

identical when different batches of anti  $T_3$  serum were used. Heterogeneity of the anti  $T_3$  serum which reacts with free  $T_3$ , conjug-

ated  $T_3$  or thyroid hormone metabolites in urine (or serum) may cause the different results of  $T_3$  RIA.

## **Studies on $^{131}\text{I}$ -Thyroxine Binding Protein Using Single Radial Immunodiffusion and Ouchterlony Method**

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In 1967, I reported the first familial thyroxine-binding globulin (TBG) deficiency in Japan. The evidence of TBG deficiency is based on the defect of the distribution of radioactivity due to  $^{131}\text{I}$ -thyroxine in inter-alpha globulin region by electrophoresis of the serum and  $^{131}\text{I}$ -thyroxine mixture. Recently, I have an opportunity for studying with anti-TBG rabbit serum (Behringwerke) for purpose to clarify the nature of TBG protein fraction in TBG deficient serum more exactly. Two different immunological methods are used, i.e. single radial immunodiffusion in antigen-contained gel layer and Ouchterlony method combined with radioautography. In the former procedure, the sample sera (0.1 or 0.2ml) are mixed with  $^{131}\text{I}$ -thyroxine ( $20\mu\text{Ci}$ ,  $55\mu\text{g}/\text{dl}$  of serum) and thereafter 7.0ml of 1.2% agarose solution are poured into the mixture. After making the agar plate, 12 or 27 wells for anti-

sera application are punched out with cutter.

As the result of the former method, the radioautogram showed the distribution of radioactivity around some wells into which are applied anti-sera to TBG, prealbumin,  $\beta$ -lipoprotein and hemopexin, corresponding to the protein precipitation rings respectively. I emphasize the probability of hemopexin to be one fraction of the thyroxine-binding proteins besides previously identified.

From the second part of these studies, I draw the conclusion that the TBG protein itself is defect in the sera of the TBG deficient patients instead of the disturbance of binding activity of TBG to  $^{131}\text{I}$ -thyroxine, because there is the precipitation line between normal serum and anti-TBG serum, while the line between TBG deficient sera and anti-TBG serum can not be detectable.

## **Activities of Thyroid Stimulator in the Fractions from the Serum of Graves' Disease Eluted Through the Acid Sephadex Column**

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In the present paper, activities of thyroid

stimulator in the fractions from the serum of

normal subjects and Graves' disease eluted through the acid Sephadex column were investigated by the slightly modified McKenzie method. In the normal subjects, bioassay responses of the untreated serum, IgG and "TSH fraction" which was found by our preliminary experiment were within normal limits. In the hyperthyroid patients without infiltrative ophthalmopathy, bioassay responses of each fraction were indistinguishable from those of normal subjects.

In the hyperthyroid patients with infiltrative ophthalmopathy, activities of thyroid stimula-

tor in the "TSH fraction" from the serum which showed below 149% of 2hr assay response were not detected, whereas, in the cases showed above 150% of 2hr assay response of the untreated serum, bioassay responses of "TSH fraction" were all LATS like activity regardless of the existence of LATS in the untreated serum or IgG fraction.

From the results obtained, it seems reasonable to assume that, in some cases with infiltrative ophthalmopathy, LATS like activity is found in the "TSH fraction" eluted through the acid Sephadex column.