Solubilized Nuclear "Receptors" for Thyroid Hormones

PHYSICAL CHARACTERISTICS AND BINDING PROPERTIES, EVIDENCE FOR MULTIPLE FORMS*

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Tissues regulated by thyroid hormones contain chromatin-localized "receptors" that may be involved in the actions of these hormones. In this report, we describe some properties of these receptors after their solubilization from rat liver nuclei and their separation from nucleic acids and basic proteins.

The nuclear extract and partially purified preparations contain a dominant class of binding sites which have a high affinity for triiodothyronine (3,5,3'-triiodo-L-thyronine, $K_d \sim 1$ nM) and for the biologically potent isopropyl diiodothyronine (3,5-diiodo-3'-isopropyl-L-thyronine, $K_d \sim 1$ nM) and also bind thyroxine (3,5,3',5'-tetraiodo-L-thyronine, $K_d \sim 5$ nM) and reverse triiodothyronine (3,3',5'-triiodo-L-thyronine, $K_d \sim 20$ nM). This binding activity elutes on Sephadex G-100 in an included peak which has a Stokes radius of 35 Å and sediments on glycerol gradients at 3.5 S. From these data a molecular weight ratio of 50,500 and a frictional ratio of 1.4 were calculated, suggesting that the receptor is somewhat asymmetrical. There was a sharp decline in triiodothyronine binding by this component above pH 8.7 (optimum around pH 7.6) where there is marked dissociation of the 4' phenolic hydroxyl of triiodothyronine ($pK_a \sim 8.5$). A similar decrease in thyroxine ($pK_a \sim 6.7$) binding with pH increases in this range was not observed. Thus, ionization of the phenolic hydroxyl may influence binding.

The solubilized preparations can also contain a minor specific-binding component that can be identified by binding analyses, and by G-100 or quaternary aminoethyl Sephadex chromatography. This component has a much lower affinity for triiodothyronine and isopropyl diiodothyronine than for thyroxine as compared to the major component. It probably has a pH optima around 6.0 and demonstrates an apparent tendency to aggregate. The minor component was not always identified by direct Scatchard analysis and may be generated in part from the major component as it was more commonly observed after storage or purification of the nuclear extract.

Thus, at least two thyroid hormone-binding components can be present in extracts of purified rat liver nuclei; the minor component may be an altered form or subunit of the major component. The relative binding activities of triiodothyronine, isopropyl diiodothyronine, and thyroxine by the major component, similar to those in intact nuclei, parallel the biological potencies of these compounds, and suggest that the dominant binding is by biologically relevant receptors. Since ionization of the phenolic hydroxyl may influence binding, the lower activity of thyroxine relative to triiodothyronine may in part be due to the fact that at physiological pH, the phenolic hydroxyl of thyroxine is more dissociated than that of triiodothyronine. The finding that this receptor is somewhat asymmetrical provides an indication of the shape of an intrinsic chromatin protein implicated in specific gene regulation. Finally, the finding that reverse triiodothyronine binds to the major component with 25% the affinity of thyroxine suggests that this product of thyroxine metabolism can directly influence thyroid hormone responses as an agonist or antagonist.

Thyroid hormones play a fundamental role in regulating mammalian development and metabolism, and have been shown to regulate the levels of several gene products in certain target tissues (1-10). However, very little is known about the molecular mechanism of action of these hormones. Recently, attention has focused on thyroid hormone-binding proteins found in the nuclei of hormone-responsive cells which may be actual receptors involved in thyroid hormone action (10-19). The identification of these thyroid hormone-binding proteins...
as tightly associated and intrinsic chromatin proteins (12, 20–24) also has considerable interest since few proteins which regulate specific gene function and which are actual chromatin proteins have been identified.

The available data relating to these nuclear binding sites is strongly suggestive that they mediate some of the cellular responses to thyroid hormones. There is a good correlation between the binding of triiodothyronine, thyroid, and a number of thyroid hormone-active analogs to nuclear receptors and the activity of these compounds in eliciting thyroid hormone responses (15, 18, 22, 25). There is some correlation between the concentration of receptors in responsive tissues and the degree of responsiveness (10). In addition, these receptors are not found in the invertebrate drosophila which is not known to respond to thyroid hormone (12).

High affinity and limited capacity thyroid hormone-binding components have also been identified in the cytosol (26–31), mitochondria (32), and other cellular fractions (26). The cytosol binders show considerable variation in different tissues in their levels and binding properties (26–31). For example, the cytosol binding proteins in cultured pituitary cells have a higher affinity for thyroxine than for triiodothyronine (17), whereas the major binding reported in the liver has a slightly higher affinity for triiodothyronine than for thyroxine (30). The binding sites in other cellular fractions have not been studied in as much detail; more information is needed before their role in thyroid hormone action can be understood. In summary, there is significant evidence to suggest that the nuclear binding sites may be actual hormone receptors, and equally convincing data has not been presented with respect to the binding sites in other cellular fractions. However, there is no proof that the nuclear sites are the only receptors.

To understand the nature of the nuclear binding sites, we have begun to purify and characterize these proteins. Solubilization of the receptors from nuclei has been achieved by ourselves and others, and some binding characteristics have been reported (17, 20–22, 30, 34). The binding activity in such extracts is sensitive to proteolytic but not other hydrolytic enzymes (17, 20). Several investigators have found that triiodothyronine bound to a nuclear extract elutes with proteins which are both excluded and included on Sephadex G-100 (21, 34). DeGroot and Strausser found that bound triiodothyronine migrates with a sedimentation constant less than 4 S on sucrose gradients (24). The reported affinity of extracted nuclear receptors for triiodothyronine varies considerably (17, 22). Finally, we found that the solubilized receptors bind to DNA and have suggested that this reaction may be the mechanism for receptor localization in chromatin (33). In the present studies, we have begun characterization of the solubilized proteins. We find that some purification is necessary to achieve a preparation which is reasonably stable. Further, these partially purified fractions allow a much better quantification of binding characteristics than can be made using the crude nuclear extract. In the course of these studies, we have also found evidence for multiple molecular forms of the nuclear thyroid hormone-binding components. However, one major form accounts for more than 90% of the triiodothyronine binding and does, as previously suspected, appear to be the actual receptor. We report some preliminary physical characterization of this receptor.

