and interconvert oestrone and oestradiol is of interest in view of the high circulating concentrations of oestrone both in the male and the female (Baird, Horton, Longcope & Tait, 1968).

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Enzymic Methylation of 2-Hydroxyoestrogens to the Corresponding 2- and 3-Monomethyl Ethers by Human Liver *in vitro*

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When 2-hydroxyoestradiol-17 β is incubated with human liver slices in the presence of *S*-adenosylmethionine, 2-methoxyoestradiol-17 β is formed as metabolite (Breuer, Pangels & Knuppen, 1961). More recently it has been shown that, in the 105 000g supernatant of rat liver, not only the 2-hydroxyl group, but also the 3-hydroxyl group, of 2-hydroxyoestradiol-17 β is methylated to a considerable extent (Knuppen & Breuer, 1966). The present study was undertaken (1) to find whether human liver is also capable of forming the 2- and 3-monomethyl ethers of 2-hydroxy oestrogens and (2) to investigate the quantitative aspects of this reaction by using various steroid substrates.

Fresh human liver was homogenized in 3 vol. (w/v) of ice-cold sucrose (0.25M); the cytosol fraction was obtained by centrifugation at 150 000g for 2h. The enzyme preparation (0.5ml) was incubated with 0.2 μ mol of 2-hydroxyoestrone, 2-hydroxyoestradiol-17 β or 2-hydroxyoestriol in the presence of *S*-adenosyl[Me-¹⁴C]methionine (1.0 μ mol), MgCl₂ (2 μ mol) and 0.5ml of phosphate buffer, pH 7.6, for 60min at 37°C under N₂. The incubation mixtures were extracted with ether-chloroform (3:1, v/v) and the combined extracts evaporated. Portions of the residues were chromatographed on formamide-impregnated paper; the isomeric monomethyl ethers of 2-hydroxyoestrone and 2-hydroxyoestradiol-17 β were separated with cyclohexane, and those of 2-hydroxyoestriol with chloroform. The 2- and 3-monomethyl ethers were identified by microchemical reactions and measured quantitatively by direct counting of the radioactivities from the paper.

All 2-hydroxylated oestrogens studied yielded the corresponding 2-monomethyl as well as the 3-monomethyl ethers. However, the ratio of the 2-methoxy to the 3-methoxy compounds depended

on the 2-hydroxylated substrates. It was 1.3 for the oestrones, 2.3 for the oestradiols and 2.8 for the oestriols. From these results it may be inferred that substitution at ring D has a directive influence on the methylation of the phenolic hydroxyl groups in ring A. The ratio of the isomeric methyl ethers of 2-hydroxyoestradiol-17 β depended also on the time of incubation: after 5min it was 1.0, after 30min 1.8, after 60min 2.3, after 90min 2.9 and after 120min 2.1. On the basis of these findings it is concluded that the two phenolic hydroxyl groups are indistinguishable functionally, as suggested by Fishman, Miyazaki & Yoshizawa (1967).

This investigation was supported by the Deutsche Forschungsgemeinschaft.

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Effect of 2-Substituted Oestrogens on the Enzymic Methylation of Catecholamines in Human Liver

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It has previously been shown that the addition of small amounts of 2-hydroxylated oestrogens to the cytosol fraction of rat liver inhibits the methylation of adrenaline and noradrenaline by the *S*-adenosylmethionine-catechol *O*-methyltransferase (EC 2.1.1.6) and thereby the formation of 3-*O*-methyladrenaline and 3-*O*-methylnoradrenaline (Breuer, Lubrich & Knuppen, 1968; Breuer, Breuer, Dahm, Knuppen & Lehmann, 1969; Knuppen, Lubrich, Haupt, Ammerlahn & Breuer, 1969). The present investigation was carried out to find out whether this interaction between oestrogens and catecholamines also occurs in human liver.

Fresh human liver was homogenized in 3 vol. (w/v) of ice-cold sucrose (0.25M). The cytosol fraction, obtained by centrifugation at 150 000g for 2h, was incubated with DL-[carbinol-¹⁴C]adrenaline hydrogen DL-tartrate, (–)-*S*-adenosyl-L-methionine iodide and MgCl₂ in phosphate buffer, pH 7.6, for 60min at 37°C under N₂. The incubations were stopped by the addition of conc. HCl and portions of the incubation mixtures were directly chromatographed on paper in the system butan-1-ol-acetic acid-water (4:1:5, by vol.). Adrenaline and 3-*O*-methyladrenaline were determined by counting the radioactivities from the paper with a radiochromatogram scanner.

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.

This investigation was supported by the Deutsche Forschungsgemeinschaft.

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Steroid Glucuronyltransferases from the Cytosol Fraction of Human Liver

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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.

This investigation was supported by the Deutsche Forschungsgemeinschaft.

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

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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