Increased Thyroxine Turnover and Thyroidal Function after Stimulation of Hepatocellular Binding of Thyroxine by Phenobarbital

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ABSTRACT Administration of phenobarbital to rats in a dosage schedule previously demonstrated to increase hepatocellular binding of thyroxine results in increased hormonal turnover, due both to increased deiodination and to fecal disposition of thyroxine iodine. The rate of biliary excretion of thyroxine iodine is roughly proportional to the hepatic content of exchangeable thyroxine. The enhanced peripheral disposition of thyroxine appears to lead to increased thyroidal function, as measured by isotopic iodine studies, and the maintenance of a normal nonradioactive serum PBI. On the other hand, thyroidectomized animals maintained on a constant replacement dose of L-thyroxine and treated with phenobarbital exhibit a marked fall in serum PBI. These findings suggest that increased thyroxine flux in phenobarbital-treated animals is secondary to primary stimulation of hepatocellular binding. Exchangeable intracellular thyroxine may thus be an important determinant of hormone turnover and, possibly, of hormonal action.

INTRODUCTION

A number of studies have now established that the liver contains substantial quantities of thyroxine in rapid equilibrium with plasma proteins (1–5). A similar exchange occurs between thyroxine bound to plasma proteins and cellular thyroxine in the kidney (5), and it appears likely that other tissue pools are also in equilibrium with plasma thyroxine. The cellular mechanism responsible for retaining thyroxine in competition with plasma proteins has not been defined but may be regarded from a kinetic standpoint as cellular binding.

Previous studies have indicated that injection of phenobarbital and chlordane stimulates hepatocellular binding of thyroxine (6). In these experiments, phenobarbital and chlordane were administered in a dosage regimen known to induce the formation of a variety of drug-metabolizing enzymes (7). Augmented liver binding resulted both from an increase in total hepatic mass and from increased binding per gram of liver. This was demonstrated by a shift of thyroxine-125I (T4,125I) from plasma to liver in the absence of demonstrable alterations in plasma binding of thyroxine. Despite the redistribution of isotopic thyroxine the concentration of nonradioactive PBI in plasma remained unchanged.

The present study was undertaken in order to elucidate the effect of augmented hepatocellular binding on the peripheral disposition of thyroxine. It was felt that the results of this investigation might provide a broader understanding of the factors governing the net thyroxine flux in the whole organism.

METHODS

Male rats of the CD strain (Charles River, Boston) weighing between 150 and 250 g were used. In order to facilitate cannulation of the bile ducts larger male rats of the same strain (300–420 g) were used in determination of biliary excretion. In testing the effect of pheno-
barbital on the serum PBI in thyroidectomized animals, female CD rats (150–200 g) were used because the sup-
plier could not provide male thyroidectomized animals at the time of these studies. Control studies had indicated that female rats and older rats were equally suitable for demonstrating the effect of phenobarbital on hepatocellular binding. Animals were housed in animal quarters under constant temperature conditions and fed a Wayne Laboratory diet containing approximately 1 μg of I per g of diet.

Plasma disappearance studies were performed in a manner similar to that used by Gregerman (8). Animals were injected under light ether anesthesia through the tail vein with 0.5 μc of Tr-131 (less than 0.04 μg), dis-
solved in 50% propylene glycol (Abbott Laboratories, North Chicago, Ill.). All animals received intraperi-
toneal injections of 1 mg NaI twice a day in order to pre-
vent thyroidal accumulation of 131I. Plasma samples were
obtained from freely flowing blood from the cut tail 6, 24, 30, and 48 hr after injection. Plasma samples were pro-
cessed and assayed for radioactivity as previously described (5). 6 hr after injection of the tracer the plasma disap-
ppearance curve approached a straight line on a semilog-
rithmic plot (Fig. 1). Fractional removal rate (k) was
determined by the method of least squares. The thy-
xine distribution space (TDS) was calculated from the
reciprocal of the zero-time extrapolation. The meta-
bolic clearance was calculated as the product of k and
TDS.

