the livers of rats treated with progesterone (4mg or 8mg daily), oestradiol (20 μ g daily), or a combination of the two steroids for 28 days. Progesterone caused a significant decrease in tryptophan oxygenase activity both when given alone and in combination with oestradiol (D. P. Rose & R. R. Brown, unpublished work).

In an attempt to investigate this problem further, the effect of additions of various steroids *in vitro* on tryptophan oxygenase activity in rat liver supernatant preparations was studied. Progesterone and deoxycorticosterone, at a concentration of 0.1mM, produced a significant inhibition of enzyme activity, as did the synthetic oestrogen ethinyloestradiol. However, mestranol, the 3-methyl ether of ethinyloestradiol, did not inhibit the enzyme, even when present at a concentration of 1mM. The 11 β -hydroxylated steroids cortisol, corticosterone and fluoxymesterone (11 β , 17 β -dihydroxy-9 α -fluoro-17 α -methylandro-4-en-3-one) did not inhibit tryptophan oxygenase despite their structural similarity to progesterone and deoxycorticosterone in being Δ^4 -3-oxo steroids. The importance of a double bond in ring A is indicated by the finding that methenolone (17 β -hydroxy-1 α -methyl-5 α -andro-1-en-3-one) inhibits the enzyme, whereas mesterolone (17 β -hydroxy-1 α -methyl-5 α -andro-2-en-3-one) is without effect. Studies with various other steroids indicated that substituents remote from ring A at C-17, C-20 and C-21 are unimportant with respect to the inhibition of tryptophan oxygenase.

The enzyme inhibition by progesterone at a concentration of 0.1mM was reversed by increasing the concentration of haematin in the assay system, and evidence obtained from Lineweaver-Burk plots was consistent with competitive inhibition. The inhibitory mechanism may involve the haem cofactor of the enzyme either by the formation of a complex with the steroid or by competition for sites on the apoenzyme. Oelkers & Dulce (1964) have suggested a similar mechanism for the inhibition of tryptophan oxygenase *in vitro* by oestrone sulphate.

We are grateful to Dr M. H. Briggs, Schering Chemicals Ltd., for gifts of methenolone and mesterolone.

- Auricchio, S., Rigillo, N. & Di Toro, R. (1960). *Minerva paediat.* **12**, 1463.
 Greengard, P., Kalinsky, H. J. & Manning, T. J. (1968). *Biochim. biophys. Acta*, **156**, 198.
 Oelkers, W. & Dulce, H. J. (1964). *Hoppe-Seyler's Z. physiol. Chem.* **337**, 150.
 Rose, D. P. (1966). *Clin. Sci.* **31**, 265
 Rose, D. P. (1969). *Lancet*, **i**, 321.

The Interconversion of Oestrone and Oestradiol by Human Endometrium and Human Benign Prostatic Hyperplasia in Organ Culture

By T. A. MABIN, M. J. McMAHON and G. H. THOMAS (introduced by J. M. CROCKER). (*Department of Anatomy, University of Birmingham, Birmingham 15, U.K.*)

Interest in this laboratory has centred on the use of organ culture in the study of the peripheral metabolism of the sex hormones. The rabbit uterus in culture is able to interconvert oestrone and oestradiol, achieving an equilibrium favouring oestradiol (Russell, Macdougall & Thomas, 1969; Crocker & Thomas, 1970). These studies have now been extended to include human endometrium and human benign prostatic hyperplasia.

Irrespective of whether human endometrium cultured in the presence of labelled oestrone or oestradiol, the major radioactive product extracted from the tissue was oestrone. For example, endometrial curettings (day 11 of the cycle) cultured for 24h with 100nM-, 10nM- and 1nM-[6,7- 3 H]oestrone gave oestrone/oestradiol ratios of 12 ± 2 , 12 ± 3 and 17 ± 5 respectively; the corresponding ratios for tissues cultured with [6,7- 3 H]oestradiol at these concentrations were 8.0 ± 0.4 , 7.4 ± 0.7 and 7.7 ± 0.9 . Thus the ratios were not markedly affected by the concentration of oestrogen used, but were consistently higher in the oestrone experiments than in the oestradiol experiments. This difference was also noted with explants at other stages of the cycle (from day 5 to day 26) and also when the endometrium was cultured for 2 days with unlabelled oestrogen before 1-day culture with the labelled steroid.

Human benign prostatic hyperplasia also was able to interconvert the two oestrogens, but compared with the above results there was a greater divergence between the ratios obtained for tissues cultured with oestrone and oestradiol. Oestrone/oestradiol ratios of 9.4 ± 1.4 and 13.9 ± 0.5 were obtained after culture for 24h with respectively 100nM- and 1nM-[6,7- 3 H]oestrone, and 1.2 ± 0.1 and 1.7 ± 0.2 in the corresponding experiments with [6,7- 3 H]oestradiol. Essentially the same results were obtained when the tissue was first cultured for 24h with unlabelled oestrogen.

Although the ratios in this work showed satisfactory consistency between experiments, the uptake of radioactivity by both tissues was more subject to variation. These variations may reflect indigenous differences in the tissue architecture or the degree of success in preserving the explants during culture.

The fact that both tissues are able to concentrate

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).

Baird, D., Horton, R., Longcope, C. & Tait, J. F. (1968). *Perspect. Biol. Med.* **11**, 384.

Crocker, J. M. & Thomas, G. H. (1970). *Biochem. J.* **117**, 24P.

Russell, S. L., Macdougall, B. R. M. & Thomas, G. H. (1969). *Biochem. J.* **115**, 45P.

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

By R. KNUPPEN, O. HAUPT and H. BREUER.
(*Institut für Klinische Biochemie, Universität Bonn, Bonn, Germany*)

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.

Breuer, H., Pangels, G. & Knuppen, R. (1961). *J. clin. Endocr.* **21**, 1331.

Fishman, J., Miyazaki, M. & Yoshizawa, I. (1967). *J. Am. chem. Soc.* **89**, 7147.

Knuppen, R. & Breuer, H. (1966). *Hoppe-Seyler's Z. physiol. Chem.* **346**, 114.

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

By H. BREUER, R. KNUPPEN and O. HAUPT.
(*Institut für Klinische Biochemie, Universität Bonn, Bonn, Germany*)

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.