The addition of equimolar amounts of 2-hydroxy-oestradiol-17 β , 2-hydroxyoestrone or 2-hydroxy-oestriol to the incubations inhibited the enzymic formation of 3-*O*-methyladrenaline by 96%. Even at an adrenaline/2-hydroxyoestradiol-17 β molar ratio 12:1 the methylation of adrenaline by the cytosol fraction of human liver was inhibited by 93%. Similar results were obtained when noradrenaline was used as substrate. The competitive nature of this inhibition was indicated by kinetic experiments. The methylation of 2-hydroxyoestradiol-17 β to the corresponding 2- or 3-monomethyl ethers (Knuppen & Breuer, 1966) by the catechol *O*-methyltransferase of human liver was not inhibited by adrenaline or noradrenaline. Even a 20-fold molar excess of the catecholamines was without any effect.

When 2-methoxy oestrogens were added to the incubations the methylation of adrenaline to 3-*O*-methyladrenaline was also inhibited; however, this inhibition was a non-competitive one. Oestrone, oestradiol-17 β and oestriol had no effect on the methylation of adrenaline or noradrenaline.

The results obtained indicate that 2-hydroxy oestrogens, which are among the main metabolites of oestrogens in man, may have a regulatory influence on the biological inactivation of vasoconstricting substances.

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Steroid Glucuronyltransferases from the Cytosol Fraction of Human Liver

By G. S. RAO, M. L. RAO and H. BREUER. (*Institut für Klinische Biochemie, Universität Bonn, Bonn, Germany*)

UDP-glucuronate glucuronyltransferases (EC 2.4.1.17) catalyse the transfer of glucuronic acid from UDP-glucuronic acid to a large number of acceptors, resulting in the formation of water-soluble compounds.

The present investigation deals with glucuronyltransferases in the cytosol fraction (158000g) of human liver that conjugate the 16 α -hydroxyl group of oestriol and the 17 β -hydroxyl group of testosterone. Fractionation with ammonium sulphate

and filtration through Sephadex G-200 purified the oestriol-conjugating enzyme 100-fold and the testosterone-conjugating enzyme 50-fold. The two activities could not be separated from one another by conventional methods. To find out whether the two activities are due to one enzyme or to two different enzymes, several studies were carried out with the partially purified enzymes (Sephadex G-200 fraction). (1) Controlled heating (Dixon & Webb, 1964; Linn & Lehman, 1965) inactivated the two enzymic activities at the same rate. (2) Freezing and thawing also inactivated both enzymic activities at the same rate. (3) The oestriol 16 α -glucuronyltransferase had optimum pH 8.0 in tris-HCl buffer, whereas the testosterone 17 β -glucuronyltransferase did not show a well-defined optimum in the different buffers used. (4) The temperature optimum for the oestriol 16 α -glucuronyltransferase was found to be 37°C; the testosterone glucuronyltransferase revealed a plateau between 32°C and 45°C. (5) The activation energy for the glucuronidation of oestriol was 11.1 kcal/mol and that of testosterone 5 kcal/mol. (6) Oestriol did not inhibit the glucuronidation of testosterone and vice versa. (7) The mixed-substrate method (Dixon & Webb, 1964) indicated that the rate of conjugation of the different substrates when incubated together is approximately equal to the sum of the rates of the conjugation when the substrates were incubated separately. (8) The K_m value for oestriol was 11.4 μ M and that for testosterone 6.4 μ M. (9) With oestriol as substrate the K_m for UDP-glucuronic acid was 100 μ M and with testosterone as the substrate 25 μ M.

These results indicate the possible existence of two separate enzymes conjugating oestriol and testosterone. Further studies are required to characterize the two enzymic activities.

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Role of Endogenous Hormones in Uterine Binding of Oestradiol-17 β

By R. J. B. KING, A. W. STEGGLES, J. GORDON and JOANNA MARX. (*Imperial Cancer Research Fund, Lincoln's Inn Fields, London W.C.2, U.K.*)

Cytoplasm from mature uteri contains a 4S oestradiol-17 β receptor that is not present in immature animals or ovariectomized animals (Steggles & King, 1970). This indicated that oestradiol-17 β binding is influenced by endogenous

hormones and this has been studied from two aspects: (1) the ability of nuclei to accept cytoplasmic receptor; (2) the number of cytoplasmic receptor sites.