Complete collections of urine and feces were obtained in
metabolic cages. Collections were made at 24-hr intervals,
at the end of which the cage and collecting syphon were
carefully washed to remove residual radioactivity. Washes were pooled with the urine. Fecal collections were
homogenized and digested in concentrated KOH. Ali-
quots of urine and fecal digests were counted in an Auto-
gamma spectrometer well. Urinary and fecal clearances
were calculated as the products of the metabolic clear-
ance and the fraction of total radioactivity excreted via
the urinary or fecal route. At the end of each study the

![Graph](image)

Figure 1 Mean plasma disappearance curves of 11 con-
trol animals and 13 animals treated with phenobarbital.
Vertical bars indicate ± se.

The effect of phenobarbital treatment on serum PBI
(BioScience, Van Nuys, Calif.) in a group of thyroidecto-
mized animals maintained on a constant dose of L-thyro-
Xine was assessed. 10 thyroidectomized animals supplied by
Charles River were injected subcutaneously daily with 2
μg of thyroxine per 100 g of body weight and were main-
tained on 1% calcium gluconate in their drinking water.
Starting on the 5th day, five animals were injected addi-
tionally with phenobarbital (100 mg/kg). The remain-
ing five animals received daily thyroxine injections and
saline alone. On the 10th day a combined dose of albu-
min-131I, and Tr-131 was injected intravenously as previ-
ously described (5). 35 min afterwards the animals were
sacrificed. Liver and plasma radioactivity were measured,
and calculations of tissue uptake of thyroxine were performed as previously indicated (5).

The thyroid gland was assayed both for \(^{131}I\) to measure thyroidal accumulation of \(^{131}I\), and for \(^{131}I\) to measure the thyroid: serum iodide concentration ratio (T/S ratio). 24 hr before sacrifice, 10 \(\mu\)g of carrier-free Na\(^{131}I\) was administered intraperitoneally. 90 min before sacrifice, 15 mg of propylthiouracil was injected subcutaneously. 45 min later 20 \(\mu\)g of \(^{131}I\) was injected. The animal was sacrificed by exsanguination. The thyroid gland was carefully dissected, weighed, and assayed for radioactivity. The concentration of \(^{131}I\) in serum was measured, and the concentration of PB\(^{131}I\) was determined after precipitation of serum proteins with 20% trichloracetic acid.

**RESULTS**

*Turnover studies (Table I and Fig. 1).* In agreement with previous findings (6) administration of phenobarbital resulted in an increased hepatic mass with a proportionately greater increase in hepatic thyroxine distribution volume. At the same time the metabolic clearance of thyroxine increased 62% above levels in control animals. The increased metabolic clearance was due both to a 33% increase in fractional removal rate and a 19% increase in total thyroxine distribution volume. Since the concentration of nonradioactive PBI does not change significantly under these conditions (6), these results imply an increased absolute turnover of thyroxine.

Thyroxine iodine is excreted via both fecal and urinary routes. In control animals 49.8% of excreted radioactivity was found in the urine and 50.2% in the stool. Administration of phenobarbital resulted in a significant \((P < 0.05)\) change in the partition of excreted radioactivity between fecal and urinary routes. An average of 43.5% of excreted radioactivity was found in the urine and 56.5% in the fecs of phenobarbital-treated animals. Recovery of radioactivity was similar in experimental and control animals (control, 84.9%; phenobarbital, 85.2%).\(^1\) The fecal clearance

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**Table I**

*Effect of Phenobarbital on Peripheral Metabolism of Thyroxine*

<table>
<thead>
<tr>
<th>Animal wt</th>
<th>Liver wt</th>
<th>TDS</th>
<th>k</th>
<th>MC</th>
<th>UC</th>
<th>FC</th>
<th>Liver space</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g/mL 100 g</td>
<td>day(^{-1})</td>
<td>mL/100 g per day</td>
<td>mL/100 g per day</td>
<td>mL/100 g per day</td>
<td>mL/100 g per day</td>
</tr>
<tr>
<td>C</td>
<td>P</td>
<td>g</td>
<td></td>
<td>mL/100 g</td>
<td></td>
<td>mL/100 g</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>205</td>
<td>211</td>
<td>8.71</td>
<td>15.51</td>
<td>15.6</td>
<td>18.6</td>
<td>1.10</td>
</tr>
<tr>
<td>SE</td>
<td>3.6</td>
<td>7.8</td>
<td>0.30</td>
<td>0.65</td>
<td>0.69</td>
<td>0.81</td>
<td>0.04</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

