Bilirubin-Binding Properties of Plasma and Liver Cytosol Preparations from Rats

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In plasma, bilirubin is known to be bound to albumin (Bennhold, 1932). Recent studies (Meuwissen *et al.*, 1966; Grodsky, 1967; Levi *et al.*, 1969) have revealed an association of the pigment with soluble macromolecular factors, probably proteins, present in rat liver cytosol preparations.

The bilirubin-binding capacity (Meuwissen & Heirwegh, 1970) of both preparations obtained from rats has been measured. A modification of this batch method, based on distribution of the pigment between an insoluble adsorbent (Sephadex G-10) and soluble binding factors, also permits measurement of the bilirubin-binding affinity of these samples. Total bilirubin concentration was measured by the method of Van Roy *et al.* (1971).

With these methods it was demonstrated that, in both plasma and cytosol, bilirubin is subjected to reversible association with soluble binding factors.

The total bilirubin-binding capacity of rat plasma is about 4×10^{20} binding sites/l without significant difference between Gunn and Wistar rats. As for human albumin (Jacobsen, 1969) evidence has been obtained for the existence of one strong binding site/molecule of rat albumin. The affinity constant of this site is about $2 \times 10^7 \text{ M}^{-1}$, irrespective of the strain of the animal.

In contrast, bilirubin-binding parameters of liver cytosol show much more variability. Bilirubin-binding capacity of Gunn-rat liver cytosol is about twice the value measured in similar preparations from Wistar rats. Irrespective of the strain of rat, cytosol obtained from fed animals has a binding capacity at least twice that found for starved litter mates. Depending on the feeding status and on the strain of the rat, the liver cytosol binding capacity ranges between 2 and 10% of the plasma binding capacity. The latter is not affected by feeding status. In both plasma and cytosol, native bilirubin concentrations are always lower than the bilirubin-binding capacity.

In cytosol different classes of binding sites are present, as indicated by a strong positive concentration-dependence of the measured binding affinity. Only apparent association constants could therefore be calculated, the values of which are of the same order of magnitude as the association constant of plasma.

Additional evidence that rat liver cytosol is heterogeneous in its content of bilirubin-binding sites was obtained by physical separation of the binding factors (Meuwissen *et al.*, 1972).

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Uptake and Binding of ^{125}I -Labelled Prolactin by Mammary Tissue

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In the rabbit prolactin plays a key role in initiating lactation, and the mechanism by which this hormone acts in stimulating the extensive changes in mammary biosynthetic activity during lactogenesis is therefore of interest. The experiments described here form part of a comprehensive study of the biochemical and morphological consequences of the action of prolactin on mammary tissue (Fiddler & Falconer, 1970; Fiddler *et al.*, 1971).

Prolactin (NIH PS8) was iodinated with ^{125}I (Yalow & Berson, 1969), and used within 14 days of preparation. Slices of mammary tissue (0.5 mm thick) were prepared from 12–15-day-lactating rabbits by using a Mickel tissue chopper. Incubation was carried out in Tyrode's solution, pH 7.4, at 37°C with gentle shaking. Uptake of ^{125}I -labelled prolactin into the tissue occurred rapidly over 60 min, with a smaller increase between 60 and 180 min. Addition of an increased quantity of ^{125}I -labelled prolactin or of 100 ng or more of normal prolactin decreased the proportion of ^{125}I -labelled prolactin bound. The addition of KI to the incubation medium had no effect. A low rate of degradation (2%/h) of ^{125}I -labelled prolactin by mammary slices was observed.

A comparison of uptake of ^{125}I -labelled prolactin by slices of liver and muscle showed active uptake by both tissues, the highest uptake occurring in liver.

Homogenization of mammary slices after incubation, followed by centrifugal fractionation (7000g-min and 90000g-min), was employed to study the progressive increase in labelling of subcellular particles. The radioactivity (c.p.s./g) of the heavy-particle and light-particle fractions and of the cell

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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