THYROID HORMONE RECEPTORS: BINDING CHARACTERISTICS, AND LACK OF HORMONAL DEPENDENCY FOR NUCLEAR LOCALIZATION

by

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SUMMARY

Thyroid hormone(s) has diverse effects on growth and metabolism. Specific "receptor" proteins which bind triiodothyronine (T_3) and other biologically active analogs and which may be involved in thyroid hormone action have been recently found in nuclei of responsive tissues. This report presents studies of these receptors in rat liver nuclei. Confirming previous reports, a Scatchard analysis of the binding data suggests the reaction: T_3 + specific receptor $\rightleftharpoons$ T_3-receptor complex, with an apparent equilibrium dissociation constant (K_d) at 22° of about 1.9×10^{-10} M and a capacity of about 1 pmole of T_3-binding sites per mg DNA.

The kinetics of the binding were also examined. T_3-receptor complex formation is second order and dissociation is first order. The apparent association (k_1) and dissociation (k_{-1}) rate constants at 22° are respectively 4.7×10^7 M^{-1} min^{-1} and 7.6×10^{-3} min^{-1}. The apparent K_d, estimated from the ratio of the rate constants (k_{-1}/k_{+1}), was about 1.7×10^{-10} M, similar to that determined from the equilibrium data. These data support the expression written above for the interaction of thyroid hormone with its receptor. Additional kinetic experiments indicate that some of the T_3 binding by cell-free nuclei is to sites previously occupied by hormone in the intact animal, providing further evidence that the intact cell and cell-free reactions are the same.

It was previously found that nuclear-bound T_3 is localized in chromatin. We found that isolated chromatin retains specific binding activity similar to that of isolated nuclei. Thus, binding may not require cytoplasmic, nucleo-plasmic or nuclear membrane factors. These findings may imply that chromatin localization of the receptor does not depend on the hormone. This idea is supported by an earlier finding that binding activity is present in nuclei from thyroidectomized animals. However, many stimuli such as steroid hormones, bacterial inducers and adenosine 3':5'-monophosphate in bacteria influence regulatory proteins at the gene level by promoting the protein's addition to or removal from chromatin. Thus, we studied the effect of thyroid hormone on the nuclear content of receptors under assay conditions of receptor stability and reversible binding. Receptor levels in hypothyroid animals are identical to those in euthyroid animals. These data suggest that the hormone does not influence the nuclear localization of receptors. Thus, the basis for thyroid hormone action may be to regulate the activity of receptors resident in chromatin rather than to promote receptor addition to or removal from chromatin.

INTRODUCTION

Thyroid hormones regulate numerous metabolic processes and profoundly influence differentiation and development (1-4). Several groups have recently found nuclear "receptor" proteins which bind the active hormones triiodothyronine (T_3)[1] and thyroxine (T_4) with high affinity (5-18). Several lines of evidence suggest that these proteins mediate some of the hormonal responses. The receptors are generally present in thyroid hormone-responsive tissues (12). They have a higher affinity for T_3 than for T_4 (5,8,14). The concentrations of T_3 and T_4 required for receptor binding and for eliciting the response are similar (5). Further, there is a good parallel between the biological potency of a number of analogs with varied activity and the analog's ability to inhibit T_3-binding by the receptor (14).

The receptors appear to be salt extractable, acidic, chromatin proteins (7,15,16). They have been detected after T_3 was administered *in vivo* (6,8,15), incubated with cultured cells (5), incubated with isolated nuclei (5,9,11,17) or bound in nuclear extracts (13,16). The nuclear content of sites, and the properties of the binding reaction suggest that the hormone is binding to similar sites in the intact cell and cell-free conditions (5,8,17,18).

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[1]Abbreviations used are: T_3, l-triiodothyronine; T_4, l-thyroxine; Tricine, N.tris-(hydroxymethyl) methylglycine.

We have recently presented evidence that the T_3 receptor is a DNA-binding protein (19). Further, it appears to be preferentially concentrated in the chromatin fractions which contain most of the endogenous RNA polymerase, contain most of the template activity for in vitro transcription and have a lower protein to DNA ratio (19). T_3 receptors and RNA polymerase also share the common property of bein among the few non-histone proteins which can be fixed to chromatin by formaldehyde treatment (20). These properties suggest the receptor may regulate gene function through a mechanism which involves the DNA component of chromatin.

Regulatory signals in both prokaryotic (21) and eukaryotic (22,23) systems which directly influence gene activity through their binding to regulator proteins, usually act by influencing the protein's addition to or removal from DNA or chromatin. Thus, inducers may promote the dissociation of bacterial repressors from the DNA (21). Conversely, cyclinc adenosine 3':5'-monophosphate in prokaryotes (21) and steroid hormones in eukaryotes (22,23) promote the addition of a regulatory protein to the genome. Since the thyroid hormone receptor may be a DNA-associated chromatin protein, we investigated whether the hormone itself exerts any influence on the nuclear content of these chromatin receptors. To do so, we further characterized T_3-receptor interaction.[2]

[2]Some of these results have been reported in preliminary form (19,20).

MATERIALS AND METHODS

Many of the procedures were performed using modifications of techniques previously described by Samuels and Tsai (5,9).

Materials - (^{125}I)T_3 (Abbott, 496 mCi/mg) was about 92% pure as determined by Sephadex column chromatography (24), and was either purified by this technique or used without further purification. Identical nuclear binding results were obtained with either preparation. Further, over 98% of the specific radioactivity bound to nuclei was extracted as described elsewhere (5) and found to migrate with authentic T_3 in the Sephadex column (24). Nonradioactive T_3 purchased from Sigma, and made in solution at 5 mM with 0.1 M NaOH, was used without further purification. Dithiothreitol was purchased from Sigma. Triton X-100 was purchased from Nuclear Chicago.

Preparation of Nuclei - Male Sprague-Dawley rats were used. Thyroidectomized animals were maintained for a minimum of two weeks post-surgically prior to use. The serum level of T_4 in thyroidectomized animals was found to range from 0 to 0.55mg/100 ml serum (25).