In control (C) group, 11 animals were studied; in phenobarbital (P) group there were 13 animals. TDS, total thyroxine distribution space; \(k\), fractional turnover by plasma; MC, metabolic clearance; UC, urinary deiodinative clearance; FC, fecal clearance; liver space, total hepatic tissue radioactivity: plasma concentration of thyroxine-\(^{131}I\) 48 hr after injection. TDS, MC, UC, FC, and liver space are expressed in terms of 100 g of body weight.

**Table II**

*Effect of Phenobarbital on Biliary Excretion of Thyroxine*

<table>
<thead>
<tr>
<th>Animal wt</th>
<th>Liver wt</th>
<th>Liver space</th>
<th>Bile flow</th>
<th>Bile:plasma conc. ratio</th>
<th>Biliary clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g/mL per day</td>
<td>mL/hr</td>
<td>g/mL/hr</td>
<td>mL/hr</td>
</tr>
<tr>
<td>C</td>
<td>P</td>
<td>g</td>
<td>mL</td>
<td>g/mL</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>360</td>
<td>369</td>
<td>13.2</td>
<td>19.4</td>
<td>10.6</td>
</tr>
<tr>
<td>SE</td>
<td>11</td>
<td>11</td>
<td>1.0</td>
<td>1.3</td>
<td>0.47</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Biliary clearances determined 24 hr after injection of T\(_4\)-\(^{131}I\) in seven control (C) and six phenobarbital-treated (P) animals.

\(^1\) Recovery was calculated as the percentage of administered radioactivity excreted in urine and feces plus estimated residual body radioactivity (TDS \(\times\) terminal thyroxine-\(^{131}I\) concentration in plasma). In calculating the fecal and urinary clearance the assumption was made that the losses of radioactivity were partitioned in the same proportion between urinary and fecal routes as the recovered excretion of radioactivity.

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**Phenobarbital Effect on T\(_4\) Flux** 1401
creased 88%, and urinary degradative (deiodinative) clearance increased 40% as a result of phenobarbital treatment.

Biliary clearance (Table II). The increased fecal clearance observed as a result of phenobarbital treatment could have resulted either from increased biliary excretion of thyroxine, diminished reabsorption from the gut, or both. Direct measurements of biliary clearance indicated a 137% increase above control levels. The increase in hepatic distribution space in these animals was 151%. The increase in biliary excretion was due both to an increase in the biliary flow and to an increase in the bile:plasma concentration ratio.

For technical reasons measurements of biliary excretion were made in larger animals than those employed in the turnover studies. Moreover, biliary measurements were performed shortly after surgical trauma and under ether anesthesia. Therefore, absolute biliary clearance cannot be easily compared to the fecal measurement performed in the smaller and unanesthetized animals. Nevertheless, the marked relative increase in biliary clearance induced by phenobarbital would appear to adequately account for the observed increase in fecal disposition of thyroxine.

Thyroid function (Table III). The finding of increased peripheral thyroxine disposal under steady-state conditions implies increased thyroidal release of hormone. Results of thyroid function tests supported the concept of increased thyroidal activity in animals treated with phenobarbital. Although turnover studies were not performed in chlordane-treated rats it seems reasonable to suppose that the increased thyroidal activity in these animals was secondary to previously demonstrated hepatic effects (6). Percentage increases over control animals in the phenobarbital-treated group were: 24 hr uptake, 80; PB131I, 37; and T/S ratio, 51. Corresponding percentage increases in the chlordane-treated group were: 24 hr uptake, 54; PB131I, 28; and T/S ratio, 38. Only slight increases in the thyroid weight were noted, 9.5% in the case of phenobarbital and 14.5% in the case of chlordane. The increase in weight was statistically significant (P < 0.005) in the case of phenobarbital but not so in the case of chlordane.