The extrathyroidal metabolism of thyroxine involves a deiodination to either 3,5,3'-triiodothyronine (triiodothyronine) or to 3,3',5'-triiodothyronine (reverse triiodothyronine). Of particular interest recently has been the finding that there are marked fluctuations in the rate of production and in the plasma level of reverse triiodothyronine relative to triiodothyronine in various clinical and physiological states (35–38). The concentrations of reverse triiodothyronine can be particularly high in the fetus (up to 300 ng/dl) and in the amniotic fluid (up to 600 ng/dl) compared with normal adults (about 40 ng/dl). These observations have led investigators to suspect a physiological role for reverse triiodothyronine, but to date the data have not yielded a clear answer. In earlier studies, an anti-thyroid activity was reported for this hormone (39, 40). More recently, some data has suggested that this compound may inhibit the conversion of thyroxine to triiodothyronine (41) and that reverse triiodothyronine could, like triiodothyronine and thyroxine, inhibit the release of thyroid-stimulating hormone by thyrotropin-releasing factor (42). Samuels and co-workers did find that reverse triiodothyronine, like triiodothyronine, stimulates the consumption of glucose in cultured pituitary cells, but suggested that this effect was due to contamination of the hormone preparation with triiodothyronine (18). Previous investigators have reported that reverse triiodothyronine was essentially not bound by the nuclear receptors (15, 19) and proposed that any biological actions of this hormone must occur through other mechanisms (15). In the present investigations, we reexamined the binding of reverse triiodothyronine and found that this compound can competitively inhibit all of the triiodothyronine binding by the putative nuclear receptors. These findings suggest that reverse triiodothyronine may have actual thyroid hormone-like agonist or antagonist properties.

MATERIALS AND METHODS

Thyroid Hormones – L-[3'-125I]Triiodothyronine (500 mCi/mg), L-[3',5'-125I]Thyroxine (1200 mCi/mg), and L-[2',5'-125I]Reverse triiodothyronine were obtained from Abbott Laboratories. Stock solutions or incubations containing [125I]triiodothyronine or [125I]thyroxine were routinely assayed for [125I]iodide, [125I]triiodothyronine, and [125I]thyroxine content by the following procedure modified from Greens (44). Samples were diluted 1:1 with 0.030 N NaOH, 1.0 M NaCl and incubated at 37° for 10 min. A 0.1-mil portion was loaded onto a Sephadex G-25 (fine) column (bed volume, 2.0 ± 0.05 ml, in a Pasteur pipette with a cotton plug) equilibrated in 0.015 N NaOH, 0.5 M NaCl, and eluted at ambient room temperature in 0.25-ml fractions with column equilibration solution. Protein-associated [125I], [125I]iodide, and [125I]triiodothyronine elute as sharp peaks at 1.0, 2.0, and 3.25 ml, respectively. [125I]Thyroxine elutes at a broad peak at 5.25 ml. Fresh stock solutions of [125I]triiodothyronine contained 12.4% iodide and no detectable thyroxine. Solutions of [125I]thyroxine contained 4.3% iodide and 0.9% triiodothyronine.

Nonradioactive triiodothyronine and thyroxine (Sigma Chemical Co., St. Louis, MO)

2 It is particularly important in binding experiments using [125I]thyroxine to know how much contaminating triiodothyronine is present. Since triiodothyronine has an affinity which is about 10-fold higher than thyroxine for the major class of nuclear binding sites. According to the manufacturer (Abbott Laboratories), [125I]thyroxine contains 3% [123I]thyroxine, 4% [35I]thyroxine and no [129I] associated with positions 3 or 5. The radioactive decay of [125I] to the stable nuclide [127I] results, in effect, in deiodination since tellurium disassociates from the hormone (44). It follows that after 25 days (when 25% of the [125I] has decayed to [127I]), the solution would contain about 9% nonradioactive triiodothyronine, a level that would significantly reduce the [125I]thyroxine binding. Competition. It is therefore, particularly critical in studies of [125I]thyroxine binding to monitor triiodothyronine contamination and to purify, when necessary, just prior to experimentation.
Nuclear Thyroid Hormone "Receptors"

All triiodothyronine lots were found to contain less than 0.01% contaminating thyroxine. Thyroxine was used without further purification. The functional group was less than 0.01% cross-reactivity with antibody to triiodothyronine. 3,3',5'-Triiodothyronine (reverse triiodothyronine) and 3,5-diido-3'-isopropyl thyronine (isopropyl diiodothyronine) were generously provided by Dr. Eugene C. Jorgensen, and were used without further purification. Reverse triiodothyronine contained less than 0.01% cross-reacting activity to the triiodothyronine antibody and migrated as a single peak ($R_s = 0.26$) by thin layer chromatography on silica gel plates (plate No. 5052, Quantum Industries, Fairfield, N.J.) using hexanex-amylalcohol:methanol:4 N NH₄OH (volume ratios 1:6:2:1). Reverse triiodothyronine was resolved in this system from 3,3'-diiodothyronine ($R_s = 0.36$), triiodothyronine ($R_s = 0.43$), thyroxine ($R_s = 0.28$), and 3'-isopropyl diiodothyronine ($R_s = 0.50$). Due to intrinsic antibody binding, reverse triiodothyronine could not be measured for thyroxine contamination. However, the synthesis schemes for reverse triiodothyronine (45) and the 3'-isopropyl analog (46) exclude triiodothyronine and thyroxine as potential contaminants. The labeled reverse triiodothyronine contained about 10% $[^{125}]$iodide and less than 10% other radioactive contaminants as analyzed by the thin layer chromatography system described above.

Preparation of Nuclear Extract—Female Sprague-Dawley rats (200 to 220 g, 8 weeks) were sacrificed in the afternoon as supplied by the vend or (Simonsen). Animals were lightly anesthetized with chlo-roform before cervical dislocation, blood was removed by cardiac puncture, and excised livers were immediately stored in liquid nitrogen. Nuclei were prepared using a procedure modified from a method developed by M. Goldberg for extraction of RNA polymerase. Frozen livers were pulverized with a cold pestle and quickly thawed by addition, with stirring, of warm (35°C) Solution A (0.34 mM sucrose, 15 mM MgCl₂, 0.24 mM spermine), 2 ml/g of tissue, to give a final temperature of about 0°C for the thawing. The liver vesicles were then draped using coarse nylon mesh, and added to ice cold Solution B (3.1 mM sucrose, 6.5 mM MgCl₂, 0.1 mM spermine), 3 ml/g of tissue. The mixture was then homogenized for three 1-min periods, separated by one minute of cooling using a Tekmar homogenizer (Cincinnati, Ohio) at maximum speed. This preparation, when stained with 1% crystal violet, was observed by light microscopy to contain cell debris, intact nuclei, and no whole cells. Nuclei were pelleted through 2.1 M sucrose, 0.36 M MgCl₂, pH 7.6) containing 0.5% Triton X-100, and repelleted (500 x g, 10 min). The nuclear pellet was washed by gentle suspen-sion in 25 volumes of Buffer C (20 mM Tricine, 2 mM MgCl₂, 0.1 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol), and saturated (4.1 M) ammonium sulfate was added to make 0.5 M. This suspension was then sonicated (4 tumors/100 ml buffer) in a Branson Sonifier B and centrifuged (15,000 x g for 20 min) at 4°C. The supernatant medium was loaded onto a QAE-Sephadex (strongly acidic cation-exchange) column (1.0 ml sample/1.0 ml bed volume) equilibrated in Buffer E, and eluted with one bed volume of Buffer E followed by two bed volumes of Buffer F (0.2 mM (NH₄)₂SO₄, 25 mM citric acid, 50 mM sodium phosphate, 1.0 mM dithiothreitol, pH 5.7). Protein concentration was measured by precipitation of 0.1 ml of each fraction with an equal volume of 7% trichloroacetic acid in a standard assay (47). Aliquots (50 μl) of each fraction were assayed for binding activity as described below, except that 1.0 nM hormone was used in the incubation mix. The fractions corresponding to peaks of hormone binding activity (see "Results") were separately pooled and precipitated by the addition of 0.3 ml/g of (NH₄)₂SO₄ (Schwarz-Mann, enzyme grade) and then centrifuged after 4 h (5000 x g for 10 min). The supernatant medium was discarded and the pellet, containing about 80% of the nuclear extract binding activity, was resus-pended in 10 mM Tris (pH 8.5), 10 mM dithiothreitol, 0.6 M NaCl (1 ml for each 3 ml of starting nuclear extract) with negligible loss in binding activity. The resuspended mixture was centrifuged (25,000 x g, 20 min), and the resulting supernatant medium containing all of the binding activity was stored in liquid nitrogen.

