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## Effect of Bilirubin on Binding of Thyroxine by Human Serum Albumin.* (30074)

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The binding of thyroxine to serum albumin can be attributed in part to the electrostatic interaction of anionic portions of the thyroxine molecule with cationic lysyl $\epsilon$-amino groups on the protein(1). It was shown previously that various anionic compounds such as unesterified fatty acids, 2,4-dinitrophenol and salicylate have the ability to displace thyroxine from binding sites on serum albumin(2). Bilirubin is an anionic compound that appears to be bound principally to albumin while circulating in human blood(3). It seemed of interest to study the binding of thyroxine in the presence of bilirubin to note whether the latter compound is capable of displacing thyroxine from albumin.

*Materials and methods.* $I^{131}$-L-thyroxine was obtained from Abbott Laboratories, Oak Ridge. Nonradioactive L-thyroxine was purchased from Aldrich Chemical Co. Bilirubin was purchased from Mann Research Laboratories, Inc. Human serum albumin (Cohn Fr. V, Batch No. 1984) was obtained from the American Red Cross through the courtesy of Dr. James H. Pert.

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Equilibrium dialysis with $I^{131}$-L-thyroxine was used to study binding as described previously(1,2,4). All experiments were performed at 30° in 0.06 M potassium phosphate buffer, pH 7.4. Further details about the dialysis method are given in the legend to Fig. 1. The bilirubin solutions were made up fresh in 0.04 N NaOH before each experiment. The binding data were analyzed in the usual way(1,5) by plotting $\bar{v}$ *vs* $\bar{v}/(A)$ as shown in Fig. 1. The experimental data plotted in Fig. 1 are the means of duplicate determinations.

*Results.* Fig. 1 shows the effect of bilirubin on the binding of thyroxine by human serum albumin at pH 7.4 and 30°. Each intercept on the $\bar{v}/(A)$ axis in Fig. 1 (the $\sum n_i k_i$ value(1,5)) represents the overall affinity of the protein for thyroxine under the conditions of the particular experiment. It can be seen from Fig. 1 that thyroxine binding to albumin is reduced in the presence of bilirubin. For example, bilirubin, at a molar ratio to albumin of 1/1 reduces the $\sum n_i k_i$ value for thyroxine binding from $7.6 \times 10^5$ to $5.9 \times 10^5$, a reduction of 22% in the binding affinity of the protein for thyroxine. Table I gives the reduction in thyroxine binding produced by each of the concentra-

TABLE I. Ability of Bilirubin to Displace Thyroxine from Albumin.

| Molar ratio bilirubin / albumin | Calculated serum bilirubin* | Decrease in thyroxine binding |
|---|---|---|
| | (mg/100 ml) | (%) |
| 1/1 | 35 | 22 |
| 2/1 | 70 | 54 |
| 3/1 | 105 | 66 |

* Based on a concentration of $6 \times 10^{-4}$ M (4 g/100 ml) for albumin in human serum.

tions of bilirubin investigated. Each molar ratio of bilirubin studied (Fig. 1) can be considered to represent a specific bilirubin level in serum and this is given in column 2 of Table I.

*Discussion.* In common with the other anions studied previously(2), bilirubin can displace thyroxine from binding sites on serum albumin. Compared to the other anions investigated(2), bilirubin is about as effective as linoleic acid in reducing thyroxine binding by albumin (Fig. 2, Ref. 2).

Albumin would normally bind about 15% of the total thyroxine circulating in human serum(6). The remainder of the thyroxine would be bound to thyroxine-binding globulin (TBG) and thyroxine-binding prealbumin (TBPA)(6,7). Bilirubin in serum appears to be bound solely to albumin(3) and would not be expected to affect thyroxine binding by either TBG or TBPA. From the results given in Fig. 1 and Table I it is estimated that bilirubin at a serum level of 35 mg/100 ml (Table I) would reduce the total protein-bound thyroxine in serum by about 3%, *i.e.*, 22% of the 15% bound to albumin. A bilirubin level in serum of 35 mg/100 ml is highly unphysiological and is encountered only rarely in some diseased states(8,9). From this it appears that under ordinary conditions bilirubin would cause little interference in the transport of thyroxine in blood.