To obtain liver, rats were anesthetized with an intraperitoneal injection of 1 ml/100 gm body weight of a 3.6% solution of chloral hydrate, or by cervical dislocation. After removing cardiac blood for T_4 analysis, 15 ml of ice-cold PBS (0.025 M potassium phosphate, 0.1 M NaCl, pH 7.6) was injected intracardially

to perfuse and chill the liver, which was then placed in ice-cold Buffer A (20 mM Tricine, 0.25 M sucrose, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5% glycerol, 0.1 mM diithiothreitol, pH 7.6). All subsequent procedures were at 0-4°. After weighing and mincing, the tissue was homogenized in four volumes (w:v) of Buffer A with five strokes at 700 rpm of a motor driven teflon pestle. All volumes cited are based on the original liver weight. After successively straining through one, two and four layers of cheesecloth, the homogenate was centrifuged at 1000 x g for 7 min. The pellet was washed by first resuspending it in two volumes of Buffer A containing 0.5% Triton, then by adding more Buffer A containing 0.5% Triton to a final volume to weight ratio of 5:1, and finally centrifuging the resuspended mixture at 800 x g for 7 min. The wash was repeated twice. The "purified" nuclear pellet was resuspended to 0.2 g/ml (unless specified otherwise) in Buffer B (20 mM Tris, 0.25M sucrose, 1 mM $MgCl_2$, 0.1 mM dithiothreitol, 5% glycerol, pH 7.6) and used in the binding experiments. This resuspended mixture contained 276 mg (± 20 S.E.M.) DNA/ml as measured by the technique of Giles and Meyer (26). When stained with 0.1% Azure C and examined under a light microscope, this preparation contained intact nuclei, with some debris and a few cytoplasmic tags.

<u>T_3 Binding by Rat Liver Nuclei</u> - Unless stated otherwise, the incubations were at 22° for 4 hours. The reaction mixture (total volume of 1 ml) contained: 0.5ml of the nuclear suspension; 20 mM Tris (pH 7.6); 0.25 M sucrose; 1 mM $MgCl_2$; 2,2 mM EDTA; 0.1 mM dithiothreitol; 50 mM NaCl; 5% glycerol; and 2 x 10^{-10}M (^{125}1)T_3.

Parallel tubes were identical except that they also contained 10^{-6} M nonradioactive T_3 as a competitor for specific $(^{125}I)T_3$ binding. Following incubation, samples were chilled in an ice bath and centrifuged at 800 x g for 7 min. When appropriate, a portion (0.2 ml) was removed to determine the concentration of free radioactivity in the supernatant medium. The remaining supernatant medium was then decanted. The pellet was washed by resuspending in 2 ml of Buffer B containing 0.5% Triton X-100, vortexing for 5 sec., centrifuging at 800 x g for 7 min., and then decanting the supernatant medium by inversion. This was repeated. The final pellet was assayed for radioactivity in a gamma spectrometer (efficiency 42-50%). Specific binding was determined by subtracting the bound cpm in the incubation with excess competing T_3 (nonspecific binding) from the cpm bound in the incubation without competitor (total binding).

RESULTS

Characteristics of the Assay - To compare receptor levels in nuclei from hypothyroid and euthyroid animals, we wanted reaction conditions in which receptors are stable enough to minimize possible thyroid hormone influence on receptor stability and to allow time for dissociation of endogenously-bound hormone. We also wanted to know whether any endogenously-bound hormone was dissociating in the reaction conditions and that the $(^{125}I)T_3$ could rebind to these same sites.

Like Samuels and Tsai (5,9), we found specific T_3 binding by nuclei at 37°. We also found similar levels of maximal binding at either 0°, 22° or 37°, but greater instability was observed at 37° than at the lower temperatures (shown in Fig. 1 for 22°). Most experiments were performed at 22°, since as discussed below the binding kinetics were found to be more rapid at this temperature than at 0°.

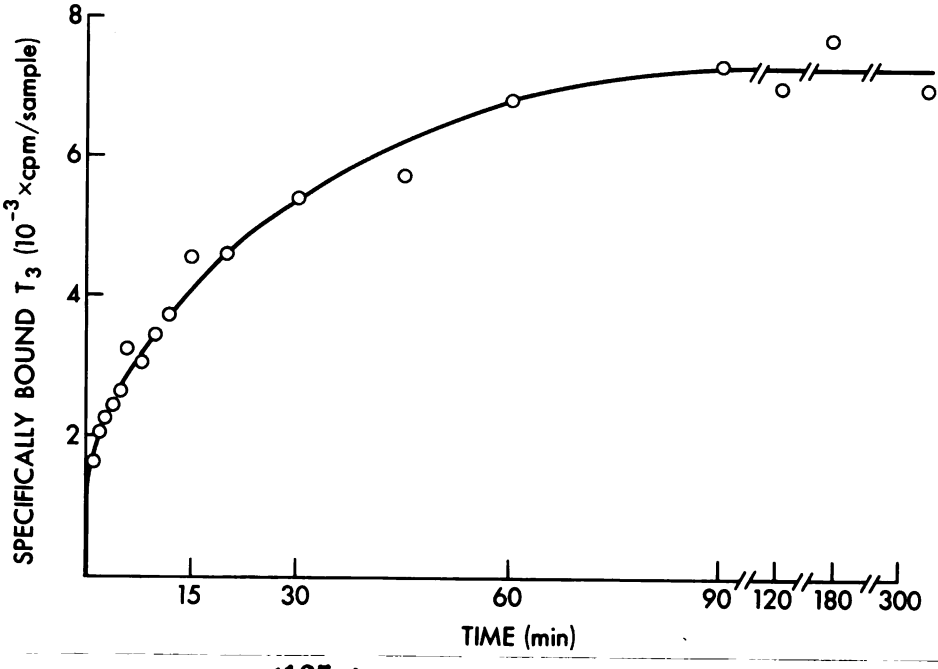

Fig. 1. Kinetics of $(^{125}I)T_3$ association with rat liver nuclei from hypothyroid animals. Specific binding was determined as described in the Methods except that the final concentration of $(^{125}I)T_3$ was 2.6×10^{-10} M.