These results suggested that increased thyroidal secretion of thyroxine was responsible for maintaining a normal level of serum PBI in phenobarbital-treated animals (6). Additional evidence favoring this conclusion is the result of experiments in which thyroidectomized animals were maintained on constant doses of L-thyroxine (2 μg/100 g of body weight) (Table IV). Pheno-}

**Table III**

<table>
<thead>
<tr>
<th></th>
<th>Animal wt</th>
<th>Liver wt</th>
<th>24 hr uptake</th>
<th>PB131I</th>
<th>T/S</th>
<th>Thyroid wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C X</td>
<td>C X</td>
<td>% dose</td>
<td>% dose/ml</td>
<td></td>
<td>mg</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>248 238</td>
<td>9.8 10.8</td>
<td>6.8 12.3</td>
<td>0.19 0.26</td>
<td>19.6 29.7</td>
<td>6.56 7.19</td>
</tr>
<tr>
<td></td>
<td>2.5 2.5</td>
<td>0.2 0.3</td>
<td>0.6 1.0</td>
<td>0.02 0.04</td>
<td>1.7 3.3</td>
<td>0.24 0.18</td>
</tr>
<tr>
<td></td>
<td>NS &lt;0.025</td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.025</td>
<td></td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Chlordane</td>
<td>214 208</td>
<td>8.2 9.2</td>
<td>5.8 8.8</td>
<td>0.19 0.25</td>
<td>20.8 28.8</td>
<td>6.62 7.59</td>
</tr>
<tr>
<td></td>
<td>6.7 11.1</td>
<td>0.4 0.6</td>
<td>0.7 0.8</td>
<td>0.01 0.01</td>
<td>1.4 2.7</td>
<td>0.41 0.49</td>
</tr>
<tr>
<td></td>
<td>NS NS</td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.02</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

Six animals were studied in each group. C, control groups; X, experimental groups; T/S, thyroid: serum iodide concentration ratio.

**Table IV**

<table>
<thead>
<tr>
<th></th>
<th>Animal wt</th>
<th>Liver wt</th>
<th>Liver space</th>
<th>Serum PBI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C P</td>
<td>C P</td>
<td>ml</td>
<td>μg/100 ml</td>
</tr>
<tr>
<td>Mean</td>
<td>174 176</td>
<td>6.28 9.11</td>
<td>7.4 11.4</td>
<td>4.96 2.60</td>
</tr>
<tr>
<td></td>
<td>5.1 4.9</td>
<td>0.30 0.26</td>
<td>0.82 0.71</td>
<td>0.31 0.08</td>
</tr>
<tr>
<td></td>
<td>NS &lt;0.001</td>
<td>&lt;0.02</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Five control (C), five phenobarbital-treated (P) animals.
The results of these studies clearly indicate that increased deiodination and biliary clearance of thyroxine accompany the enhanced hepatocellular binding of thyroxine induced by phenobarbital. It is tempting to suggest that these phenomena are causally related, i.e., that the hepatocellular content of exchangeable thyroxine governs the rate of hepatic deiodination and biliary transport. This hypothesis is most readily tested by measurements of biliary clearance rather than total deiodinative clearance. The liver is the only organ directly involved in biliary transport of thyroxine, whereas a number of tissues contribute to the over-all deiodination of the thyroid hormones. The similarity in the relative increase in hepatic thyroxine space (2.5-fold) and the relative increase in clearance (2.4-fold) induced by phenobarbital supports the concept of intracellular exchangeable thyroxine as an important determinant of the rate of cellular metabolism. The enhanced biliary excretion of thyroxine also adequately accounts for the increased disposition of thyroxine via the fecal route.

The increased total deiodinative clearance probably reflects enhanced metabolism of thyroxine in the stimulated liver. In previous studies (6) it was shown that 35 min after the intravenous injection of tracer, the increased total volume of distribution of T₄₁²³I could be attributed exclusively to the increased hepatic distribution volume. Similarly, in the present experiments the differences in the total thyroxine distribution volume between the phenobarbital-treated and control animals was 7.2 ml, whereas the difference between hepatic thyroxine distribution volumes was 7.5 ml. These findings strongly suggest that the phenobarbital effect on peripheral thyroxine metabolism is confined to the liver.