Binding Assay—The binding assay mix consisted of a limiting number of binding sites per cell (in the nuclear extract or QAE-purified procedure), 5 nM $[^{125}]$iodothyronine or $[^{125}]$thyroxine, and Buffer G (50 mM sodium phosphate (pH 7.6), 0.03 M ammonium sulfate, 1.0 mM EDTA, 0.1 mM dithiothreitol, 5% glycerol) to make a total volume of 0.5 ml. To measure "nonspecific" binding (48), parallel tubes were prepared that were identical except that they also contained unlabeled triiodothyronine or thyroxine, respectively, at 1000-fold excess. Incubations were for 60 min at 37° or 90 min at 35° based on kinetic studies, see "Results". Following incubation, samples were chilled on an ice bath and 0.4 ml was chromatographed at 55°C on a small Sephadex G-25-50 column (bed volume, 6.0 ± 0.05 ml) prepared from a Pasteur pipette. The G-25 was equilibrated in Buffer G. The excluded peak, containing macromolecular-bound hormone, was collected, and the amount of $[^{125}]$iodide determined with an Auto-gamma spectrometer (efficiency ~76%). Specific binding (i.e. high affinity, limited capacity) (48) was cal- culated by subtracting nonspecific binding (incubation containing excess triiodothyronine or thyroxine) from total binding (without competitor). When necessary, the free hormone concentration was calcu-lated by subtracting the total binding from the total hormone concen-tration in the mixture.

Competition Studies—Various concentrations of triiodothyronine, thyroxine, reverse triiodothyronine, and 3'-isopropyl diiodothyronine were incubated with 1 nM $[^{125}]$triodothyronine or 1 nM $[^{125}]$thyroxine and hormone-binding sites from the nuclear extract described above. The amount of $[^{125}]$iodothyronine or $[^{125}]$thyroxine was determined after 3.5 h of incubation at 35°C as decribed above. Unlabeled hormones were prepared as 0.1 mM solutions in 1-propanol and diluted to the desired molarity in Buffer G. It was determined that the presence of 1% or less 1-propanol in the reaction mix had no effect on triiodothyronine or thyroxine binding.

Binding to Purified Enzymes—Purified enzymes were incubated with 2 nM $[^{125}]$triiodothyronine (2 hr) under conditions that were optimal for enzymatic activity, and were then assayed for specific binding as described under "Methods." The enzymes and buffer conditions used are as follows. DNA polymerase (E.C. 2.7.7.7, 1.0 mg/ml), 20 mM Tris (pH 8.0), 0.1 mM dithiothreitol, 8 mM MgCl₂, 10% glycerol, 125 μg/ml egg albumin; terminal deoxynucleotidyltransferase (2.5 μg/ml), 50 mM potassium phosphate (pH 7.0), 4 mM 2-mercaptoethanol, 8 mM MgCl₂, 125 μg/ml of egg albumin; poly(A) polymerase (E.C. 2.7.7.7, 0.5 μg/ml), 200 mM Tris (pH 8.3), 4 mM 2-mercaptoethanol, 0.5 mM MnCl₂, 100 μg/ml of egg albumin; RNA polymerase I (0.16 mg/ml), 50 mM Tris (pH 7.5), 2.5 mM MgCl₂, 40 mM (NH₄)₂SO₄. Parallel incubations containing radioactive enzyme substrates instead of $[^{125}]$triiodothyronine or nuclear extract instead of enzyme were performed and showed that the enzymes were active under these conditions and exhibited active binding to the receptor. DNA polymerase (49), terminal deoxynucleotidyltransferase (50) and poly(A) polymerase (51) were provided by Dr. F. J. Bollum (University of Kentucky Medical School). RNA polymerase I (52) (see Footnote a) was provided by Dr. M. Goldberg (National Institute of General Medical Sciences). These enzymes were assayed as described in the respective references.
**RESULTS**

Molecular Sieve Chromatography and Density Gradient Sedimentation of Macromolecular Bound Triiodothyronine —

The elution of macromolecular bound \[^{131}I\]thyroid hormone on Sephadex G-100 (Fig. 1) shows a minor peak which is excluded from the gel, and a major peak that is included and which elutes in a position corresponding to a Stokes radius of 35 Å when compared to standard proteins (53). This finding is similar to those reported previously (21, 34). The data in Fig. 1 also demonstrate that the binding corresponding to the included peak is specific (by the criteria of limited capacity and high affinity) since it is not observed when the extract is incubated with an excess of nonradioactive triiodothyronine in addition to the radioactive triiodothyronine. However, the binding of \[^{131}I\]thyroid hormone exhibited by radioactivity in the excluded peak is apparently due to both specific binding and to other interactions which are of lower affinity and higher capacity since competition with excess nonradioactive triiodothyronine partially inhibited, but did not abolish the peak. These data indicate that there is heterogeneity of the binding in the nuclear extract and that a determination of only the eluted radioactivity as previously reported (21, 34) does not reflect an accurate profile of specifically bound hormone.