The data in Figs. 2 & 3 delineate characteristics of the binding assay. Fig. 2 shows the influence of nuclear washing on the assay results. Specific binding and total nuclear-bound radioactivity in either competitor-free or competitor-containing incubations is shown. There is some loss of specifically-bound radioactivity with continued washing (as much as 30%); however, the amount of nonspecifically-bound radioactivity decreases much more. Thus, for the best estimate of total receptor content an extrapolated value (to zero washes) should be used; however, since results obtained after two washes were highly reproducible and yielded a low "background" of nonspecific binding, the amount of binding after two washes was used for comparative experiments and for rate and affinity analyses.

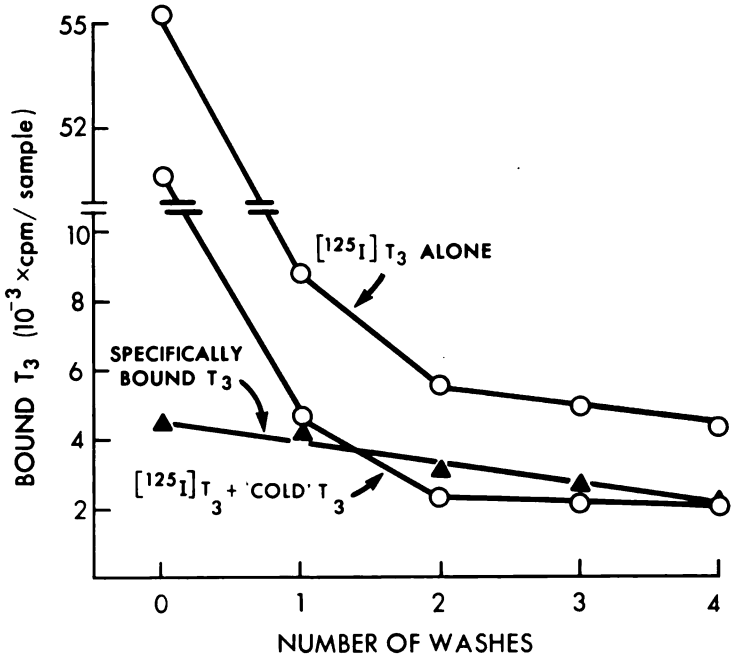

Fig. 2 Effect of washing on nuclear binding. Nuclei from livers of euthyroid rats were incubated as described in the Methods except that the time of incubation was 5.5 hours, NaCl was omitted, and the final $(^{125}I)T_3$ concentration was 9.5×10^{-10} M. Shown is binding: A, in the absence of competitor $(^{125}I)T_3$ alone); B, in the presence of competitor ($(^{125}I)T_3$ + 'cold' T_3); and the specifically bound T_3(A minus B).

When, nuclei from 0.1 or less of liver are incubated in 1 ml, the assay is linear with respect to the concentration of added nuclei (Fig. 3). Although the departure from linearity above 0.1 g/ml is partly explained by a decrease in free T_3 during the reaction period due to binding, it does not explain all of the deviation (see legend to Fig. 3). Thus, to study binding under conditions in which there is linearity, nuclei from 0.1 g or less of liver per 1 ml of incubation were used.

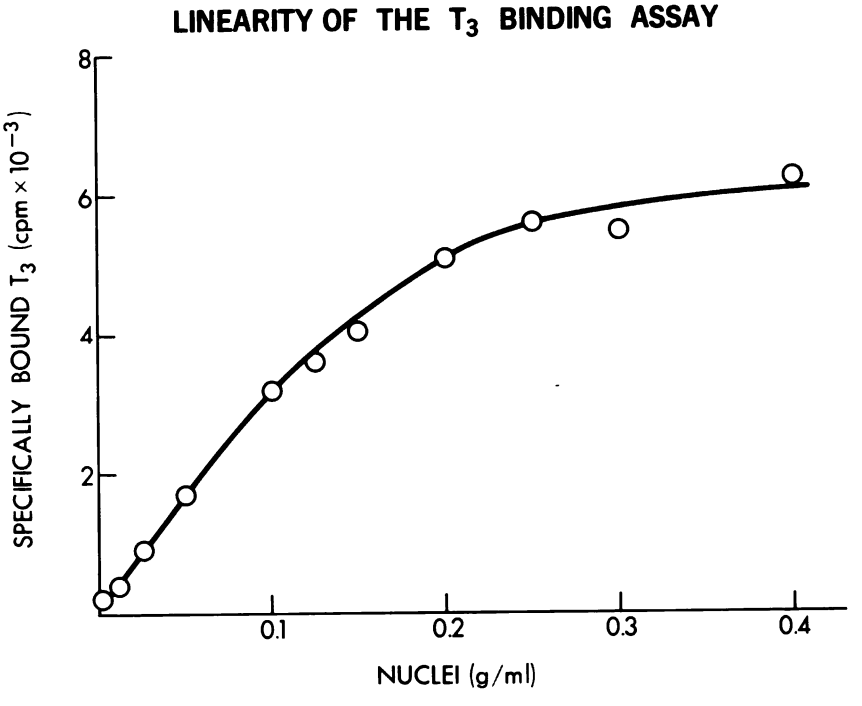

Fig. 3. Linearity of the assay. Binding was assayed in nuclei from euthyroid rats as described in the Methods except the final incubation concentration of (^{125}I)T_3 was 3.7 x 10^{-10} M, and the amount of nuclei (based on the weight of liver from which they were obtained) was varied. The free T_3 concentration determined from the supernatant medium varied from 3.7 x 10^{-10} M at lower concentration points, to 2.9 x 10^{-10} M at higher concentrations of nuclei. This decrease in free T_3 only partially explains the deviation from linearity at higher concentrations of nuclei.

Nuclear binding is highly sensitive to pH (Fig. 4) and to salt (Fig. 5), and binding was ordinarily performed at the optima for these two variables. The salt sensitivity is not observed with receptors which have been extracted from nuclei (16; also confirmed by us).

