decreased type I and type II hydrogen approximately equally. 11-Deoxy-17-hydroxycorticosterone, progesterone and testosterone decreased type I hydrogen, but had hardly any effect on type II hydrogen.

Such analyses may provide a means for demonstrating how steroids can modify the production of tissue hydrogen. The present results support the notion of two types of NADPH hydrogen, since each type can be affected differently.

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Differential Effects of Reduced Nicotinamide-Adenine Dinucleotide Phosphate on Cytoplasmic-Mitochondrial Interactions

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The study of interactions between oxidation in the cytoplasm and the mitochondria is facilitated by using intact tissue sections. It was shown (Butcher, 1969) that mitochondrial hydrogen transport from the oxidation of succinate is greatly enhanced if NADPH is oxidized simultaneously in the cytoplasm. The NADPH may be generated in the sections by the addition of NADP⁺ and either glucose 6-phosphate or 6-phosphogluconate, or it may be added exogenously.

Sections of rat liver (10 μ m) were incubated at 37°C for 30 min in air in media containing neotetrazolium (as hydrogen acceptor) and polyvinyl alcohol (20%, w/v, as stabilizer) in glycylglycine buffer, pH 8.0. To some media (a) only succinate was added; to others (b) a source of NADPH; others received both these components to test whether the resultant activity was greater than the sum of (a) and (b) tested separately. The same tests were done in the presence of either pregnenolone or cortisone (0.1 mM). Pregnenolone decreased the activity caused by oxidation of glucose 6-phosphate by about 30% but had no effect on that found when 6-phosphogluconate was the substrate. Cortisone had no inhibitory effect. Neither of these steroids affected the simple oxidation of succinate. Moreover, the inhibition of glucose 6-phosphate dehydrogenase activity, although small, resulted in 90–100% inhibition of the enhanced mitochondrial hydrogen transport, i.e. the interaction between

oxidation in the cytoplasm and the mitochondria. In contrast, decreasing the glucose 6-phosphate oxidation in the absence of pregnenolone had little effect.

The amount of hydrogen produced from glucose 6-phosphate oxidation in the presence of phenazine methosulphate, i.e. type I+type II hydrogen as described by Altmann & Chayen (1970), was diminished by pregnenolone to give the same value as was obtained in the absence of phenazine methosulphate (type I hydrogen). It seems possible that pregnenolone could be inhibiting type II hydrogen generated from glucose 6-phosphate dehydrogenation specifically and that it is this fraction of hydrogen, or of NADPH, that is responsible for the cytoplasmic-mitochondrial interactions. Alternatively the interaction could require NADPH formed from 6-phosphogluconate dehydrogenation, or added exogenously, and that pregnenolone is inhibiting the formation of 6-phosphogluconate from glucose 6-phosphate (Altmann & Robertson, 1970). Either way these results strongly suggest that NADPH is not treated as a single entity in tissues.

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Inhibition of Tryptophan Oxygenase in Rat Liver By Steroid Hormones

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Pregnant women and normal human subjects treated with oestrogens show evidence of an increased capacity for the conversion of L-tryptophan into nicotinic acid ribonucleotide. This has been attributed to an increased activity of liver tryptophan oxygenase (EC 1.13.1.12) brought about either by the direct action of oestrogens, or indirectly via elevated corticosteroid concentrations (Rose, 1966, 1969). Tryptophan oxygenase activity is elevated in the liver of pregnant rats (Auricchio, Rigillo & Di Toro, 1960), but treatment with a combination of oestrone and progesterone failed to produce an increase in the activity of the enzyme (Greengard, Kalinsky & Manning, 1968). In a further study tryptophan oxygenase activity was determined in supernatant fractions prepared from

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.

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

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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