The major portion of the bound triiodothyronine in the nuclear extract sediments through glycerol gradients at 3.5 S (when compared to proteins of known sedimentation velocities (Fig. 2)). By utilizing the data obtained from the molecular sieve and density gradient approaches, a molecular weight of 50,000 and frictional ratio of 1.4 can be calculated (55) if a partial specific volume of 0.725 cm³/g is assumed (56). A frictional ratio greater than 1.0 can result from a combination of bound water and nonspherical shape; however, bound water alone can only account for a maximum ratio of about 1.1 (67). Thus, the ratio of 1.4 attributed to the major form of the thyroid hormone binder, suggests that the receptor is somewhat asymmetric (58).

The specific binding component of the excluded peak on G-100 could be made up of aggregates of the included binder, a higher molecular weight binder, or as suggested by Surks and co-workers, triiodothyronine associated with the nucleoprotein complexes (34). To examine the last possibility, samples of nuclear extract were incubated with DNase and filtered on agarose 0.5 M (Fig. 3). As shown, the excluded peak is essentially abolished after the DNase treatment, whereas no specific DNase effect was observed on the included peak. Thus, even though heterogeneity of the excluded peak can be identified by the competitive experiment shown in Fig. 1, at least part of the peak from the crude extract may be due to DNA-protein complexes or aggregates. These data also suggest that the predominant triiodothyronine-binding activity is due to the component that is included on Sephadex G-100.

Partial Purification with Removal of Histones and DNA —

Previous evidence suggested that the nuclear thyroid hormone receptor is a non-histone protein (17, 22, 34). As an initial approach to purification, we therefore, chose chromatography on QAE-Sephadex (a strongly basic ion exchanger, see "Materials and Methods") because of its potential for binding neutral and acidic proteins (and not histones) at high pH. In addition, the nuclear extract was titrated to pH 9.2 and dialyzed to low ionic strength prior to chromatography ("Materials and Methods"). The step results in a visible precipitation of basic proteins and nucleic acids, but there is no loss of binding activity from the supernatant medium. The binding activities eluted from QAE-Sephadex in two peaks (Fig. 4). Peak A containing 5% and 75%, respectively, of the original binding activity. They were separately pooled, precipitated with ammonium sulfate, and resuspended for analysis as described under "Materials and Methods." The binding in Peak

![Fig. 1](left). Sephadex G-100 fractionation of nuclear extract bound to \[^{131}I\]thyroid hormone. Nuclear extract (175 μg of protein) was incubated in a standard 0.5-ml reaction mix in Buffer G containing 5 nM \[^{131}I\]thyroid hormone alone (I) or with 1 μM unlabeled \[^{131}I\]thyroid hormone (C) for 20 min at 25°C. The samples were chilled to 4°C and 0.5 ml was loaded onto separate Sephadex G-100 columns (1.5 × 90 cm; bed volume, 133 ml) equilibrated in Buffer G at 4°C. Fractions (1.6 ml) were collected at 0.33 ml/min and the amount of \[^{125}I\]thyroid hormone was determined in each fraction. Free thyroid hormone begins to elute at Fraction 90. From 60 to 80% of the radioactive thyroid hormone bound prior to G-100 filtration is recovered in the two macromolecular peaks.

![Fig. 2](right). Glycerol gradient sedimentation. Nuclear extract was incubated with 2 nM \[^{131}I\]thyroid hormone for 2 h at 25°C in Buffer G and free triiodothyronine was removed with a standard G-25 assay column (see "Materials and Methods"). Of the excluded volume, 0.1 ml was loaded onto a 3.5-ml, 10 to 30% linear glycerol gradient and centrifuged at 160,000 × g for 18 h at 4°C. Two-drop fractions were collected by tube puncture and the amount of \[^{125}I\]thyroid hormone determined in each fraction. Standard proteins of known sedimentation constants (54) lysozyme, 7.0 S; hemoglobin, 4.3 S, cytochrome c, 1.17 S) were run on parallel gradients and their final gradient position determined by optical absorption at 280 nm.

![Fig. 3](right). Agarose gel filtration and DNase treatment of nuclear extract. Nuclear extract was prelabeled with \[^{131}I\]thyroid hormone as described in the legend to Fig. 1 and incubated at 37°C in Buffer G containing 10 μM MgCl₂ and 10 μM CaCl₂ with (O) or without (C) 426 units (2130 units/ml) DNase 1 ( Worthington, DPF) before chromatography. The column dimensions and elution rate were as described in Fig. 1 except that 2.0-ml fractions were collected.
A was not retained by increasing the relative volume of ion exchanger (QAE-Sephadex) and therefore is not due to an overloading of the column by the sample. Thus, at least two thyroid hormone-binding components are apparent after the overloading of the column by the sample. Thus, at least two does triiodothyronine, whereas the reverse is true for Peak B the finding that thyroxine binds more avidly to Peak A than does triiodothyronine, whereas the reverse is true for Peak B (Fig. 4).

The thyroid hormone-binding activity in Pool A, labeled with either 1 nm [125]Itriiodothyronine or [125]Ithyroxine eluted only in the excluded volume from Sephadex G-100 (data not shown). However, the triiodothyronine binding in Pool B also showed a major included peak on Sephadex G-100 which is identical in elution position to the included peak from nuclear extract (Fig. 5). Since Pool B does not contain detectable DNA (Table I), it is likely that the minor excluded peak (Fig. 5) represents protein aggregates rather than nucleoprotein complexes. Thus, even though DNA-protein associations may cause receptors to be excluded from the gel in the nuclear extract, other aggregations in the absence of DNA can also occur.

Fig. 5 also shows the elution profile on Sephadex G-100 of Pool B equilibrated with [125]Ithyroxine. Of note is that the ratio of triiodothyronine/thyroxine bound by each peak at this concentration is quite different (excluded peak ratio: 1.1/1, included peak ratio: 11/1). These data indicate that more than one binding form may be present, even in Pool B.

The binding activities in Pool B represent over 90% of the total triiodothyronine-binding activity eluted from the QAE-Sephadex column (Fig. 4). Since triiodothyronine has much greater hormonal activity in the rat than thyroxine (about 10-fold higher) and the major binding activity in Pool B shows a higher affinity for triiodothyronine than for thyroxine, the binders in this fraction are more likely candidates for further study as receptors. Pool B also has several advantages for receptor characterization. In contrast to nuclear extract, Pool B shows a linear hormone binding over a wide range of protein concentrations (Fig. 6). Also, this fraction is more stable during incubations at 37°C (Fig. 7) than is the crude extract. These results suggest that studies based solely on nuclear extract could lead to a major underestimation of the binding activity. Pool B was also depleted in histones when compared to nuclear extract by acrylamide gel electrophoresis (59) (data not shown), and contained very low levels of DNA (Table I).

