L-Triiodothyronine Binding to Serum Protein. I. Competition by Thyronine.*† (29072)

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Several investigations to determine the nature of the binding of L-triiodothyronine and L-thyroxine to serum proteins have been carried out with thyroxine analogs and other compounds which have been demonstrated to be competitors(1-3). The present work was undertaken in an attempt to study the properties of the binding sites of human serum protein with respect to triiodothyronine and thyroxine. DL-thyronine was selected for this study because it has the carbon skeleton, the functional groups and many of the properties of thyroxine. While thyronine has not been detected in the thyroid gland and its utilization in direct iodinations seems unlikely(4) its appearance in extra-thyroidal tissue following the de-iodination of L-thyroxine or L-triiodothyronine has been substantiated (5).

It will also be shown in this study that thyronine and some of its analogs do compete with triiodothyronine and, to a certain extent, with thyroxine for the binding sites on serum proteins. Monoiodo and diiodotyrosine were tested; they exhibited no such effects(6).

Materials and methods. L-triiodothyronine labeled with I-131 in a concentration of 0.0065 mg per ml and a specific activity of 33.1 millicuries per mg, and L-thyroxine labeled with I-131 in a concentration of 0.008 mg per ml and a specific activity of 24.5 millicuries per mg were purchased from Abbott Laboratories. One-tenth ml of the stock (I-131-T-3) solution was diluted to 50 ml with M/15 phosphate buffer, pH 7.3. This working dilution, used for all the experiments, contained 2×10^{-12} moles of T-3 per 0.1 ml. L-thyroxine I-131 labeled was dissolved and used in the same manner. The final dilution contained 2.06×10^{-12} moles of L-thyroxine per 0.1 ml. DL-thyronine (T-O), purchased from Mann Laboratories, was utilized in a solution of 1.092 mg dissolved in 10 ml of 50% propylene-glycol in phosphate buffer M/15. From this stock solution working solutions of T-O were prepared in concentrations of 4×10^{-12}, 8×10^{-12}, 12×10^{-12}, 16×10^{-12} and 20×10^{-12} moles of DL-thyronine per 0.1 ml. Monoiodotyrosine and Diiodotyrosine from Nutritional Biochemicals were treated in solutions of 4×10^{-12} and 8×10^{-12} molar in 0.1 ml.

Sephadex-G-25 (filter gel) was obtained from the Pharmacia Corporation. Into each of five 10 ml Erlenmeyer flasks were added 2 ml of normal human serum and 0.2 ml of DL-T-O solution in a concentration that corresponds to 2, 4, 6, 8 and 10 times the concentration of the I-131-T-3 to be added later to the reference serum. The flasks were shaken in an incubator bath for 30 minutes at 37°C. Following this, 0.2 ml of the diluted I-131-T-3 solution was added to each flask (0.1 ml per ml of serum), and the flasks were again incubated for 30 minutes at 37°C.

At the same time, the reference material without T-O was prepared, which consisted of 2 ml of serum from the same individuals mixed with 0.2 ml of diluted I-131-T-3 solution and 0.2 ml of M/15 phosphate buffer solution (to give the same volume in all samples) and this reference flask was incubated along with the other samples.

After incubation, 1 ml of the reference serum was set apart for assay of total activity. One ml of all samples including the reference was then subjected to gel filtration

* Abbreviations used: T-3 = L-3,5,3′-triiodothyronine: T-4 = L-thyroxine; T-O = DL-thyronine; 3′-isopropyl-T-2 = DL-3′-isopropyl,3,5-diiodothyronine.