Although the mechanism of action of thyroxine is not known, recent studies indicate that the thyroid hormone stimulates the incorporation of amino acids into tissue protein(10,11). Campbell *et al*(12) have investigated the effect of various thyroxine analogs on amino acid incorporation into liver protein in an *in vitro* cell-free liver preparation. They report the following order of effectiveness of different analogs in stimulating amino acid incorporation into liver

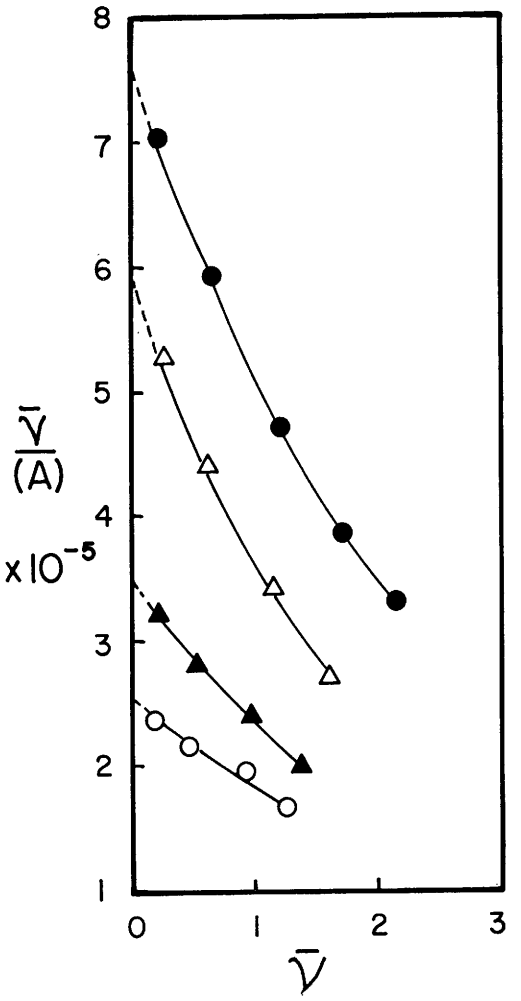


FIG. 1. Effect of bilirubin on binding of thyroxine to human serum albumin at 30° in 0.06 M potassium phosphate buffer, pH 7.4. ●, thyroxine binding, no bilirubin added; △, 1 mole of bilirubin added per mole albumin; ▲, 2 moles of bilirubin added per mole albumin; ○, 3 moles of bilirubin added per mole albumin. Equilibrium dialysis with $I^{131}$-L-thyroxine was performed in a total volume of 10 ml with 5 ml volumes inside (protein side) and outside the dialysis bag. Each tube contained 0.0725 μmole albumin and 6.8 μmoles EDTA. The molar ratio of thyroxine to albumin was varied from 0.31/1 to 2.3/1 (0.0224 to 0.168 μmole thyroxine) while a fixed quantity of bilirubin was added as given above (see also Table I). Total dialysis time was about 6½ hr. Extent of thyroxine binding was determined by counting aliquots taken from both sides of dialysis membrane (1,4). $\Sigma n_i k_i$ value (the intercept on the $\bar{\nu}/(A)$ axis) was obtained for each experiment by extrapolation, as shown by dotted lines.

protein *in vitro*(12): tetraiodothyroacetic acid > L-thyroxine > 3,5,3′-triiodothyropropionic acid > 3,5,3′-L-triiodothyronine > DL-thyronine, with D-thyroxine being equally as effective as L-thyroxine. It is of interest to note that this order of effectiveness is the same as the order of relative binding affinities exhibited by these analogs for albumin† (4). It may be that thyroxine must first be bound at a specific site in a similar way to its binding to albumin in order for the hormone to exert its stimulatory action in the amino acid-incorporating system from liver (12). This suggests the possibility that, in addition to bilirubin toxicity itself(13,14), high intracellular concentrations of bilirubin may interfere with an action of thyroxine on protein biosynthesis.

*Summary.* The effect of bilirubin on the binding of thyroxine by human serum albumin has been studied by means of equilibrium dialysis. The results show that bilirubin can displace thyroxine from binding sites on albumin.

† Tetraiodothyroacetic acid exhibits the same relative binding affinity as tetraiodothyropropionic acid (4) for thyroxine-binding sites on albumin.

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## Hematocrit Changes in Endotoxin Shock.* (30075)

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It is well established that endotoxin shock in dogs is accompanied by a marked rise of the hematocrit of venous or arterial blood(1, 2). The purpose of the present experiments was to study the mechanisms underlying such an increase of blood cell percentage (H) caused by endotoxin. Thus the role of splenic contraction was investigated by comparing the arterial cell percentage ($H_a$) with the portal venous cell percentage ($H_p$) in dogs with intact spleen and by comparing the $H_a$ of such dogs with the $H_a$ of splenectomized dogs. The influence of transcapillary fluid exchange in various regions was evaluated by comparing the cell percentage of various venous blood samples with $H_a$ and by following the plasma protein concentrations. The effect of acidosis on erythrocyte size and hence the H of various blood samples was studied by measuring the changes in blood $pCO_2$ and pH and by determining the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC).

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*Methods.* Forty-five healthy mongrel dogs, including 33 with intact spleens and 12