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Chopra I J. A radioimmunoassay for measurement of thyroxine in unextracted serum. *J. Clin. Endocrinol. Metab.* 34:938-47, 1972.
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A simple, rapid, sensitive, and precise radioimmunoassay method for measuring thyroxine in small volumes of unextracted serum is presented. It is adaptable to automation and has been applied to diagnosis of thyroid disease and mass screening for neonatal hypothyroidism. [The *SCI*® indicates that this paper has been cited in over 615 publications since 1972.]

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Until 1971, the best available method for measurement of thyroxine (T_4) in human serum employed (1) a time-consuming extraction of serum with ethanol followed by evaporation of the ethanol extracts to dryness, and (2) quantitation of T_4 in the dried residue by a competitive protein-binding assay (CPBA) using human serum thyroxine-binding globulin (TBG) as a binding protein.[1] Our studies in 1969-1970 had suggested that it was convenient to produce T_4 antibodies in rabbits by immunization with thyroglobulin, a large-molecular-weight thyroid protein, from which thyroid hormones are naturally synthesized.

T_4 antibodies were first used in a radioimmunoassay (RIA) of T_4 in dried ethanol extracts of sera (in a manner very similar to CPBA) except that T_4 antibody was used in place of TBG. This T_4 RIA was more sensitive and specific than the CPBA of T_4. The RIA also permitted measurements in larger numbers of samples at a time than the CPBA. However, T_4 still had to be extracted from serum by organic solvents such as ethanol.

I hoped that a compound might be available that would inhibit the binding of T_4 to normal serum proteins but not to T_4 antibody. Such an agent would be required to free T_4 completely from serum proteins to make it available for reaction with T_4 antibody, thus rendering it measurable in an RIA of unextracted serum. A number of agents had this appropriate property, but their solubility, toxicity, and/or cost were important limitations for general use.

The breakthrough occurred in May-June 1971, when I found that an easily soluble, relatively inexpensive fluorescent dye, 8-anilino-1-naphthalene sulfonic acid (ANS), is a potent inhibitor of T_4 binding by human serum TBG in low concentrations, which have little or no effect on T_4 binding by rabbit T_4 antibody. Murphy and Pattee had already provided the full description of an RIA of T_4 in unextracted serum using this reagent.

The RIA described in this paper still remains the most popular T_4 RIA used routinely for the diagnosis of thyroid disease. The reasons for its popularity include simplicity, specificity, precision, reproducibility, great sensitivity, and amenability to automation. One very important application was pioneered by Jean Dussault.[2] The great sensitivity of the T_4 RIA made it possible to detect T_4 in a small spot of blood on filter paper, allowing him to screen newborns for neonatal hypothyroidism. This screening for neonatal hypothyroidism is now done very widely all over the world. The T_4 RIA described in this report, or a minor modification thereof, continues to be a standard procedure for studying thyroid physiology in health and disease.[3-5]

My work on T_4 RIA was conducted in the laboratory of David H. Solomon who was then the chairman of the Department of Medicine at Harbor General Hospital, an affiliated campus of the UCLA School of Medicine. I was fortunate to have the collaboration of Solomon and Gildon N. Beall, an immunologist at Harbor, who provided valuable help and guidance in the production of T_4 antibodies in rabbits.

1. **Murphy B E P & Pattee C J.** Determination of thyroxine utilizing the property of protein-binding. *J. Clin. Endocrinol. Metab.* 24:187-96, 1964. [See also: **Murphy B E P.** Citation Classic. *Current Contents/Life Sciences* 27(21):20, 21 May 1984.]
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3. **Lee W N P, Golden M P, Van Herle A J, Lippe B N & Kaplan S A.** Inherited abnormal thyroid hormone-binding protein causing selective increase of total serum thyroxin. *J. Clin. Endrocrinol. Metab.* 49:292-9, 1979.
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5. **Shen D-C, Wu S-Y, Chopra I J, Huang H-W, Shian L-R, Bian T-Y, Jeng C-Y & Solomon D H.** Long term treatment of Graves' hyperthyroidism with sodium ipodate. *J. Clin. Endocrinol. Metab.* 61:723-7, 1985.