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FIGURE 1

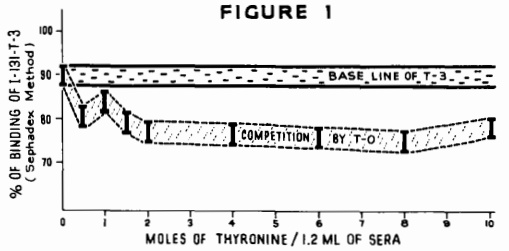


FIGURE 2

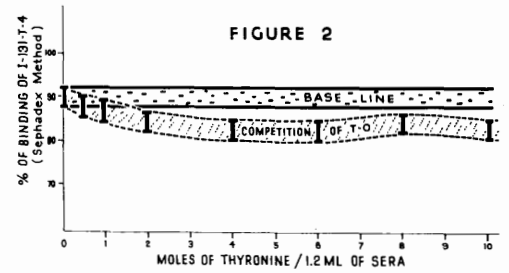
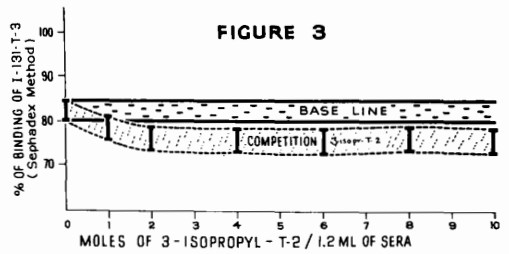


FIGURE 3



through a column of Sephadex-25 in order to estimate the *in vitro* uptake of labeled L-triiodothyronine (or radio-L-thyroxine), by the serum proteins as described previously(7,8). By these means it is possible to separate 3 active fractions from each sample: radioactive T-3 or T-4 that remains in the column: protein-bound T-3 (I-131) [or as protein-bound T-4 (I-131)] that is collected in the first ml of the eluate after the hold-up volume; and free I-131 that collects in the last 6 ml portion of the eluate. The activity of the free iodide is subtracted from the total activity of the standard to obtain the activity due to the radioactive T-3 or T-4 alone. The amount of radioactive T-3 or T-4 that is bound by the serum proteins is then compared to the corrected reference, and the percentage of uptake of T-3 by the protein under assay is calculated as described.

Results. The data (Fig. 1) obtained from 5 patients (not under treatment for thyroid diseases) reveals that there is a significant depression in the I-131-T-3 "uptake" (binding to the protein) when increasing concentrations of DL-thyronine are added to the serum. This competition lowered the "uptake" by 12-18% with an average of 15%. In the experiments performed using radio-L-thyroxine (I-131), DL-thyronine acted as a mild competitor (Fig. 2), causing a depression of the radio-L-thyroxine (I-131) "uptake" by the protein in the range of only 4-7%. In Fig. 3 are depicted the results obtained from the competition of 3,5-diiodo, 3′-isopropyl-thyronine(9) (available from Smith Kline & French Laboratories) for the binding sites of T-3. The lowered "uptake" was only 2-5%, indicating that this compound is a very mild competitor of T-3. While T-O and 3,5-diiodo, 3′-isopropyl-thyronine showed depression of "uptake," monoiodo and diiodotyrosine showed no effects.

The curves in the 3 Figures indicate the upper and lower limits of the % of binding for each study. The baselines for Fig. 1 and 2 were from the same group of patients. Each baseline was obtained from the average of 15 determinations using the pooled serum of 5 euthyroid patients. Baseline for Fig. 3 included 3 patients from the previous group and 2 other euthyroid patients. The % average range from control was 3.6 with $P<.001$ for each point. The curves for the competitive studies were obtained from averages of 10 determinations for each concentration. In Fig. 1, a % average range from control of 4.7 with $P<.01$ was obtained for each point. In Fig. 2, 4.8 with $P<.01$. In Fig. 3, 5.1 with $P<.01$.

