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The Form of Retinol-Binding Protein in Human Serum

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Retinol-binding protein as isolated from human serum by chromatography on DEAE-Sephadex (Alvsaker *et al.*, 1967; Kanhai *et al.*, 1968; Peterson, 1971) and on arginine-Sepharose (Kirby *et al.*, 1971) is found in association with thyroxine-binding prealbumin in the same protein fraction. It has been shown that these two proteins as isolated can form a 1:1 molar complex (Raz & Goodman, 1969) in solution under conditions of pH, temperature and ionic strength that suggest that retinol-binding protein may be present as such in the original serum. However, its behaviour with changes in ionic strength and pH under electrophoresis, as reported (Raz *et al.*, 1970; Peterson, 1971) and in our own experiments to be discussed, is sufficiently variable to indicate that the association is far from simple and may arise as an artifact during isolation, and not necessarily exist as such in normal serum. Human serum has therefore been examined directly by several mild physicochemical techniques to examine whether retinol-binding protein is free or combined with thyroxine-binding prealbumin.

Immunoelectrophoresis was conducted on Agarose gel with 0.05 M buffer systems at pH 5.5, 7.5 and 8.9. In every case two separate and distinct zones corresponding to free retinol-binding protein and thyroxine-binding prealbumin were revealed by precipitin formation against the corresponding antisera. Immunodiffusion was also carried out on Ouchterlony plates with either whole serum alone or serum admixed with different concentrations of purified rabbit antiserum to either retinol-binding protein or thyroxine-binding prealbumin. In each test retinol-binding protein and thyroxine-binding prealbumin were observed to diffuse as if separate entities. Again, serum diluted twofold with physiological saline was subjected to ultrafiltration through an Amicon UM20 and PM30 membranes. The ultrafiltrate was examined and found to contain retinol-binding protein but not thyroxine-binding prealbumin by immunological reaction against the appropriate specific rabbit antisera.

This evidence, together with the findings of free retinol-binding protein secreted into urine of patients with tubular proteinuria (Peterson & Berggard, 1971), indicates that retinol-binding protein is largely in the free form in human serum.

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The Function of Cortisone Reductase with 20-Oxo Steroid Substrates Differing in Ring A

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Kinetic measurements are described for the reduction of certain 20-oxo steroids by cortisone reductase (EC 1.1.1.53). Changes in the structure of the substrate that altered the shape of the A ring (particularly the region of the A/B ring junction) caused changes in the apparent K_m and apparent V_{max} , but particularly in the former. On the other hand, the introduction of highly polar and bulkier groups at C-3 by conversion of 3 β -hydroxypregn-5-en-20-one into the sulphuric acid or succinic acid half ester had little effect on the apparent K_m and somewhat decreased the apparent V_{max} . Of the substrates in this study, the best (pregn-4-ene-3,20-dione, K_m about 1 μ M, V_{max} about 6.1 μ mol/min per mg at 25°C at pH 7.0 with 150 μ M-NADH) was utilized by the enzyme about 8 times as efficiently as the worst (3 β -hydroxy-5 β -pregnan-20-one, K_m about 4.9 μ M, V_{max} about 3.4 μ mol/min per mg at 25°C at pH 7.0 with 150 μ M-NADH). It is suggested that in the enzyme-bound steroid the A ring may lie at the extremity of a binding site.

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