cytoplasm increased with incubation time, with the light-particle fraction gaining proportionately more radioactivity at the expense of the cytoplasm. The particulate fractions obtained from mammary slices incubated for 60 min with ^{125}I -labelled prolactin have been incubated with trypsin, collagenase, deoxyribonuclease I or ribonuclease and the displacement of ^{125}I has been measured. Trypsin caused the largest displacement from both total and heavy-particle fractions, but collagenase and deoxyribonuclease I also had a significant effect on the heavy-particle fraction.

Cytoplasm from these experiments has been fractionated on columns of Sephadex G-200, to identify the binding of intracellular ^{125}I -labelled prolactin by soluble cell proteins. Radioautography is also being used to locate ^{125}I -labelled prolactin in mammary tissue slices after incubation.

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Some Properties of Colchicine-Binding Protein(s) ('Microtubule Protein') in Soluble and Particulate Preparations of Mammalian Brain

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The kinetic properties and stability of colchicine-binding proteins present in soluble extracts of brain have been extensively studied (Borisy & Taylor, 1967a,b; Weisenberg *et al.*, 1968; Wilson & Friedkin, 1968; Wilson, 1970). In contrast, little is known about the properties of membrane-bound colchicine-binding activity, which accounts for a considerable proportion of that present in homogenates of immature and adults brain (Lagnado *et al.*, 1971a,b; see also Feit & Barondes, 1970).

Further work shows that Mg^{2+} (final concn. 1–10 mM) markedly enhance colchicine-binding activity in both soluble and particulate fractions derived from rat brain homogenates prepared in PMS buffer (0.32 M-sucrose in 1 mM-sodium phosphate buffer, pH 6.5) after high-speed centrifugation. However, the colchicine-binding activity of both soluble and particulate fractions prepared in 20 mM-phosphate buffer, pH 6.8, containing 100 mM-sodium glutamate was less than half that seen in PMS buffer, even though the stabilizing effects of the phosphate-glutamate buffer seen after storage of the preparations at -10°C (cf. Wilson, 1970) was confirmed. The protective effects of GTP on colchicine-binding

activity was especially evident for the soluble fraction in the absence of added Mg^{2+} .

The kinetic properties and stability of colchicine-binding activity of samples prepared in PMS buffer were studied at various temperatures (between 2° and 55°C). Maximum values for colchicine-binding activity were obtained after incubation for 1.5–2.5 h at all temperatures tested for both soluble and particulate preparations, highest values occurring at 37°C . However, stability studies showed that the half-life of colchicine-binding activity for particulate preparations was at least twice that seen for soluble preparations at several temperatures. It was also found that preincubation of both soluble and particulate preparations at 1°C (before assay at 37°C) was accompanied by an initial increase of colchicine-binding activity, reaching a maximum of about 200% of control values at 3 h.

Relatively low concentrations (1–50 μM , final) of vinblastine or vincristine stabilize colchicine-binding activity in soluble extracts of chick-embryo brain (Wilson, 1970). Similar results are found for both the soluble and particulate preparations of rat, guinea-pig and rabbit brain. Moreover, inclusion of these alkaloids (at a final concn. of 50 μM) in the initial homogenizing medium (PMS buffer) caused a remarkable redistribution of colchicine-binding activity after high-speed centrifugation: about 95% of the activity was recovered in the particulate fraction, as compared with values of 30–45% in the absence of alkaloid. This phenomenon was not seen in the absence of added Mg^{2+} and was not due to the preferential activation of the particulate colchicine-binding activity. Indeed, the addition of vincristine (50 μM) to a high-speed supernatant containing Mg^{2+} also induced the precipitation of colchicine-binding activity after recentrifugation.

The present results, together with additional observations on the properties of membrane-bound colchicine-binding proteins, are consistent with the view that particulate colchicine-binding activity may represent a stabilized form of microtubule protein (Lagnado *et al.*, 1971b). The possible significance of these findings in relation to the role of microtubules in nerve tissue will be discussed.

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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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