by radiogaschromatography before and after glucuronidase hydrolysis. In the rat different concentrations of T(10^{-6}–10^{-3} M) were metabolised in liver slices and microsomes to 5α- and 5α,3β-hydrogenated metabolites and in cytosol to 3α,5β-hydrogenated metabolites. In man high concentrations of T(10^{-5}–10^{-2} M) were metabolised in liver slices and cytosol, mainly to 3α,5β-hydrogenated compounds, in microsomes only to androst-4-enedione and hydroxylated metabolites. Glucuronidation of metabolites in all compartments was low. Low and physiological concentrations of T(10^{-8}–10^{-6} M) were metabolised in human liver slices to 3α,5β-hydrogenated and to a lower extent to 5α-hydrogenated compounds, in cytosol to 3α,5β-metabolites and in microsomes to 5α- and 3α,5α-metabolites. Glucuronidation of metabolites was high in tissue slices and microsomes, low in cytosol. (Supported by the DFG.)

49. Modification of testosterone metabolizing enzymes by neonatal estradiol benzoate

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Five day old rats injected with 125 μg of estradiol benzoate dissolved in 0.05 ml of corn oil (EB), or oil alone (controls) were used. At the age of 60 days each animal was injected i.v. (barbiturate anesthesia) with 5 μCi of [^{3}H]-testosterone dissolved in 50% aqueous ethanol (0.25 ml); blood was taken 1, 2, 4, 8, 16, 32, 64 and 128 min later. Plasma was separated and extracted 3 times with 5 vol. of ethyl acetate. After the combined solvent was evaporated, the residue was dissolved in methanol (5 ml) and an aliquot (1 ml) used to determine radioactivity ("free steroids"). Plasma remainder was diluted with methanol to a 70% sol. The precipitated proteins were removed by centrifugation and radioactivity was determined ("conjugated" fraction). Total metabolic clearance rate (MCRT) in the control group was 2.11 ± 1.05 min (±S.E.M.; $N = 4$) and 135.7 ± 23.9 min, respectively. Neonatal EB treatment ($N = 5$) significantly decreased the second $t_{1/2}$ which was 45.9 ± 3.9 min; the first $t_{1/2}$ was not influenced (2.44 ± 1.03 min). The disappearance of 'conjugated' fraction from blood was also more rapid. The treated/control ratio decreased from 0.95 at 1 min intervals to 0.15 at 128 min. Purification of the free fraction (two t.l.c. systems) gave testosterone fraction (about 50% total radioactivity-time dependent curve). Testosterone MCR pattern indicated a three-compartment model. Our results show that estrogen treatment very shortly after birth alters some of the enzymes concerned with metabolism of testosterone which persists until sexual maturity.

50. Medroxyprogesterone acetate metabolism by cultured rat caecal contents

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The susceptibility of MPA (17α-acetoxy-6α-methyl-4-pregnene-3,20-dione) to metabolism by intestinal bacteria has been studied. Rat caecal contents were incubated at 37°C in brain–heart infusion medium (Difco) for 3–5 days under anaerobic conditions. Thereafter, MPA (100 μg) and [1,2-^{3}H]-MPA (1.5 × 10^5 c.p.m.) were added and the incubation continued for a further 48 h. Under these conditions MPA was completely metabolised, as judged by thin-layer chromatography. Gas chromatography–mass spectrometric (GC–MS) analysis of the metabolites revealed the presence of two tetrahydro-MPA derivatives which did not form an O-methyloxime but were readily silylated to mono-trimethylsilyl ethers. This strongly suggests them to be 3 (α or β)-hydroxy-5 (α or β)-reduced MPA metabolites. Mammalian liver may also metabolise MPA by ring A reduction. Dihydro-MPA derivatives, probably 3-hydroxy-compounds were detected by GC–MS in dog bile after oral MPA administration. To what extent MPA reduction by the intestinal microflora influences MPA absorption is not known. However, preliminary studies of MPA absorption in dogs after oral administration, as judged by plasma MPA radioimmunoassay, showed ampicillin which is known to impair intestinal bacterial steroid metabolism, to have no effect on MPA absorption. (Supported by the World Health Organization and the Ford Foundation.)

51. The metabolism of norethisterone and ethinyloestradiol by rat gut wall

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Only part of an oral dose of norethisterone (N) or ethinyloestradiol (EE) reaches the peripheral circulation of rats, rabbits or women and this 'First pass' effect could be partially due to gut wall metabolism. N-[^{3}H] was administered intraduodenally *in vivo* to rats and blood samples collected from the hepatic portal vein (HPV) and carotid artery (CA). The concentration of various metabolites was higher in HPV than in CA blood and 14% of the administered dose was metabolised by the gut. As a further test of the site of metabolism, N-[^{3}H] and EE-[^{3}H] were incubated *in vitro* with everted rat gut sacs. Both steroids accumulated within the sacs and were extensively metabolised. The N metabolising system was not saturated by the addition of 170 μg unlabelled N and its capacity was not demonstrably enhanced by prior phenobarbitone treatment (80 mg/kg/day for 5 days). Over 50% of the EE was conjugated as a glucuronide. These results demonstrate that in the rat, gut wall metabolism plays a significant role in reducing the oral bioavailability of such steroids.

52. Changing proportion of spare LH(HCG) receptors in testes of rats in different stages of sexual maturation

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To understand the mechanism of increasing testosterone (T) levels during sexual maturation of rats in the absence of parallel changes in serum LH, [^{125}I]-HCG binding, cAMP and T production by the Leydig cells in response to varying concentrations of HCG were studied at days 21, 30, 45, 60 and 90. The number of HCG binding sites, 0.21 × 10^{-13} mol/mg protein at day 21 gradually increased to 0.80 × 10^{-13} mol/mg protein at day 60. A greater responsiveness of Leydig cells for cAMP was noted at days 30 and 45. Both basal and maximal T production increased as age advanced. Although the maximal T production was not different at days 45, 60 and 90, the HCG concentration needed for the purpose decreased from 27.03 pM to 6.76 pM suggesting an increase in Leydig cell sensitivity with progress in age of rats. At days 21 and 30, no apparent dissociation was observed between the testosterone response curve and [^{125}I]-HCG binding curve, though at later periods (days 45, 60 and 90) dissociation between them was clearly evident. The percent receptor occupancy for maximal T production decreased from 36.5% at day 30 to 3.5% at day 90. Thus, the proportion of spare receptors increased during sexual maturation. From these studies it appears that the spare receptors play an important role in modulating Leydig cell sensitivity to gonadotropins during sexual maturation.