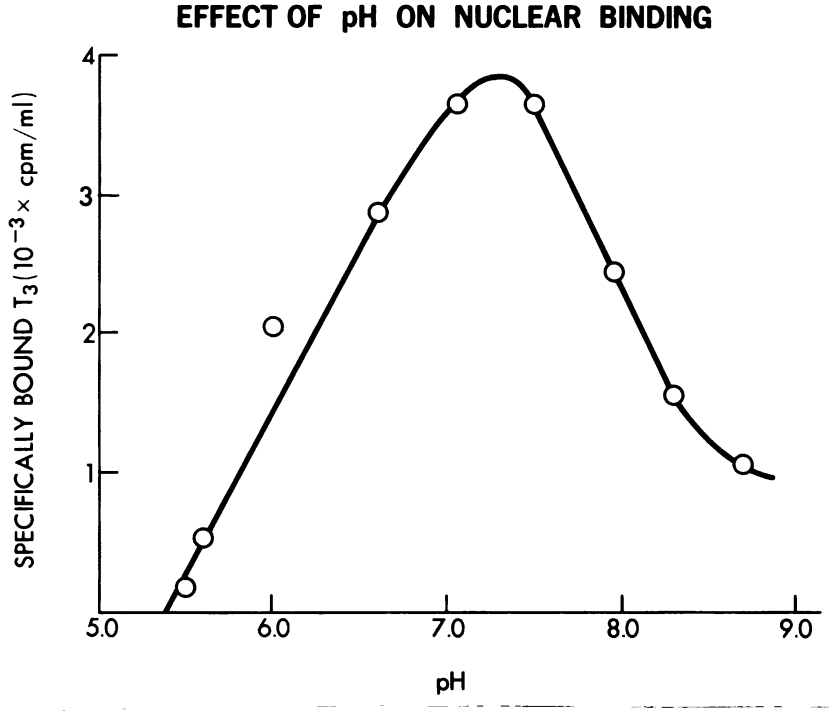

Fig. 4. Effect of pH on nuclear binding. Nuclei from euthyroid rats were prepared and binding was assayed as described in the Methods except that pH was varied.

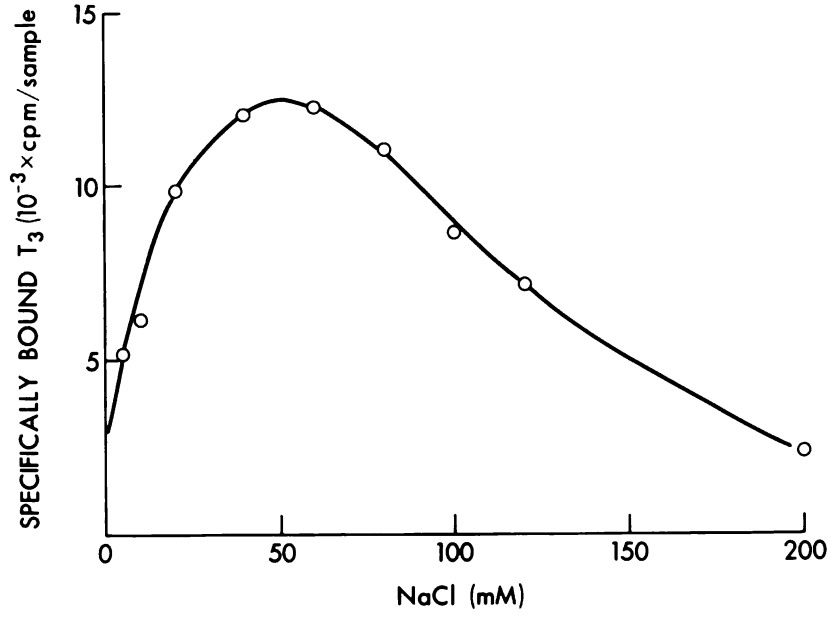

Fig. 5. Effect of NaCl on nuclear binding. (^{125}I)T_3 binding by nuclei from hypothyroid rats was measured as described in Methods except that NaCl was varied.

Kinectic Analysis of the Binding - The data in Fig. 6 indicate that the cell-free binding by isolated nuclei is reversible. In this experiment a portion of nuclei which were bound and equilibrated with $(^{125}I)T_3$ received a "chase" of excess nonradioactive T_3 to prevent subsequent $(^{125}I)T_3$ binding. Dissociation is indicated by the progressive decline in specifically bound radioactivity after the "chase" as compared with control incubations which remained equilibrated with $(^{125}I)T_3$. In three experiments, the time required for 1/2 dissociation ranged from 82 to 140 min (mean = 108 min), and more than 80% of the bound complexes had dissociated by four hours.

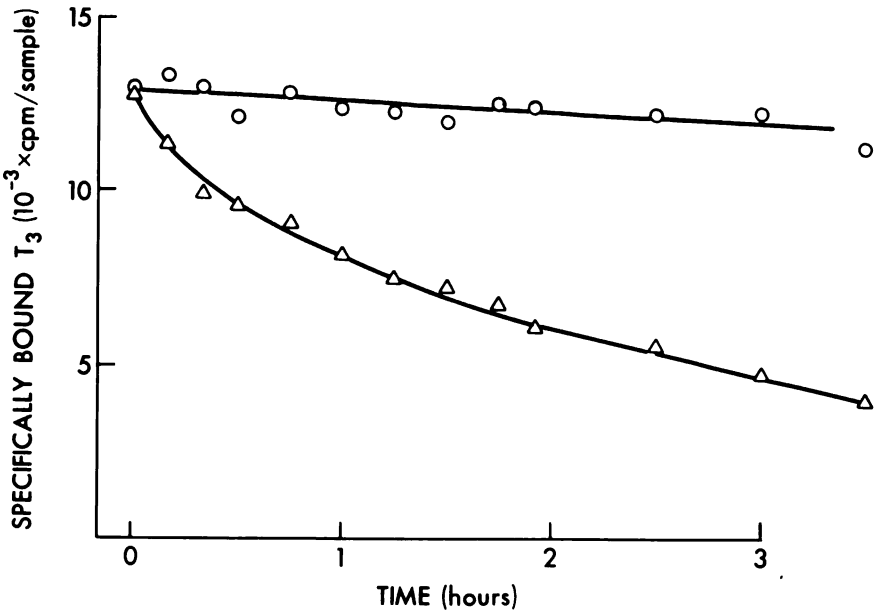

Fig. 6 Rate of dissociation of specifically-bound T_3. Nuclei were incubated with $(^{125}I)T_3$ (3×10^{-10} M) for 90 min at which time half of the tubes each received a "chase" of 20 ml of 5×10^{-5} M nonradioactive T_3 in buffer B to make the final T_3 concentration 10^{-6} M. The incubation at 22° was continued and at the time points indicated, binding in "control" (0) and "chase" (Δ) incubation tubes was measured.