Effect of pH on Binding—An analysis of the effect of pH on binding activity in the Pool B fraction (Fig. 8) shows a major optimum for triiodothyronine around pH 7.6 (7.4 to 8.0) and a second minor optimum around pH 6. By contrast, optimal thyroxine binding is around pH 6. The two optima may reflect the two components known to exist in Pool B with the minor component (which binds thyroxine more avidly than does the major component) having the lower pH optima. The possibility that at pH 6 most of binding is by the minor component is further supported by Sephadex gel filtration data. At this pH, essentially all of the bound triiodothyronine in Pool B elutes in the excluded fraction (data not shown) similar to the binding in QAE Pool A (see above) or the minor excluded component identified at pH 7.6 (Fig. 5). The more avid binding of thyroxine by the minor component may obscure a decline in binding of this hormone to the major component which would parallel the observed decline in triiodothyronine binding below pH 7.

The profile observed in Fig. 8 could be entirely due to the response of the receptor proteins to pH. However, pH influences on the ligand should also be considered since dissociation of the 4'-hydroxyl of triiodothyronine (pKₐ = 8.45 (60)) and thyroxine (pKₐ = 6.7 (60)) is also pH-dependent and could contribute to the pH profile if an intact hydroxyl is important for the hormone-receptor association (e.g. by hydrogen bonding (61)). In fact, there is a sharp decline in triiodothyronine binding around pH 8.5 to 9.0 which is in the region where increasing pH results in major dissociation of the 4'-hydroxyl. If an intact phenolic hydroxyl is important for the hormone's association with the receptor, then a dramatic effect on thyroxine binding would not be expected near pH 8.5 to 9.0 because the 4'-hydroxyl of thyroxine (pKₐ = 6.7) becomes mostly dissociated at a lower pH. This is the case (Fig. 8). This hypothesis further predicts a sharp decline in thyroxine binding around the pKₐ for this compound. This is observed, however, data in this portion of the curve are more difficult to interpret since most of the binding is probably due to the minor component. Hormone Binding—The binding of increasing concentrations of triiodothyronine and thyroxine by the nuclear extract, shown in Fig. 9 in the form of a Scatchard plot (62), conforms
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Fig. 5 (left). Sephadex G-100 fractionation of Pool B bound to \(^{125}\text{I}\)triiodothyronine (■) or \(^{125}\text{I}\)thyroxine (○). A sample of Pool B containing 140 µg of protein was incubated for 60 min at 37° in a standard 0.5 ml reaction mix containing 5 nM hormone. The sample was chilled to 4° and 0.5 ml was chromatographed as described in the legend to Fig. 1 and the amount of radioactivity was determined in each fraction.

Fig. 6 (right). Linearity of the binding assay. Increasing amounts of nuclear extract (○) or Pool B (■) were incubated for 120 min at 25° with 1.0 nM \(^{125}\text{I}\)triiodothyronine and then assayed for specific binding and protein as described under "Materials and Methods."

Fig. 7. Kinetics of binding. Samples of nuclear extract (○, ●, △) or Pool B (□) were incubated for various lengths of time at 0° (○, ●), 25° (□), and 37° (△, □) in Buffer G containing 2 nM \(^{125}\text{I}\)triiodothyronine. Incubations were terminated by chilling (5 min in an ice bath) and were then assayed for specifically bound hormone as described under "Materials and Methods."

by this criterion to a single class of sites with an approximately 5-fold higher affinity for triiodothyronine than for thyroxine. However, in some preparations (data not shown) a minor component with a lower affinity for triiodothyronine was also observed. The latter was more commonly observed in extracts which had been stored for a longer period of time. A Scatchard analysis of the Pool B-purified preparation also shows the presence of at least two binding components. The data (Fig. 10) for triiodothyronine can conform to two straight lines and could be due to a major component with a higher affinity for triiodothyronine than for thyroxine, and a minor component with a higher affinity for thyroxine than for triiodothyronine. This interpretation seems even more likely when taken together with the Sephadex gel filtration data shown in Fig. 5.

The indications based on the Scatchard analysis are further supported by competition data shown in Fig. 11 which also show the effects of two other analogs, isopropyl diiodothyro-

Fig. 8. Effect of pH on \(^{125}\text{I}\)triiodothyronine and \(^{125}\text{I}\)thyroxine binding to Pool B. Standard 0.5 ml incubation mixes contained Pool B (50 µl, 70 µg of protein), 5 nM \(^{125}\text{I}\)triiodothyronine (○), or \(^{125}\text{I}\)thyroxine (■) and were buffered with either 25 mM citric acid/50 mM sodium phophate (pH 4.8 to 5.4), 0.1 M sodium phophate (pH 5.4 to 7.5), 0.05 M Tris (pH 7.5 to 8.4), or 0.1 M sodium carbonate/bicarbonate (pH 8.4 to 10.3). All the buffers were adjusted to 0.6 ionic strength with either (NH₄)₂SO₄ or NaCl to match Buffer G. The binding profiles observed are not due to buffer inhibition since different buffers having the same pH showed identical binding. Reaction mixtures were incubated for 60 min at 37° and the pH was measured in each reaction tube just prior to assaying for hormone binding. Parallel incubations containing excess unlabeled triiodothyronine or thyroxine were used as described under "Methods" to determine nonspecific binding at each pH. The data shown therefore represents only specific binding.

Fig. 9 (left). Scatchard analysis of \(^{125}\text{I}\)triiodothyronine or \(^{125}\text{I}\)thyroxine binding by nuclear extract. Nuclear extract (50 µl, 175 µg of protein) was incubated in a standard 0.5 ml reaction mix in Buffer G containing from 0.1 nM to 100 nM \(^{125}\text{I}\)triiodothyronine (○) or \(^{125}\text{I}\)thyroxine (■). Specifically bound hormone was assayed after incubation for 120 min at 25° as described under "Materials and Methods."

Fig. 10 (right). Scatchard analysis of \(^{125}\text{I}\)triiodothyronine and \(^{125}\text{I}\)thyroxine binding to Pool B. A sample of Pool B (50 µl, 70 µg of protein) was incubated in a standard 0.5 ml reaction mix in Buffer G containing from 0.1 nM to 100 nM \(^{125}\text{I}\)triiodothyronine (○) or \(^{125}\text{I}\)thyroxine (■). Specifically bound hormone was assayed as described under "Materials and Methods" after incubation for 120 min at 25°.
In the crude extract all of the binding of [¹²⁵I]triiodothyronine is much more readily inhibited by nonradioactive triiodothyronine than by thyroxine (Fig. 11A). With [¹²⁵I]thyroxine most of the binding is more readily inhibited by triiodothyronine than by thyroxine; however, some of the [¹²⁵I]thyroxine binding is more readily inhibited by thyroxine than by triiodothyronine. These data again suggest that there is a minor component which binds thyroxine more avidly than triiodothyronine. As mentioned above, this lower affinity triiodothyronine binding component is not always apparent on a direct Scatchard plot of triiodothyronine binding (Fig. 9). This heterogeneity is also not apparent in the competition studies using [¹²⁵I]triiodothyronine (Fig. 11 A, and C) since more than 90% of the binding in nuclear extract at the low concentration of [¹²⁵I]triiodothyronine used is by the major component (which has a higher affinity for triiodothyronine than for thyroxine). By contrast, a higher proportion of the binding of a low concentration of [¹²⁵I]thyroxine by nuclear extract is due to the minor form of the receptor. The heterogeneity would therefore be more readily observed in the competition analysis using [¹²⁵I]thyroxine. A similar pattern of competition inhibition is observed using the Pool B-purified material. With [¹²⁵I]triiodothyronine, all of the binding is more readily inhibited by triiodothyronine than by thyroxine, whereas some heterogeneity is again suggested when [¹²⁵I]thyroxine is used.

