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

with granulocytes and lymphocytes of healthy subjects androstenedione was converted to testosterone (2·7–4·3%), 5α-androstanedione (2·8–6·6%) and androsterone (·6–1·9%). Degradation was similar in normal and leucaemic cells. Testosterone was in every instance oxidized to androstenedione (1·3–3·4%) but it failed to serve as a substrate for the formation of 5α-dihydrotestosterone in the leucocytes of normal subjects. Only in cells of patients with leucaemia was 5α-dihydrotestosterone (1·0–2·4%) obtained as an additional metabolite of testosterone. The results indicate a different content of steroid metabolizing enzymes in normal and pathological cells.

101. *In vitro* androgen bioconversion by normal and cryptorchid rat testes

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We have studied in rats the pubertal changes in the testicular steroid enzyme activities responsible for androgen production. This study included normally developing animals as well as animals made cryptorchid surgically, during multi-phase pubertal development till adulthood. The *in vitro* bioconversion of the androgen precursors through the Δ4 pathway by testicular homogenates was used and demonstrated the effects of age in both groups of animals. In addition the kinetics of the metabolism of the androgen precursors demonstrated an early period of 30 min with rapid bioconversion, which slowed down later. The sexually immature rats had higher levels of androstenedione than testosterone in contrast to the findings in the adult animals. C-5-reduced androgens, like androstanediol and androsterone were favourably catabolized in prepubertal rats in contrast to the sexually mature animals. Animals kept cryptorchid for 4 days until sacrifice, at different stages of pubertal development showed similar patterns of *in vitro* androgen bioconversion to normal prepubertal and adult animals. Surprisingly, the percentage conversion to testosterone was elevated in these testicular tissues in comparison to the normal animals. This could be due to an enhanced pituitary gonadotropic effect, as shown in our laboratory previously after a 4-day cryptorchid period. All kinetic studies in normal rats showed a period of rapid bioconversion of the androgen precursors during the first half hour of incubation and a slowing down after that period. The age-dependent profile of kinetics was evident for both normal as well as cryptorchid testes.

102. On lipophile sulfoconjugates of DHEA

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Lipophile steroid sulfoconjugates were biosynthesized by incubation of 7α-^{3}H-DHEA sulfate or cytidine phosphosulfate 7α-^{3}H-DHEA with mitochondria or erythrocytes. The enzyme, governing the conversion of steroid sulfate into sulfatide, possessed a K_m-value of $6{\cdot}6 \times 10^{-6}$M, a pH-optimum near 8·0, and depended mainly on CTP as coenzyme and Mg^{2+} as cofactor. The diglyceride transferase, catalyzing the transformation of cytidine phosphosulfate steroid into sulfatide, exhibited a K_m-value of $3{\cdot}6 \times 10^{-6}$M, a pH-optimum of 7·6, and appeared to be independent of coenzymes. Using 7α-^{3}H-DHEA ^{35}S-sulfate and ^{14}C-carboxyl 1,2-dipalmitin for substrates a triple-labelled steroid sulfatide was obtained, the molar ratio of incorporated ^{3}H-steroid ^{35}S-sulfate to ^{14}C diglyceride approximating unity. By analysis the ratio C_{19}-steroid : sulfate : glycerol : fatty acid in isolated steroid sulfatides came close to 1 : 1 : 1 : 2. The IR- and mass-spectra of highly purified steroid sulfatides resembled those of synthetic DHEA dipalmitoyl glycerosulfate, the fragmentation pattern indicating the formation of a steroid sulfate ion, not observed in the mass spectrum of ammonium DHEA sulfate. By diffusion studies with BIO-beads the molar weight of biosynthetic steroid sulfatides was found to exceed 400.

4B. Steroid catabolism: Estrogens

103. The metabolism of ethynyl estradiol in women

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17 μCi of [9,11-^{3}H]-EE_2 and 8 μCi of [4-^{14}C]-EE_2 was administered orally and i.v. to postmenopausal or castrate individuals. The urine was collected over boric and ascorbic acids. The samples were pooled and after extraction on Amberlite XAD-2, the conjugates were purified using gel filtration on LH-20 with a $CHCl_3$: MeOH (1 : 1) 0·01M NaCl solution. Following sequential enzymatic hydrolysis of the conjugates, metabolite profiles were provided by gel filtration on LH-20 using a benzene : MeOH (85 : 15) solution. An alkylated LH-20 column with a reverse phase system completed the metabolite separation. Ethynyl metabolites were separated from non-ethynyl compounds on a silver-sulfoethylcellulose column and identification was completed using reverse isotope crystallization and/or gas–liquid chromatography—mass spectroscopy. Four conjugate peaks resulted from the LH-20 purification and were designated I, II, III, and IV in regard to their order of elution. Peak I was the dominant conjugate and was 85% hydrolyzed by Ketodase. Its metabolite profile included 2-methoxy-EE_2, 16β-OH-EE_2, 2-OH-EE_2 and EE_2, the major metabolite. Peak II was 88·8% hydrolyzed by Ketodase and its metabolite profile was similar to Peak I. The non-ethynyl metabolites of Peak I and II were four in number and represented 14·3 and 23·6% of the radioactivity, respectively. Peak III was hydrolyzed 33·3% by Ketodase and 35·8% by *Helix pomatia* sulfatase. The major metabolite in each case was 2-methoxy-EE_2. Peak IV was 40·8% hydrolyzed by *Helix pomatia* sulfatase. EE_2 was the primary metabolite.

104. The metabolic fate of mestranol and ethynylestradiol in humans

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Since it has been shown previously that mestranol (ME) can act as an estrogen exclusively after demethylation to ethynylestradiol (EE), the aim of the present work was (1) to examine extent and kinetics of the demethylation of ME in humans and (2) to accumulate data concerning the oxidative breakdown of EE in humans. (1) The demethylation of ME has been followed after i.v. administration of methoxy-^{3}H-ME. If demethylated, the tritium of this preparation is transferred to HTO and equilibrates with the body water. The