In previous studies, a linear Scatchard plot of the nuclear binding data was obtained (5,9,17). Thus, the binding may conform to the reaction: T_3 plus receptor $\rightleftharpoons$ T_3-recepter complex. If this is true, then the rate of T_3-recepter complex formation should be second order, proportional to the concentration of hormone and unoccupied receptor. Further, the rate of dissociation should be first order, proportional to the concentration of bound complex. Both of these predictions are verified by the kinetic analysis. Fig. 7 shows the second order plot (27) of the log of the ratio of free hormone to free recepter at very early time periods (when the proportion of bound receptors and thus the contribution by dissociation is low). The linearity suggests that the reaction is second order. The apparent association rate constant (k_{+1}) determined in four experiments from the slope of the plots ranged from 3.6×10^7 to 5.3×10^7 $M^{-1}min^{-1}$ (mean = 4.6×10^7 $M^{-1}min^{-1}$).

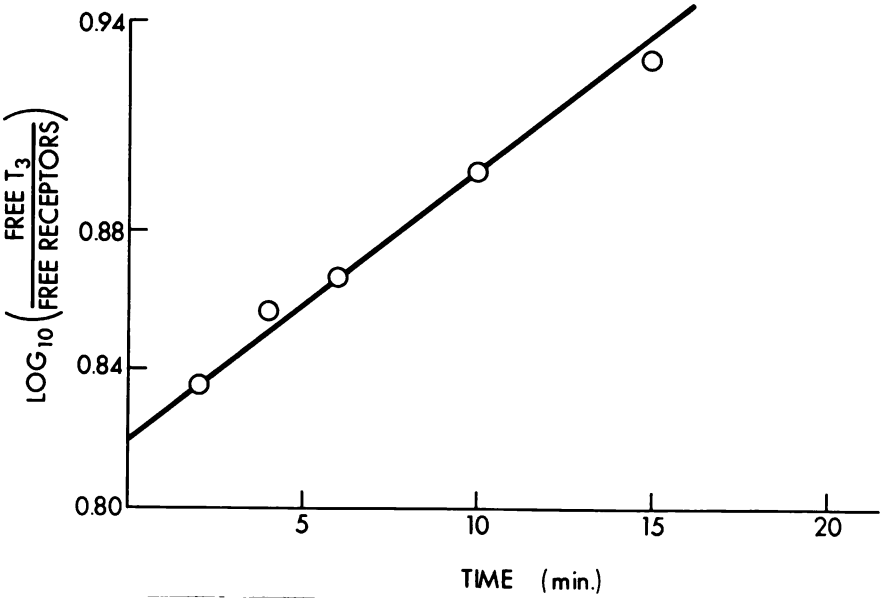

Fig. 7. Analysis of the kinetics of association. Binding by nuclei from hypothyroid rats was measured as described in the Methods except that time was varied. The final (^{125}I)T_3 concentration in the reaction mixture was 5.4×10^{-10} M. The concentration of free T_3 was determined from the supernatant medium after centrifugation of the reaction mixture. The total number of binding sites was determined from a Scatchard analysis of a parallel experiment (Fig. 10). The concentration of free receptors was determined as the total minus the bound.

The kinetics of dissociation (Fig. 8) are first order and the apparent dissociation rate constant (k_{-1}) determined in three experiments (23) ranged from $4.8 \times 10^{-3} min^{-1}$ to $8.4 \times 10^{-3} min^{-1}$ (mean = $6.7 \times 10^{-3} min^{-1}$). The apparent equilibrium dissociation constant (K_d) estimated from the ratio of the rate constants (k_{-1}/k_1) was 1.5×10^{-10} M.

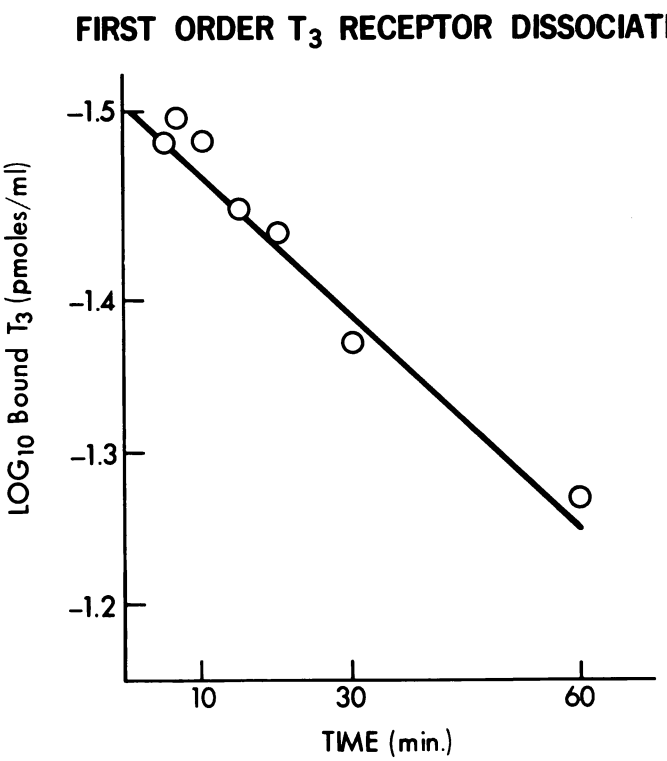

Fig. 8. Analysis of the kinetics of dissociation. Binding by nuclei from hypothyroid animals was measured as described in the Methods. After 5 hours incubation, half of the tubes received a "chase" of 50 ml of 2×10^{-5} M radioactive T_3 in buffer B so that the final nonradioactive T_3 concentration was 10^{-6} M. Then, binding in "chase" and "control" incubations was measured. After correction for denaturation (less than 5% over the 60 min period), the log of the remaining specifically bound T_3 was plotted as a function of time.

