barrier') and medium-chain fatty acids intramitochondrially (see Tubbs & Garland, 1968). This localization may enable medium-chain fatty acids (or their CoA derivatives) to compete more successfully than long-chain fatty acids with the inhibitor when high substrate concentrations are used. By contrast, this apparent chain-length specificity is not found with the much lower acylcarnitine concentrations (20–100 μM) used in polarographic experiments. It is therefore no longer necessary to assume that inhibition of β -oxidation by pent-4-enoic acid involves a stage catalysed by enzymes with different chain-length specificities (Sherratt, 1969). Indeed, preliminary results that 0.1 mM-pent-4-enoyl-CoA inhibits thiolase (EC 2.3.1.16) by about 50% with 0.03 mM-acetoacetyl-CoA as substrate may partly explain inhibition of β -oxidation.

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Characteristics and Partial Purification of the Corticosteroid-Binding Protein Extracted from Rat Liver

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Cortisol and corticosterone are believed to bind tightly to rat liver protein (Bellamy, 1967). It has been shown (Sanyal & Snart, 1968; Snart, Sanyal & Agarwal, 1970) that the 200 000g supernatant fraction obtained from rat liver contains two tight-binding receptor sites having association constants of the order 10^{10} l/mol and 10^8 l/mol. It has been suggested that these receptor sites may be physiologically active on the basis of the dose-response characteristics of respiratory and enzymic changes induced by the hormone (Dalton & Snart, 1970).

The 200 000g supernatant fraction prepared from rat liver was equilibrated with [^3H]corticosterone. Fractionation on a 5–20% sucrose density gradient revealed a single broad band of bound radioactivity in the range 5–8S. However, fractionation on a Sephadex G-200 column (0.8 cm $\times$ 100 cm) showed labelled steroid bound to three protein fractions having molecular weights of the order 200 000, 100 000 and 50 000, as estimated from their retention characteristics.

Cochromatography on DEAE-cellulose of 200 000g supernatant fraction and rat serum, preincubated with [^3H] and [^{14}C]corticosterone respectively, showed that the elution of the bound [^3H]corticosterone preceded that of [^{14}C]corticosterone bound to transcortin in the serum.

Sephadex G-200 chromatography of a supernatant fraction prepared from animals injected 1 h before removal of the liver with [^3H]corticosterone in the presence of unlabelled carrier revealed only two clear peaks of bound steroid, corresponding to fractions of molecular weights 200 000 and 100 000, most of the bound radioactivity being associated with the latter.

The protein has been partially purified by sequential treatments involving BaCl_2 precipitation of particulate material, gel filtration, $(\text{NH}_4)_2\text{SO}_4$ precipitation, hydroxyapatite, DEAE-cellulose and Sephadex G-200 chromatography, which have resulted in about 200-fold purification. The protein retained by the DEAE-cellulose would appear to correspond to the unit of molecular weight 50 000. Work is in progress to determine the interrelationship of these protein units.

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The Effects of Various Steroids on Pentose-Shunt Dehydrogenase Activity

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Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase can be quantitatively demonstrated in intact tissue sections by following the reduction of tetrazolium salts to insoluble coloured formazans, which are precipitated in the section (Altmann, 1969a,b). The NADPH hydrogen travels along the microsomal cytochrome P-450 system (Slater, 1968; Smuckler, Arrhenius & Hultin, 1967) until it reaches the electrode potential of the tetrazole being used, when reduction, and precipitation of the formazan, occur. The addition of the electron carrier phenazine methosulphate to the incubation medium results in an increased activity. Under these conditions the activity is exactly equivalent to that which is demonstrable in homogenates of the same tissue, being equal to the total hydrogen generated (Altmann, 1969c).

The following steroids were tested for their effects on glucose 6-phosphate dehydrogenase and

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