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II. Medizinische Klinik der Universität München

ISOLATION AND RADIOIMMUNOASSAY
OF THYROXINE-BINDING GLOBULIN (TGB)*

P. G. Livesey, F. Erhardt, K. Horn and P. C. Scriba

Problem: As a preliminary to investigating the peripheral control of thyroid status, it may be important to measure TBG directly. One of the difficulties in developing a radioimmunoassay lies in the need for isolating a pure preparation of TBG (1).

Methods and Results: 1. Affinity chromatography was used whereby thyroxine was coupled to agarose particles by cyanogen bromide activation. When incubated with serum at room temperature for one hour, TBG became bound to the agarose particles. These particles were washed with 0.5 M bicarbonate buffer pH 8.6 and layered into a column. After further washing the TBG was then eluted, using 0.002 M barbital buffer pH 9.3.

2. To improve the specificity of binding, a "spacer" molecule (l-lysine) was placed between the thyroxine molecule and the agarose backbone. Binding studies between $^{125}\text{I}\text{T}_4$ and TBG-extracted serum indicate the improved efficiency of agarose lysine- T_4 in extracting TBG compared with that of agarose- T_4 . Nevertheless, despite this apparent advantage, subsequent elution of TBG from agarose-lysine- T_4 proved more difficult. Preliminary studies show that this is possible with 2.5% salicylate in barbital buffer pH 9.3.

3. Electrophoresis of the freeze-dried protein material eluted from either agarose- T_4 or agarose-lysine- T_4 showed it to contain TBG together with small amounts of non-specifically bound albumin and γ -globulin. Further purification was therefore necessary on a DEAE-Sephadex A 50 column, eluting with a linear concentration gradient of NaCl in 0.06 M Tris HCl buffer pH 8.6. The TBG peak, detected by prior incubation of the protein extract with $^{125}\text{I}\text{T}_4$, was eluted at between 0.08 and 0.1 M with respect to NaCl. Electrophoresis and autoradiography showed the eluted protein to be a single inter- α -globulin, which carried $^{125}\text{I}\text{T}_4$. Furthermore it was antigenic to a highly specific anti-TBG antibody (Levy, 1971), as demonstrated by a heavy precipitin line when subjected to immunodiffusion on 1% agar.

4. With this preparation of TBG, iodination with ^{125}I was possible employing several modifications of the Hunter and Greenwood method. 50% of the labelled ^{125}I -TBG (i. e. 10,000 opm) was subsequently found to bind to the anti-TBG antibody when used in a dilution of 1:40,000 to 1:80,000. This made it possible to plot a good standard curve and from it preliminary serum measurements of TBG were possible.

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