Influence of Thyroid Hormone on the Nuclear Content of Sites - Utilizing the optimized reaction conditions, the binding capacity in nuclei from euthyroid and thyroidectomized animals is shown in Fig. 9. The concentration dependency and total levels obtained in the two states are identical. Further, a Scatchard analysis (28) of these data (Fig. 10) also suggests that the affinity and total content of sites are identical in the two states. The apparent equilibrium dissociation constant and number of sites in the euthyroid animal ranged from 1.1×10^{-10} to 2.4×10^{-10} M (mean = 1.9×10^{-10}M) and from 0.6 to 0.8 pmole/mg DNA (mean = 0.7 pmole/mg DNA) respectively in three experiments and in the hypothyroid animal ranged from 1.1×10^{-10} to 2.4×10^{-10} M (mean 1.9×10^{-10} M) and from 0.5 to 0.8 pmole/mg DNA (mean = 0.65 pmole/mg DNA) respectively in five experiments. Considering the approximately 30% loss during washing (Fig. 2), the nuclear content of T_3-binding sites may be about 1 pmole/mg DNA. These data suggest that normal levels of thyroid hormone do not have an influence on the nuclear content of receptors. Since these experiments were performed under conditions in which a minimum of 70%-90% of any endogenously-bound T_3 would have dissociated (based on the kinetic data), and in which most of the receptors are unoccupied at the start of the reaction (see below), it is likely that the comparative estimate of capacity is reasonable. We also examined binding in animals made thyrotoxic by injections of T_3 and T_4. It appeared that the number of nuclear sites was also unchanged in this condition; however, unequivocal results were not obtained.

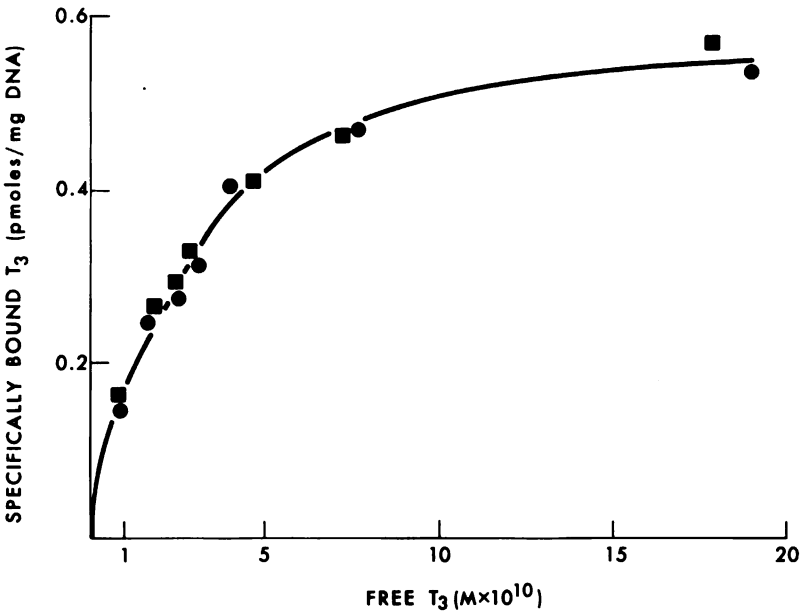

Fig. 9. $(^{125}I)T_3$ concentration dependency for nuclear binding in nuclei from hypothyroid (■) and euthyroid (●) rats. The experiments were performed as described in the Methods except that the concentration of $(^{125}I)T_3$ was varied.

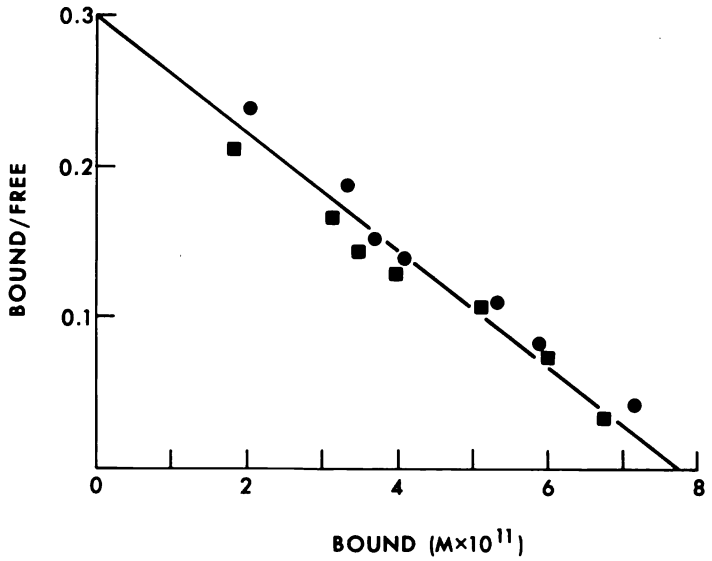

Fig. 10. Scatchard (28) analysis of the binding data from the experiment shown in Fig. 9.

Relation of the Cell-Free Sites to Those in the Animal - As mentioned earlier, it seems likely that the cell-free nuclear sites are identical to those which bind the hormone in the animal. However, conclusions derived from the comparison between euthyroid and hypothyroid animals depends on this assumption. Further, we were cautioned by findings in steroid receptor systems where cell-free nuclear binding sites for receptors were not the same ones which bound the receptor in the intact cell (29-32). We sought additional clarification of this point through a kinetic analysis. If $(^{125}I)T_3$ binds to some sites occupied by hormone in the animal (and which retained hormone through the nuclear preparation procedure), then, the initial concentration of free receptor available for $(^{125}I)T_3$ binding would be reduced due to occupancy of receptors by endogenously-bound hormone. Under these conditions, the initial rate of $(^{125}I)T_3$ binding should be slower in nuclei from euthyroid animals than in nuclei from hypothyroid animals. These kinetic differences should disappear as endogenously-associated hormone dissociates.