Of note is that with both the nuclear extract and the Pool B-purified preparation, the biologically potent isopropyl diiodothyronine is as effective as triiodothyronine in the inhibition of the major binding component. This provides an additional criterion of specificity suggesting that the major binder is a biologically active receptor. By contrast, isopropyl diiodothyronine has a lower affinity for the minor component (Fig. 11, B and D) than either thyroxine or triiodothyronine.

A surprising finding is that reverse triiodothyronine dose competitively inhibit all of the [¹²⁵I]triiodothyronine binding with an avidity that is about 25% (at 50% competition) of thyroxine and about 3% of triiodothyronine. Since these studies indicated a competitive capacity of reverse triiodothyronine which is greater than previously reported (15, 10), we felt that it was important to consider the possibility that contaminants with other thyronine compounds that have a higher affinity for the receptor could result in artifactual competition data. The synthesis scheme used for the reverse triiodothyronine used here yields only the L-isomer and has no apparent chemical mechanism to form triiodothyronine or thyroxine (45). Thin layer chromatography studies provide a further indication of the reverse triiodothyronine purity since it migrated as a single spot and was resolved from 3,3′-diiodothyronine, triiodothyronine, and thyroxine (data not shown). Further, triiodothyronine radioimmunoassay demonstrated less than 0.01% triiodothyronine contamination. Therefore, it is unlikely that contaminants in the reverse triiodothyronine preparations are responsible for the competition. However, the possibility remained that a single outer ring deiodination during the binding reaction could form 3,3′-diiodothyronine and that this product could be responsible for the observed competition of triiodothyronine binding to the receptor. This possibility was tested by incubating nuclear extract under standard binding conditions with [¹²⁵I]reverse triiodothyronine, extracting the reaction mixture with an equal volume of butanol:ethanol (3:1, v:v) and then examining the extracted products by thin layer chromatography (“Materials and Methods”). More than 96% of the extracted radioactivity migrated with authentic reverse triiodothyronine and less than 2% of the radioactivity migrated with authentic 3,3′-diiodothyronine. Therefore, there is little conversion under our reaction conditions. For the observed competition to be due to 3,3′-diiodothyronine the affinity of this compound for the receptor would have to be 20-fold higher than that of reverse triiodothyronine. This is not the case since Jorgensen and Bolger (46) found (using an identical assay system) that the binding of 3,3′-diiodothyronine is actually weaker than that of reverse triiodothyronine (61). This result has also been confirmed by us. It is also unlikely that other breakdown products of reverse triiodothyronine account for the observed competitive inhibition since further deiodination appears to reduce biologic ac-
zymes, themselves, are or contain the thyroid hormone receptor. Therefore, it is unlikely that any of these enzymes involved in nuclear function have been purified nearly to homogeneity from thyroid target tissues and were available to us, it seemed reasonable to ask if any of these have thyroid hormone-binding activity. We were unable to demonstrate triiodothyronine binding by purified DNA polymerase, terminal deoxynucleotidyltransferase, poly(A) polymerase, or RNA polymerase I. Therefore, it is unlikely that any of these enzymes, themselves, are or contain the thyroid hormone receptors. Of course, these data do not exclude the possibility that the receptors modulate the activity of these enzymes or have intrinsic activity which is similar to that of the enzymes tested.

**DISCUSSION**

In the present studies, characteristics of thyroid hormone-binding sites, solubilized from rat liver nuclei, are presented. We found that after some purification of the salt extracted receptor, a preparation could be obtained which is stable at 37°, and which has other desirable characteristics such as linearity of binding over a wide range of protein concentrations. The data with the partially purified preparations also indicate that the receptor binding of triiodothyronine and thyroxine does not require basic proteins (histones) or DNA since these components have been removed.

In the nuclear extract and partially purified preparations (Pool B) the dominant binding activity has characteristics suggestive that it is a thyroid hormone receptor. This component binds triiodothyronine with a higher affinity than thyroxine and also has a high affinity for the biologically potent isopropyl diiodothyronine. The major binding component is included in Sephadex G-100 gels (Stokes radius 35 Å) and migrates around 3.5 S in glycerol gradients. From these data a molecular weight ratio of 50,500 was estimated. The value is lower than that reported by Surks et al. (21, 34) who used only the molecular sieve approach, a method that would tend to overestimate the size of the receptor if it is asymmetrical or contains a significant amount of bound water. In fact, the present studies suggest that the thyroid hormone receptor is somewhat asymmetrical since the estimated frictional ratio of 1.4 is greater than can be attributed to bound water alone.

It is of interest to compare the proportion of the thyroid hormone receptors with those obtained for another class of regulating proteins that interact with chromatin, the steroid receptors. The latter differ from thyroid hormone receptors in that they are not ordinarily present in chromatin in the absence of the hormone. Further, even in the presence of the hormone, the steroid receptors are not as tightly associated with the chromatin as the thyroid hormone receptors (as measured by ease of extraction). The steroid receptors are also much more asymmetrical than are the thyroid hormone receptors; for instance, the progesterone receptors isolated from chick oviduct have frictional ratios of 1.7 and 1.9 (64, 65). It would appear that proteins which regulate gene expression at the level of chromatin may therefore vary considerably in their shapes: extreme asymmetry, as seen with the steroid receptors, is apparently not a general characteristic of proteins involved in genomic regulation.

