K. Thyroid and Parathyroid

Radioimmunoassay of Triiodothyronine in Serum and Tissue

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In most of the measurement of serum hormone by radioimmunoassay, it is necessary to use hormone free serum for the standard curve in order to exclude the nonspecific effect of the serum on the assay system. However, it is impossible to make hormone free tissue and the procedures to extract hormone from tissue are the necessary step for the measurement of hormone in tissue. The present experiment, therefore, was undertaken to investigate the method to extract T_3 for radioimmunoassay to determine the tissue content of T_3.

Procedures to extract T_3 investigated were the extraction of T_3 by Sephadex G-25 column or by ethanol. At first, the results of serum T_3 obtained by the above methods were compared to those obtained by the standard method in which T_3 free serum was used for the standard curve. In extraction of T_3 by Sephadex column, samples or standard T_3 containing 0.1N-NaOH were applied to the column saturated with 0.1N-NaOH, and the absorbed T_3 was eluted with 0.1% BSA in borate buffer. Antiserum was then added to a portion of the combined BSA eluate. In ethanol extraction of T_3, 0.3 ml of the ethanol extracts of samples (sample:ethanol=1:2) was directly added to the assay system, and 0.3 ml of 67% ethanol to the tubes for the standard curve. Although standard curves were different from each other, values for serum samples were not significantly different from either of the above methods indicating that these method could be employed for the determination of tissue T_3.

The thyroid tissue was homogenized and the supernatant of the 100,000g was digested with pronase, diluted with buffer, and subjected to the radioimmunoassay by the methods including the extraction of T_3 as well as by the standard method in which hormone free serum was omitted. Results of thyroidal T_3 content by the above methods agreed well and it is concluded that either of the above methods is sufficiently accurate for the determination of thyroidal T_3 content.