As shown in Fig. 11, the early kinetics of association at 22° in nuclei from hypothyroid and euthyroid animals are similar.[3] This suggests that any remaining endogenously-bound formone dissociates too quickly for kinetic differences to be observed. This impression is strengthened by an analysis of the early kinetics at 0° (when dissociation is slowed), where a clear distinction between

[3]The amount of total endogeneus hormone (T_3 (33) and T_4) in the incubation medium at the end of the reaction was determined to be negligible as compared to the radioactive T_3 concentration.

hypothyroid and euthyroid nuclei is observed (Fig. 11). The amount of binding during the first hour is less in nuclei from euthyroid animals than in nuclei from hypothyroid animals even though the amount of binding which is ultimately observed (data not shown for 0°) and the total content of sites (Fig. 10) are the same. The kinetic data at 0° are also shown in Fig. 12 in the form of a rate analysis. For this plot, the "free receptor" concentration was calculated as if no endogenously-bound hormone were present. The apparently slower rate constant (reflected by the slope) in the euthyroid nuclei probably reflects a lower concentration of free receptor at the start of the reaction.[4] It is likely that this is due to occupancy by endogenously-bound T_3 may dissociate from sites which can later bind (^{125}I)T_3. Although these data do not demonstrate that all of the endogenously-bound T_3 can dissociate from sites which may rebind, this possibility seems likely. Also, these data do not allow an estimate of the actual proportion of receptors which were occupied by T_3 (or T_4) in the animal, since the quantity of endogenously-bound material which dissociated during the nuclear preparation procedures is not known. In fact, from the data in Fig. 12, it can be calculated that less than 10% of the receptors were occupied by endogenously-bound T_3 by the start of the reaction. If the estimate by Oppenheimer and colleagues (8) is correct that a larger proportion of the receptors are occupied by hormone in the euthyroid rat, it is likely that substantial dissociation did occur during our preparation procedures.

[4]An alternate explanation would be that the association rate constant in euthyroid nuclei is actually lower. The identical affinity (Fig. 10) and dissociation rate constant (data not shown) argue against this.

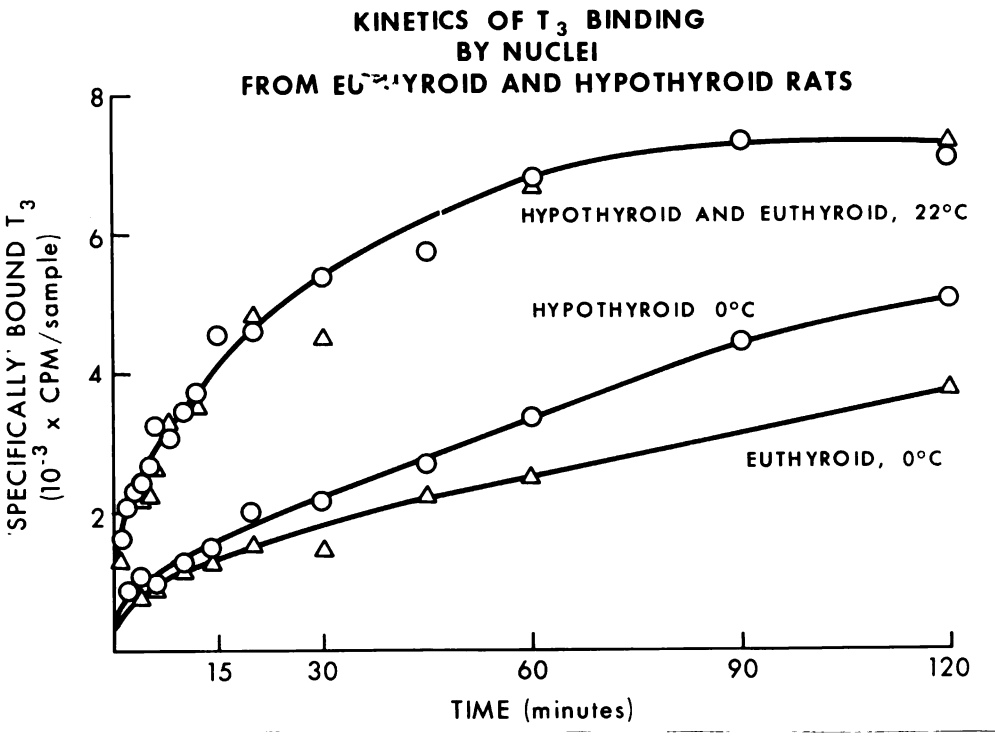

Fig. 11. Early kinetics of T_3 binding by nuclei from hypothyroid (O — O) and euthyroid (Δ — Δ) rats at 0° or 22°. Binding was performed as described in the Methods except that time was varied.

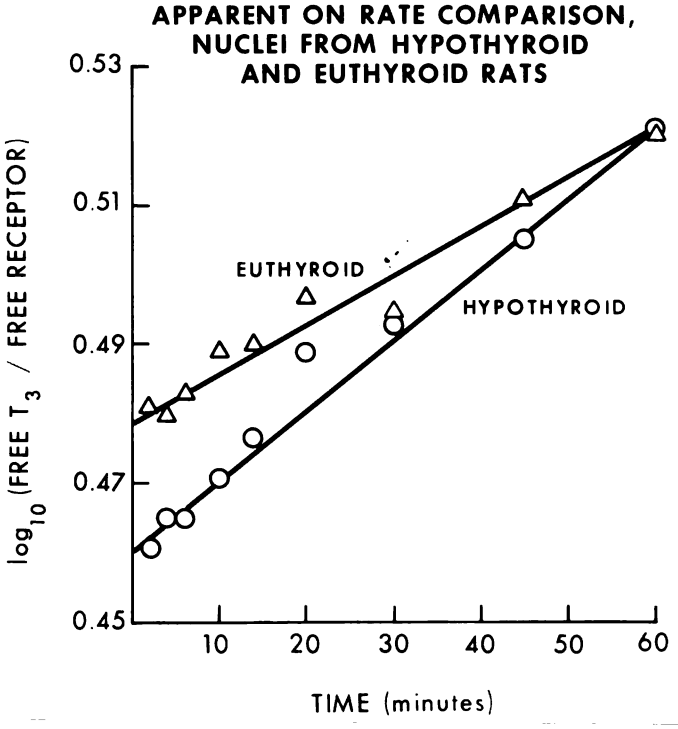

Fig. 12. Comparison of the association kinetics at 0°. The data are taken from the experiment shown in Fig. 9. The free receptor and free T_3 concentrations were determined as described in the legend to Fig. 7. The total receptor concentration was determined from the data shown in Fig. 10 which were obtained in the same experiments.

