hydroxy) 17β-hydroxysteroid ratio, 6·5–10/1 (normal 1·2–2·2/1). Analysis of pedigrees reveal inheritance as autosomal recessive. The clinical abnormality is expressed in males – one female studied shows the same biochemical defect. Carriers show a modest increase in urinary 5β/5α ratio. We postulate this condition as the distinct clinical entity of steroid 5α-reductase deficiency which may delineate the roles of T and DHT in sexual development. At a critical period in utero, masculinization of external genitalia may be DHT dependent, but Wolffian differentiation T dependent. The events at puberty may be mainly T dependent, with exception of facial hair and prostate growth which may be DHT dependent.

94. Localization of androgen metabolizing enzymes in human skin

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Human axillary skin rapidly metabolizes testosterone to the active androgen 5α-dihydrotestosterone; this may control hair growth and tissue differentiation in the axilla. Androstanediols and 17-oxosteroids are also formed. The distribution of steroid metabolizing enzymes in skin is of interest. Female axillary skin was dissected under a microscope after partial digestion with collagenase. Isolated tissue fractions were incubated with 7α-^{3}H testosterone in Krebs Improved Ringer I medium containing added cofactors. Testosterone metabolites were separated by chromatography and identified by reversed isotape dilution. The sweat gland fraction (mainly apocrine glands) contained 46% of the 17β-hydroxysteroid dehydrogenase, 90% of the 3α-hydroxysteroid dehydrogenase and 80% of the 5α-reductase. Other tissues (hairs, epidermis, sebaceous glands and residual dermis) contained lesser amounts of these enzymes. The apocrine sweat glands which are thought to be androgen controlled contain most of the testosterone metabolizing enzymes in axillary skin and form the most 5α-dihydrotestosterone. The other tissues also formed 5α-dihydrotestosterone though in the hair follicles androstenedione was the major metabolite.

95. Factors influencing the interconversion of androstenedione and testosterone in skin

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Human skin from different body sites exhibits characteristic patterns of androgen metabolism *in vitro*. Forehead skin rapidly converts testosterone to androstenedione but shows little activity in the reverse reaction; axillary skin actively converts androstenedione into testosterone. These differences could depend on the generation of NAD and NADP in the tissue or on the properties of the 17β-hydroxysteroid dehydrogenases. Incubation of slices of forehead and axillary skin under a variety of conditions expected to affect the generation of oxidized and reduced NAD and NADP did not alter the patterns of steroid metabolism. Homogenates of forehead and axillary skin only metabolized testosterone and androstenedione in the presence of added NAD^+ and $NADP^+$ (testosterone) or NADH and NADPH (androstenedione). NAD^+ and NADH were the preferred cofactors. In this system the same patterns of steroid metabolism were obtained as with whole tissue slices, suggesting that the differences in interconversion of testosterone and androstenedione may be due to the properties of different forms of 17β-hydroxysteroid dehydrogenase.

96. Studies on testosterone metabolism in liver and extrahepatic tissues of man

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The formation of hydrogenated C_{19}-steroids was investigated after incubation of (1,2-^{3}H)-testosterone (T) with tissue slices of human liver, skin, striated muscle, fat, lung, kidney, gastric mucosa and myometrium. Tissue slices of human liver, incubated without cofactors, transformed T in particular to 4-androstenedione(Δ^4-A,14-36%); 5α- or 5β-hydrogenated metabolites were formed only to a small extent. Liver slices, fortified by NAD/NADH, metabolized T to Δ^4-A(27–42%) and to 5β,3α-androsterone(5β,3α-A, 7–9%). With a NADPH-generating system T was metab-

olized to 5β,3α-A(29–41%) and 5β-androstan-3α,17β-diol (8–16%). Skin slices from the pubic area metabolized T to androsterone (4–15%), 5α-androstanedione(5–9%), 5α-dihydrotestosterone(3–17%), 5α-androstane-3α,17β-diol(2–3%) and to Δ^4-A(2–7%), but not to 5β-hydrogenated metabolites. Slices from lung and gastric mucosa as well as fat tissue transformed T only to Δ^4-A(27–70%), but to neither 5α- nor 5β-hydrogenated metabolites. Under the conditions employed, tissue slices from kidney, striated muscle and myometrium did not metabolize T to an appreciable extent. (Supported by SFB 51 of the Deutsche Forschungsgemeinschaft.)

