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 μ M) 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.

We thank the British Diabetic Association for financial support.

Holland, P. C. & Sherratt, H. S. A. (1969). *Biochem. J.* **114**, 67P.

Senior, A. E., Robson, B. & Sherratt, H. S. A. (1968). *Biochem. J.* **110**, 511.

Sherratt, H. S. A. (1969). *Br. med. Bull.* **25**, 250.

Tubbs, P. K. & Garland, P. B. (1968). *Br. med. Bull.* **24**, 158.

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

By M. K. AGARWAL, R. E. SHEPHERD and R. S. SNART. (*Department of Zoology, University of Sheffield, Sheffield S10 2TN, U.K.*)

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 [3 H]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 [3 H]- and [14 C]-corticosterone respectively, showed that the elution of the bound [3 H]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 [3 H]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 BaCl₂ precipitation of particulate material, gel filtration, (NH₄)₂SO₄ 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.

Bellamy, D. (1967). *Mem. Soc. Endocr.* **15**, 43.

Dalton, T. & Snart, R. S. (1970). *J. Endocr.* (in the Press).

Sanyal, N. N. & Snart, R. S. (1968). *J. Endocr.* **40**, xvi.

Snart, R. S., Sanyal, N. N. & Agarwal, M. K. (1970). *J. Endocr.* (in the Press).

The Effects of Various Steroids on Pentose-Shunt Dehydrogenase Activity

By F. P. ALTMANN and A. ROBERTSON. (*Division of Cellular Biology, The Kennedy Institute, London W.6, U.K.*)

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