Discussion. The results depicted in Fig. 1 indicate that DL-T-O is capable of competing with L-T-3 for some of the binding sites on the serum proteins of man. The results suggest that the binding of T-O to the protein may be in some respects similar to the one exercised by the hormone itself. This competition is not augmented by increased concentrations of T-O after an initial level of over 1½ mole equivalents of T-O was reached. Addition of a 5% solution of trichloracetic acid precipitates the serum pro-

tein still containing the T-O bound to the protein, while with n-butanol it is possible to extract most of the "bound" T-O. It is also seen (Fig. 2) that the binding sites for thyroxine may be of a slightly different type, perhaps of a more firm type than the ones of T-3; or that T-O is more like T-3 than like T-4 in its binding properties, for T-O exhibits a much smaller effect in its competition with T-4, occupying fewer of the binding sites. This might be because the binding sites on serum protein are of a different type and have different degrees of retention or capacity in their holding of the diphenyl ether structures. Studies by others have indicated(10) that the thyronine structure is required for firm binding by human serum albumin. The type of binding of T-3 and of T-4 to human serum albumin has not been fully established (whether physical or chemical) yet n-butanol extraction will remove these compounds from the protein; therefore there is sufficient evidence to suggest that the binding may be of a complex physical-chemical nature.

Current work with analogs of T-3 in which one of the iodines has been substituted by the isopropyl group has been shown to be in some physiological parameters more active than L-T-3(9). One of the more potent among these compounds, 3,5-diiodo-3′-isopropyl-thyronine, was tested and shown also to be a competitor of T-3 when studied in this system (Fig. 3).

By addition of fractions of mole equivalent of the competitor in both T-3 and T-4 "binding" testing systems it was possible to obtain a slope-curve which was mildly suggestive of titration, indicating a possible stoichiometric ratio between T-O and the binding sites capable of being occupied by T-3 and T-4.

The results obtained by comparing Fig. 1 and 2 support the contention that T-4 might be more firmly bound to the serum proteins than T-3(11,12,13).

Summary. 1. It has been shown that DL-thyronine competes for the binding sites in human serum proteins of both T-3 and T-4 to the extent of 12-18% for the former and 4-7% for the latter. 2. It thus appears that thyroxine is more firmly held to these proteins than T-3; or that T-O is more like T-3 than like T-4 in its binding properties. 3. 3,5-diiodo-3′-isopropyl-thyronine was also shown to be a competitor of T-3 for binding sites of serum proteins, although to a much lower extent (2-5%). Monoiodo and Diiodotyrosine did not show competition for these binding sites.

1. Pitt-Rivers, R., Tata, J. R., *The Thyroid Hormones,* Pergamon Press, Inc., New York, 1959.

2. Astwood, E. B., Solomon, D. M., *Mechanisms of Action of Anti-thyroid Drugs, Iodide and Other Thyroid Inhibitors, The Thyroid,* 2nd Ed., Harper and Row, N. Y., 1962, 74.

3. Hetzel, B. S., Good, B. F., Welby, M. L., Charnock, J. S., Effect of Salicylate on Plasma Protein-Bound Iodine in Various States of Thyroid Function, *Adv. Thyroid Res.,* 1961, 56.

4. Lissitzky, S., Roques, M., Benevent, M. Th., *Excerpt Med., Internat. Congress Series,* 1960, v26, 33.

5. Werner, S. C., *The Thyroid,* Hoeber Med. Division, Harper and Row Pub., Inc., 1962.

6. Barker, S. B., *The Thyroid,* Brookhaven Symposium in Biology, #7, 1955, 74.

7. Shapiro, B., Rabinowitz, J. L., *J. Nuclear Med.,* 1962, v3, 417.

8. Rabinowitz, J. L., Shapiro, B., Johnsen, P., *ibid.,* 1963, v4, 139.

9. Greenberg, C. M., Blank, B., Pfeiffer, F., *Fed. Am. Soc. Exp. Biol.,* 1963, 621.

10. Sterling, K., *Annual Meeting The American Thyroid Association, Inc.,* 1963, 38.

11. Robbins, J., Rall, J. E., *J. Clin. Invest.,* 1955, v34, 1331.

12. Larson, F. C., Albright, E. C., *Endocrinology,* 1955, v56, 737.

13. Ingbar, S. H., *ibid.,* 1958, v63, 256.

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