4A 2. Steroid catabolism: Androgens—II

97. Induction of androgen-metabolizing enzymes by testosterone in female rat liver

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There is a sex difference in androgen metabolism in the liver of rats. In the cytoplasmic fraction prepared from the liver, testosterone (T) is predominantly converted to 5β-reduced metabolites in males, whereas the formation of these metabolites is low in females. The induction of the enzymes involved in androgen metabolism in the female rat liver by T was investigated. The injection of T-propionate into female rats resulted in an increase of the production of labelled 5β-reduced metabolites when 4-^{14}C-T or 4-^{14}C-androstenedione (A) was incubated with the hepatic cytoplasmic fraction. This increase was prevented by the administration of actinomycin D or puromycin. The conversion of A to T was markedly higher in males than in females when A was used as a substrate. The injection of T-propionate into female rats increased the production of T from A, whereas actinomycin D or puromycin prevented the increased production of T induced by T-propionate. These findings suggest that the induction of Δ^4-5β-steroid reductase and 17β-hydroxysteroid dehydrogenase catalyzing the interconversion T $\rightleftarrows$ A occurred by the injection of T-propionate.

98. Testosterone and progesterone metabolism in the human prostate

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Preparations of minced or homogenized human prostatic tissue with benign hyperplasia obtained surgically were incubated with several concentrations of different steroid substrates in the presence of, or without various nucleotide phosphate cofactors.

Initially [17α-^{3}H,4-^{14}C]-testosterone incubations were carried out. Major 5α-reduction was shown in all cases with minor differences between 17β-hydroxy and 17-keto metabolites as expressed by the ^{3}H/^{14}C ratios.

Comparison of [4-^{14}C]-testosterone with [4-^{14}C]-progesterone metabolism in minced preparations of a single gland was made in several cases. Major progesterone radiometabolites were 5α-reduced and identified by crystallization to constant specific activity as 5α-pregnane-3,20-dione and 3β-hydroxy-5α-prègnan-20-one.

No significant differences in the amount of testosterone 5α-reduction or metabolism was found when samples from different parts of the human prostate gland (according to J. McNeal) were used. Since testosterone 5α-reduction and accumulation of 5α-dihydrotestosterone are intimately related with benign hyperplasia in human and canine prostate, these results suggest that progesterone may be used as a competitive inhibitor of the prostatic 5α-reductase. (Supported by a Grant from C.N.A.M.T.S. and funds from Lab. Besins-Iscovesco, Paris).

99. Structural and kinetic properties of microsomal 17β-hydroxysteroid dehydrogenase

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Because of the high 17β-hydroxysteroid dehydrogenase (17β-SDH) activity associated with microsomes from guinea-pig liver kinetic and structural studies of the enzyme from this source were undertaken. Livers were homogenized in 0·25 M sucrose. The microsomal fraction (105,000 **g**, 60 min pellet) was washed successively with 0·14 M NaCl, 1·0 M NaCl and 0·1 M Na_2CO_3–0·1 M Na HCO_3 and suspended finally in sucrose. Relative activities with testosterone (T) and estradiol (E_2) did not change during the washing steps. After fractionation by centrifugation in 1·23 M sucrose over 90 per cent of the activity was in the "smooth" microsome fraction. Activity was inhibited by 6·3 mM o-phenanthroline (67%) and 0·16 mM 1,8-ANS (59%) but not by 2,2″-bipyridine, isobutyramide or pyrazole suggesting inhibition by hydrophobic interaction at the active site rather than binding to Zn. With 1·6 nM NAD V_{max} was the same for T and E_2 and equimolar mixtures of the two substrates confirming the interaction of both steroids at the same active center. Activity with NADP was less than 10% of that with NAD. The identity of V_{max} values with T and E_2 is consistent with a reaction mechanism for the two substrates involving a common rate-limiting step. (Supported by the St. Paul-Ramsey Med. Educ. and Res. Foundation).

100. Metabolism of testosterone and androstenedione in human leucocytes

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No information is available on metabolism of androgens in human leucocytes and nothing is known about the significance of steroid degradation in normal and leucaemic blood cells. We therefore studied the *in vitro* metabolism of labelled testosterone and androstenedione in granulocytes and lymphocytes of 6 healthy subjects and of 4 patients with leucaemia. The cells ($1·85–7·5 \times 10^7$) obtained by separation with the NCI-IBM cell separator were incubated for 2 h with 500 nCi ^{14}C-testosterone or ^{14}C-androstenedione in Krebs–Ringer bicarbonate buffer (3 ml) containing an NADPH regenerating system. After incubation at 37 °C the steroids were extracted and paper chromatography performed. The radioactive metabolites were then separated as trimethylsilylethers by gas chromatography. The conversion rates (in % of the substrate) were calculated from the radioactivity of the gas fractions. In all experiments