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