The nuclear oestradiol-17 β receptor is an acidic protein, but very little is known about the acceptor molecule for this receptor. No association of nuclear receptor with DNA was detected by sucrose-density-gradient analysis. The receptor does complex with histones, and it seems likely that the acceptor is a basic protein (King, Gordon & Steggles, 1969). When uterine nuclei were incubated with [6,7- ^{3} H]oestradiol-17 β -labelled supernatant at 37°C, ^{3} H entered the nucleus and could be extracted with 0.4M-KCl. At 0°C, very little ^{3} H entered the nucleus. Liver nuclei took up less ^{3} H at 37°C than did uterine nuclei. The ability of nuclei from mature uteri to accept [6,7- ^{3} H]oestradiol-17 β (expressed per mg of either nuclear DNA or protein) under these conditions decreased within one day of ovariectomy. Liver nuclei were not affected.

Ovariectomy of mature rats also decreased the number of cytoplasmic receptors as measured by the protamine method (Steggles, Vertes & King, 1969). One week after ovariectomy the number of cytoplasmic sites either per uterus or per mg of DNA has fallen by about half. One week after hypophysectomy the value fell to 20–30% of the controls. Adrenalectomy had no effect. None of these operations affected the number of receptor sites when expressed per mg of protein, which suggested that the number of sites decreased in proportion to the loss of cytoplasmic protein and that hypophysectomy had a greater effect than ovariectomy.

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Metabolic Clearance Rates and Conversions of Oestradiol-17 β in the Sheep

By J. R. G. CHALLIS, F. A. HARRISON and R. B. HEAP. (*Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.*)

The metabolic clearance rate of oestradiol-17 β and its interconversions with oestrone have been measured in non-pregnant women (Longcope, Layne & Tait, 1968). We have begun to investigate the metabolic clearance rate of oestradiol-17 β in non-pregnant and pregnant sheep.

Tritiated oestradiol-17 β (50 and 100Ci/mmol) was chromatographically purified and infused at a constant rate (about 1 μ Ci/min) into a jugular vein of both conscious and anaesthetized sheep. The blood concentration of labelled oestradiol-17 β reached equilibrium after 2½h, and samples were taken from the opposite jugular, haemolysed and stored at –15°C. After thawing at 4°C unlabelled oestradiol-17 β , oestradiol-17 α , oestrone, 16-oxo-oestradiol-17 β and oestriol were added to the samples, extracted with diethyl ether, partitioned to obtain a phenolic fraction and separated by repeated t.l.c. Specific radioactivities were measured before and after formation of derivatives (acetates and heptafluorobutyrate; Challis & Heap, 1969).

The whole-blood metabolic clearance rate of oestradiol-17 β in a non-pregnant anaesthetized sheep was 2117 litres/day (39 litres/day per kg body wt.). In an anaesthetized pregnant sheep (day 144 of pregnancy) this value had almost doubled. Three labelled metabolites, oestradiol-17 α , oestrone and 16-oxo-oestradiol-17 β , were identified in an extract of the 'free' and 'protein-bound' oestrogens; very low radioactivity was found in the oestriol fraction. In these and two experiments on a conscious pregnant sheep the conversion ratio of oestradiol-17 β and its labelled metabolites was calculated (conversion ratio, CBB: 100 $\times$ isotope concentrations of product/precursor). In the non-pregnant sheep the conversions into oestrone and oestradiol-17 α were similar (13 and 14% respectively). In mid-pregnancy (day 92) the conversion into oestradiol-17 α had increased, but a few days before parturition it had fallen to 4.4%, whereas the conversion into oestrone had increased to 18.9%. The conversion into 16-oxo-oestradiol-17 β was greater than 15%.

These preliminary experiments indicate that in the sheep, as in the human, the metabolic clearance rate of oestradiol-17 β exceeded the estimated splanchnic blood flow, suggesting considerable extrahepatic oestrogen metabolism, particularly in late pregnancy. Significant changes were observed in the relative concentrations of circulating labelled metabolites. The ratio of CBB oestrone to CBB oestradiol-17 α was about 1.0 in the non-pregnant sheep, but greater than 4.0 just before parturition.

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