The present data also demonstrate that extracts of purified liver nuclei or partially purified receptor preparations contain thyroid hormone-binding components which differ from the major form of the receptor. One component binds the hormone nonspecifically and is not ordinarily reported because its contribution to the total binding is subtracted when specific binding is calculated. However, this nonspecific component can represent a significant part of the bound radioactivity which is excluded from Sephadex G-100 (Fig. 1). We also demonstrated a second component which binds triiodothyronine specifically but which differs from the principal form of the receptor in that it has a higher affinity for thyroxine than for triiodothyronine or isopropyl diiodothyronine. This component was evident in Scatchard analyses (Fig. 10), competition studies (Fig. 11), and was suggested by the pH studies (Fig. 8). An enrichment of this component can also be obtained by QAE-Sephadex chromatography (Fig. 4, Pool A) or by Sephadex G-100 (Fig. 5, excluded fraction). This component was not always observed by Scatchard analysis of the crude nuclear extract. Curiously, when this extract was stored on ice or was partially purified, the minor component could be clearly demonstrated by a subsequent Scatchard analysis. This apparent generation of the minor component following fractionation led us to a more extensive study in which strong evidence has been obtained that the major receptor component can undergo qualitative changes during storage or purification. Such "altered" receptors bind thyroxine more avidly than triiodothyronine and can account for the appearance of the minor component. The fact that the minor component can be generated also suggests that its presence is not due to contamination of the nuclear preparation by extranuclear binders.

An analysis of the effect of pH on binding indicates that careful attention must be given to this parameter when measuring binding activity; pH is especially critical when determining triiodothyronine-thyroxine cross-reactivity. For example, at pH values in the 7.4 to 8.2 range, this cross-reactivity reflected the biological potency of these two hormones, but at high pH, the total binding of thyroxine exceeded that of triiodothyronine. The finding of a dramatic decline in triiodothyronine binding around the pK of its phenolic hydroxyl, which was not observed in this range for thyroxine (which has a lower pK) also suggested that the integrity of the phenolic hydroxyl is important for binding. If this is true, then the higher potency of triiodothyronine (relative to thyroxine) may be attributed in part to the fact that its phenolic hydroxyl is less dissociated at physiologic pH; however, steric and other factors of the 5'-iodine may also contribute.

The competition data suggest that reverse triiodothyronine has a high affinity (K_a ~ 30 nM) for binding to the major form of the intranuclear receptor. This affinity is about 25% that of
thyroxine and about 3% that of triiodothyronine. This finding was surprising in light of previously published reports that reverse triiodothyronine was essentially not bound by nuclear sites (15, 19). As mentioned under "Results," it is unlikely that our results are due to contaminants in the reverse triiodothyronine preparations or to conversion of this compound to other analogs during the binding reaction. It is not known why the present findings differ from those obtained previously. However, in the previous studies (15, 19) the hormone was incubated with intact nuclei or was injected into the animal. Therefore, if there is any impairment to reverse T₃ uptake under these experimental conditions, an intrinsic affinity of the hormone for the receptor would be underestimated. If there were impaired uptake, then our binding data using the solubilized receptors would result in an overestimation of the actual in vivo potency of the metabolite and the binding studies by Oppenheimer and colleagues (19) would be more informative. Alternatively, the possibility that there are other intrinsic differences in the relative hormone cross-reactivity as measured by the various techniques cannot be excluded.

Nevertheless, the finding that reverse triiodothyronine binds to the nuclear receptors implies that this metabolite can have agonist or antagonist actions at the level of the thyroid hormone receptor. Since the affinity of the reverse triiodothyronine for the receptor is still only a few per cent that of triiodothyronine, even if there is adequate accessibility of the receptors to reverse triiodothyronine, the latter would have to be present in significant excess of triiodothyronine to result in significant receptor occupancy. In certain cases, the levels of reverse triiodothyronine do exceed those of triiodothyronine by more than 10-fold (in the case of the human amniotic fluid before 20 weeks (36)) and by almost 20-fold in the sera of the fetal sheep (38). The levels of reverse triiodothyronine in normal adult humans is less than that of triiodothyronine and in certain other clinical conditions such as starvation, they are only about 2-fold greater than triiodothyronine (35). Since the plasma binding of triiodothyronine and reverse triiodothyronine is approximately the same (35), the total plasma levels probably give a reasonable index of the free concentration of the two hormones. If the reverse binding constants we report here using the soluble rat receptor reflect the relative concentrations, then this metabolite could conceivably be present in significant excess of triiodothyronine to result in a significant occupancy of the receptors (relative to triiodothyronine) in the fetal cases, but would do so at a minor extent in the case of the adult. These thoughts must be considered speculative until the effects of reverse triiodothyronine are examined in each system. Of note is that Chopra et al. reported in abstract form that reverse triiodothyronine does inhibit the release of thyroid stimulating hormone in response to thyrotropin-releasing factor administration in rats with about 0.4% the activity (on a dose basis) of triiodothyronine (42). These observations are consistent with the idea that reverse triiodothyronine, if present in large concentrations, can influence thyroid hormone-regulated functions. Further, in other studies, we have examined the reverse triiodothyronine action in cultured pituitary tumor cells and have found agonist activity with respect to the thyroid hormone-regulated functions of glucose consumption and growth hormone production. However, in this case the data suggest that reverse triiodothyronine could be acting as a prohormone by being converted to 3,3'–diiodothyronine. The present data with the intranuclear receptor strongly suggest that if reverse triodo-

