6-phosphogluconate dehydrogenase activities in intact 10 μ m sections of rat liver, all at concentrations of 0.1 mM: dehydroepiandrosterone, 11-deoxy-17-hydroxycorticosterone, pregnenolone, 17 α -hydroxypregnenolone, progesterone, hydrocortisone, testosterone, corticosterone, cholesterol, cortisone, 17 β -oestradiol, oestriol, 11 α -hydroxyprogesterone. In both the absence and the presence of phenazine methosulphate the first eight steroids all depressed the activity with glucose 6-phosphate as substrate by between 20–50% over a 15 min incubation. However, none of the steroids had any effect on the 6-phosphogluconate dehydrogenase activity. The lower activities obtained in the presence of phenazine methosulphate mean that the total amount of hydrogen being generated from glucose 6-phosphate oxidation is less in the presence of the effective steroids.

The following scheme represents the oxidation of glucose 6-phosphate:

It is evident that glucose 6-phosphate dehydrogenase activity (1) will result in the production of 6-phosphogluconate after the lactone has been hydrolysed by lactonase (2). Hence the activity recorded with glucose 6-phosphate as substrate is, in part, due to 6-phosphogluconate dehydrogenase (3), since the substrate for the latter enzyme is produced during the glucose 6-phosphate dehydrogenase reaction according to the above scheme (Glock & McLean, 1953).

The steroids could be acting (a) by decreasing the activity of the glucose 6-phosphate dehydrogenase (1), or (b) by blocking the lactonase reaction (2). The latter would result in a lower activity with glucose 6-phosphate as substrate, since the 6-phosphogluconate dehydrogenase would now be deprived of its substrate. Under both mechanisms the activity of 6-phosphogluconate dehydrogenase (3), when tested separately, would be unaffected. Blocking the lactonase reaction might result in the eventual 'switching-off' of the glucose 6-phosphate dehydrogenase and hence of the whole pentose shunt pathway.

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Evidence for Two Types of Hydrogen Atom in Reduced Nicotinamide-Adenine Dinucleotide Phosphate Arising from Glucose 6-Phosphate Oxidation, Based on the Inhibitory Action of Certain Steroids

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Glucose 6-phosphate dehydrogenation results in the production of NADPH. The NADPH hydrogen can be detected in tissue sections by its capacity to reduce tetrazolium salts (Altmann, 1969a,b). The addition of phenazine methosulphate results in an increased flow of NADPH hydrogen, this higher activity being identical with that produced in homogenates of the same tissue, and equivalent to the total hydrogen being generated (Altmann, 1969c). Thus, in the absence of phenazine methosulphate, not all of the hydrogen is detected as reduced tetrazolium (formazan). We may call that hydrogen which reached the tetrazole in the absence of phenazine methosulphate 'type I' hydrogen, and the extra hydrogen which is only seen in the presence of phenazine methosulphate 'type II' hydrogen.

Altmann & Robertson (1970) have shown that in rat liver certain steroids affect the amount of hydrogen deriving from glucose 6-phosphate oxidation, but that they have no effect on the hydrogen deriving from 6-phosphogluconate oxidation, when tested over a 15 min period. These experiments have been repeated with an incubation time of 2 min, since at such short times the interference from 6-phosphogluconate dehydrogenase activity (Altmann & Robertson, 1970) does not occur (F. P. Altmann, unpublished work). In some experiments, pregnenolone, although having no effect on the activity in the absence of phenazine methosulphate (type I hydrogen), caused a significant depression of the activity in the presence of phenazine methosulphate (type I + type II hydrogen), suggesting different properties for the two types of hydrogen.

As a first approximation towards clarifying these effects, the eight effective steroids (Altmann & Robertson, 1970) were tested on glucose 6-phosphate dehydrogenase activity for various times up to 15 min and compared with controls. The amounts of type I and type II hydrogen were calculated and the differences from the control were plotted against time.

Corticosterone, hydrocortisone, dehydroepiandrosterone, pregnenolone and hydroxy pregnenolone

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