Comparison of Nuclear and Chromatin Binding - The results suggest that thyroid hormone does not influence the nuclear content of receptor. Since the thyroid receptor may be located on the chromatin component of nuclei, an implication from these results could be that the hormone does not influence the chromatin localization of receptors. If so, free chromatin and nuclear binding should have roughly similar properties. The data in Fig. 13 indicate that the binding of $(^{125}I)T_3$ by rat liver chromatin is similar to that of nuclei. Thus, there may not be a requirement of nucleoplasmic or nuclear membrane factors for binding. It is also likely that the nuclear membrane does not influence receptor accessibility by T_3. These results suggest that the findings using isolated nuclei imply similar properties with respect to chromatin.

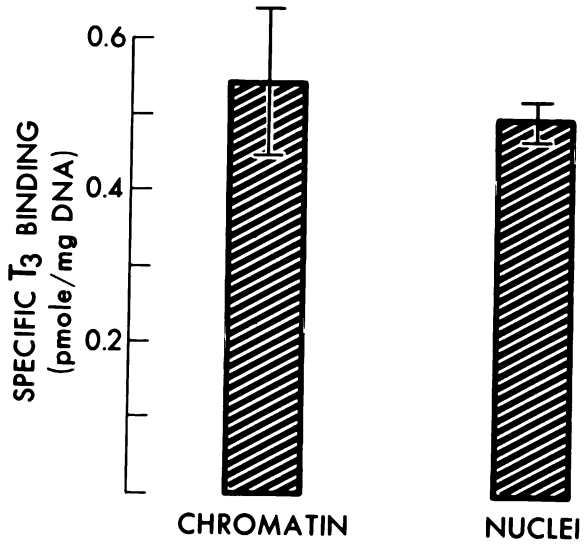

Fig. 13.

Fig. 13. Comparison of $(^{125}I)T_3$ binding by isolated nuclei and chromatin from euthyroid rats. Nuclei were prepared as described in the methods. Chromatin was prepared from the resuspended nuclei from 5 g of liver by a modification of the technique of Axel, Cedar and Felsenfeld (34). Sucrose (2 M) was added to a final concentration of 1.75 M. This mixture was then centrifuged at 22,000 x g for 20 min. The pellet was resuspended with a loose-fitting Dounce glass-glass homogenizer in 20 ml of 0.05 M Tris (pH 7.9) and the mixture was centrifuged at 12,000 x g for 10 min. The pellet was similarily resuspended and then centrifuged at 27,000 x g for 15 min, first in 20 ml of 0.01 M Tris, pH 7.9, and then in 20 ml of 0.005 M Tris, pH 7.9. The final chromatin pellet was similarly resuspended in 0.001 M Tris, pH 7.9 Specific binding of $(^{125}I)T_3$ to chromatin and nuclei was determined as described for nuclei in the methods except that the reaction did not contain NaCl, only one wash was performed after the reaction, and the chromatin was spun at 35,000 x g for 15 min after the reaction and the wash. The graph shows the mean and standard error of the mean of four chromatin incubations and the mean and range of two nuclear incubations.

DISCUSSION

In the present studies the nature of the binding reaction of triiodothyronine (T_3) by putative nuclear receptors has been characterized. The equilibrium dissociation constant and quantity of receptors from our data (at 22°) are in excellent agreement with the value determined by Samuels and Tsai at 37° (5,9). These values reflect a higher affinity than reported by Oppenheimer and colleagues (17) and by Thomopoulos and colleagues (13) determined at 0°. However, the latter workers, using nuclear extracts, did not obtain a linear Scatchard plot, probably due to lack of correction for nonspecific binding. Since the binding reaction is sensitive to such experimental conditions as temperature, pH and salt (Figs. 3-5 and 11), it is likely that differences in the apparent affinity determined in various laboratories would be observed.

Our kinetic analyses support the indications derived from the equilibrium studies. The rate of association is proportional to the concentrations of T_3 and unbound receptor, whereas the rate of dissociation is proportional to the concentration of bound complex. Further, the apparent equilibrium dissociation constant, calculated from the ratios of the rate constants of 1.5×10^{-10} M, is in excellent agreement with the value of 1.9×10^{-10} M determined by the equilibrium data. Thomopoulos and colleagues (13) found first order kinetics of dissociation at 0°, and reported an association constant of T_3 binding by nuclear extracts; however,

an analysis of the association rate data were not presented and it is not clear how this value was determined. The equilibrium dissociation constant they reported from kinetic data was about 3 times smaller than determined by equilibrium data. This constant was roughly ten times larger than we report at 22°.

The binding studies suggest that thyroid hormone, like most of the other hormones, interacts with its receptors without apparent cooperativity. However, many ligand-protein interactions involved in regulation are cooperative; in fact, it was anticipated that cooperativity in regulation might be a quite general characteristic (35). Nevertheless, the thyroid hormone response could exhibit cooperative kinetics at steps distal to the hormone-receptor interaction.

Our studies also suggest that the nuclear content of thyroid hormone receptors is not influenced by physiological levels of the hormone. This finding is in apparent contradistinction to the findings of Samuels and colleagues (5) who found that thyroid hormone administered to cultured GH_1 cells increased the quantity of subsequent cell-free binding. However, these differences may be obliterated when the cell-free nuclei and chromatin appears to be identical, it is likely that the hormone acts neither to remove receptors from nor to add them to chromatin. As indicated earlier, this is an exception to a common pattern whereby stimuli bind the regulatory protein and influence whether or not it is associated with the genome (21-23). However, there is at least one system where an inducer does not alter the concentration of its binding protein on the gene. This occurs in the regulation of the arabinose

operon in bacteria where the inducer apparently causes a translocation of the regulatory protein (*araC* gene product) from one site (where it is a repressor) to another (where it acts as a positive element) (21). Of course, no information is available in this respect regarding thyroid hormone. However, if T_3 hormone does act through binding to these chromatin "receptors", it is likely that the hormonal influence is on properties other than whether the receptor is present in chromatin.

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