REFERENCES

1. Barker, S. B. (1971) in The Thyroid (Werner, S. C., and Ingbar, S. H., eds) 3rd Ed, pp. 227–234, Academic Press, New York.
2. Hoch, F. L. (1974) in Handbook of Physiology (Greep, R. O., and Astwood, E. B., eds) Vol. 3, pp. 377–391, American Physiological Society, Washington, D. C.
3. Green, S. H. and Najjar, S. (1974) in Handbook of Physiology (Greep, R. O., and Astwood, E. B., eds) Vol. 3, pp. 377–391, American Physiological Society, Washington, D. C.
4. Tata, J. R. (1974) in Handbook of Physiology (Greep, R. O., and Astwood, E. B., eds) Vol. 3, pp. 469–478, American Physiological Society, Washington, D. C.
5. Cohen, P. P. (1970) Science 168, 533–543.
6. Frieden, E., and Just, J. J. (1970) in Biochemical Actions of Hormones (Litwack, G., ed) pp. 1–52, Academic Press, New York.
7. Edelman, I. S., and Ismail-Beigi, F. (1974) Recent Prog. Horm. Res. 30, 230–250.
8. Tsai, J. S., and Samuels, H. H. (1974) Biochem. Biophys. Res. Commun. 59, 429–432.
9. Kurt, D. T., Seppel, A. E., and Feugelson, P. (1976) Biochemistry 15, 1031–1036.
10. Oppenheimer, J. H., Schwartz, H. L., and Surks, M. I. (1974) Endocrinology 99, 389–395.
11. Sondler, B. J., MacLeod, D. M., Ring, J., and Baxter, J. D. (1975) J. Biol. Chem. 250, 4111–4119.
12. Charies, M. A., Ryffel, G. U., Obinata, M., McCarthy, B. J., and Baxter, J. D. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 1787–1791.
13. Tata, J. R., and Widnell, C. C. (1966) Biochem. J. 98, 604–620.
14. Oppenheimer, J. H., Koerner, D., Schwartz, H. L., and Surks, M. I. (1972) J. Clin. Endocrinol. Metab. 35, 330–333.
15. Oppenheimer, J. H., Schwartz, H. L., Dillman, W., and Surks, M. I. (1973) Biochem. Biophys. Res. Commun. 55, 544–550.
16. De Groot, L. J., and Torresani, J. (1975) Endocrinology 96, 357–369.
17. Samuels, H. H., Tsai, J. S., Casanova, J., and Stanley, F. (1974) J. Clin. Investig. 54, 853–865.
18. Samuels, H. H., Tsai, J. S., and Cintron, R. (1973) Science 182, 1253–1266.
19. Koerner, D., Surks, M. I., and Oppenheimer, J. H. (1974) J. Clin. Endocrinol. Metab. 38, 706–709.
20. De Groot, L. J., Refetoff, S., Brausser, J., and Barsano, C. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 4042–4046.
21. Surks, M. I., Koerner, D., Dillman, W., and Oppenheimer, J. H. (1973) J. Biol. Chem. 248, 7066–7072.
22. Thomopoulos, P., Dastuzue, B., and Defer, N. (1974) Biochem. Biophys. Res. Commun. 55, 544–550.
23. Gardner, R. S. (1975) Biochem. Biophys. Res. Commun. 67, 625–633.
24. De Groot, L. J., and Straussser, J. A. (1974) Endocrinology 95, 74–83.
25. Coerner, D., Schwartz, H. L., Surks, M. I., Oppenheimer, J. H., and Jorgensen, E. C. (1975) J. Biol. Chem. 250, 6417–6423.
26. Tata, J. R. (1975) Nature 257, 19–23.
27. Defter, N., Dastuzue, B., Sabatier, M. M., Thomopoulos, P., and Kruh, J. (1975) Biochem. Biophys. Res. Commun. 67, 995–1004.
28. Suft, S. B., Toccafondo, R. S., Malan, P. G., and Ekins, R. P. (1973) J. Endocrinol. 80, 41–52.
29. Spealding, S. W., and Davis, P. J. (1971) Biochim. Biophys. Acta 229, 579–583.
30. Dillman, W., Surks, M. I., and Oppenheimer, J. H. (1974) Endocrinology 95, 492–498.
31. Sterling, K., Saidana, V. F., Brenner, M. A., and Milch, P. O. (1974) Nature 250, 693–695.
32. Sterling, K., and Milch, P. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 3225–3229.
33. MacLeod, D. M., and Baxter, J. D. (1975) Biochem. Biophys. Res. Commun. 62, 577–583.
34. Surks, M. I., Koerner, D., and Oppenheimer, J. H. (1975) J. Clin. Investig. 55, 50–60.
35. Chopra, I. J., Chopra, U., Smith, S. R., Reza, M., and Solomon,
D. H. (1975) J. Clin. Endocrinol. Metab. 41, 1043–1049
36. Chopra, I. J., and Crandall, B. F. (1975) N. Engl. J. Med. 293, 740–743
37. Burger, A., Nicod, P., Suter, P., Vallotton, M. B., Vagenakis, A., and Braverman, L. (1974) Lancet 1, 653–655
38. Chopra, I. J., Sack, J., and Fisher, D. (1975) in Perinatal Thyroid Physiology and Disease (Fisher, D. A., and Burrow, G. N., eds) pp. 33–48, Raven Press, New York
39. Pittman, C. S., and Barker, S. R. (1959) Endocrinology 64, 468–468
40. Pittman, J. A., Tingley, J. O., Nickerson, J. F., and Hill, S. R., Jr. (1966) Metabolism 9, 983–985
41. Chopra, I. J. (1976) Clin. Res. 24, 142A
42. Chopra, I. J., Carlson, H. E., and Solomon, D. H. (1976) Clin. Res. 24, 270A
43. Green, W. (1972) J. Chromatog. 72, 83–91
44. Bagdall, K. W. (1966) The Chemistry of Selenium, Tellurium and Polonium, p. 157, Elsevier, London
45. Shiiba, T., and Cahnmann, H. (1964) J. Org. Chem. 29, 1652–1653
46. Blank, B., Pfeiffer, R., Greenberg, C., and Kerwin, J. (1963) J. Med. Chem. 6, 554–560
47. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
48. Baxter, J. D., Higginso, S. J., and Rouzeau, G. O. (1975) Methods Enzymol. 32, 240–248
49. Bollum, F. J. (1960) J. Biol. Chem. 235, 2399–2403
50. Bollum, F. J. (1974) in The Enzymes (Boyer, P. D., ed) Vol. 10, pp. 145–169, Academic Press, New York
51. Tstrapalis, C. M., Dorson, J. W., and Bollum, F. J. (1975) J. Biol. Chem. 250, 4486–4496
52. Roeder, R. G., and Rutter, W. J. (1969) Nature 224, 234–237
53. Page, M., and Godin, C. (1969) Biochim. Biophys. Acta 194, 329–331
54. Smith, M. H. (1968) in Handbook of Biochemistry—Selected Data for Molecular Biology (Sober, A. H., ed) pp. C10–C23, Chemical Rubber Company, Cleveland, Ohio
55. Siegel, L. M., and Monte, K. J. (1966) Biochim. Biophys. Acta 112, 346–362
56. Martin, R. G., and Ames, B. N. (1961) J. Biol. Chem. 236, 1372–1379
57. Roth, R., and Pollard, F. C. (1964) Molecular Biophysics, 2nd Ed, p. 98, Addison-Wesley, Reading, Mass.
58. Schachman, H. K. (1959) Ultracentrifugation in Biochemistry, p. 239, Academic Press, New York
59. Panyim, S., and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337–346
60. Robbins, J., and Rall, J. E. (1967) in Hormones in Blood, (Gray, C. H., and Bacharach, A. L., eds) 2nd Ed, p. 383, Academic Press, London
61. Jorgenson, E. C., Bolger, M. B., and Dietrich, S. W. (1976) Proceedings of Fifth International Congress on Endocrinology, in press
62. Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51, 660–672
63. Samuel, H. H., and Tsai, J. S. (1975) Clin. Res. 23, 388A
64. Sherman, M. R., Corvol, P. L., and O'Malley, B. W. (1970) J. Biol. Chem. 245, 6085–6096
65. Sherman, M. R., Tuazon, F. B., Díaz, S. C., and Miller, L. K. (1976) Biochemistry 15, 980–989
Solubilized nuclear "receptors" for thyroid hormones. Physical characteristics and binding properties, evidence for multiple forms.
K R Latham, J C Ring and J D Baxter

J. Biol. Chem. 1976, 251:7388-7397.

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