If we accept the likelihood that phenobarbital acts only upon the liver, we may calculate the contribution of hepatic deiodination to total tissue deiodination. Thus, total deiodinative clearance (Dₜ) represents the sum of hepatic deiodinative clearance (Dₜ) and the deiodinative clearance by extrahepatic tissue (Dₑ). If we assume that deiodination is proportional to the content of exchangeable tissue thyroxine,²

\[
Dₜ = αVₜ \quad \text{and} \quad Dₑ = βVₑ \quad (1, 2)
\]

in which \(Vₜ\) = hepatic thyroxine distribution volume, \(Vₑ\) = extrahepatic cellular thyroxine distribution volume, and \(α, β\) are proportionality constants. Thus,

\[
Dₜ = αVₜ + βVₑ. \quad (3)
\]

Since we assume that phenobarbital effects are limited to the liver,

\[
(Dₜ)ₚ = α(Vₜ)ₚ + β(Vₑ)ₑ \quad (4)
\]

\[
(Dₜ)ₑ = α(Vₜ)ₑ + β(Vₑ)ₑ \quad (5)
\]

in which the subscripts \(c\) and \(p\) refer to control and phenobarbital-treated groups. Solutions of simultaneous equations 4 and 5 from data in Table I will yield the following values:

\[
(Dₜ)ₑ = α(Vₜ)ₑ = 3.2 \text{ ml/100 g per day}
\]

\[
(Dₜ)ₚ = α(Vₜ)ₚ = 6.5 \text{ ml/100 g per day}
\]

\[
(Dₑ)ₑ = Dₑ)p = βVₑ = 5.2 \text{ ml/100 g per day}
\]

Thus, under control conditions the liver is responsible for approximately 38% of the thyroxine deiodinated in the animal. In the phenobarbital-treated group, the contribution of hepatic deiodination rises to 55% of the total.

In previous studies phenobarbital was found not to cause a statistically perceptible change in serum PBI despite the marked shift of isotopic T₄ between plasma and liver observed 35 min after

²It appears quite likely that hepatic thyroxine as determined from the product of the hepatic thyroxine distribution volume and the plasma thyroxine concentration is largely, if not exclusively, in the exchangeable form. After initial equilibration which takes place in the first 15 min after intravenous injection of labeled thyroxine, the plasma and hepatic radioactivity decline at nearly parallel rates over the ensuing several days (9). Since hepatic radiothyroxine has been demonstrated to be entirely exchangeable in the early phase (5), and since hepatic radioactivity is largely in the form of thyroxine, it follows that at the intervals considered in this study hepatic thyroxine is also in the exchangeable form. This conclusion is further strengthened if one compares the liver : plasma thyroxine concentration ratio 35 min after injection of T₄₁²³I, 0.6 (6) to that obtained in animals equilibrated with ᵃ¹³I, 0.5 (10).
intravenous injection of the tracer. These findings raised the possibility that the thyroid was releasing additional amounts of hormone which served to sustain the plasma PBI at normal levels. In the present series of experiments the increased thyroidal activity was confirmed by isotropic measurements of thyroid accumulation of iodine, the thyroid:plasma iodide gradient, and the concentration of PB131I in plasma. These results confirm previous reports of increased thyroidal activity in rats after barbiturate treatment (11, 12). Although it is difficult quantitatively to relate these increases to the observed augmentation of peripheral turnover, the general direction of the changes serves to support the idea that increased thyroidal secretion of thyroxine is responsible for maintaining a normal PBI. Additional data favoring this explanation were obtained in the study of thyroidectomized animals maintained on a constant dosage regimen of T4. In this group there was a marked fall in serum PBI in the subgroup treated with phenobarbital. The fall in serum PBI was somewhat greater than one would have anticipated on the basis of the observed increase in liver space, but this discrepancy can probably be attributed to limitations in interpretation imposed by the small number of animals studied.

The following sequence of events can be postulated to explain the observed effects of phenobarbital on thyroxine metabolism and thyroid function. Administration of phenobarbital causes a primary increase in hepatocellular binding, as a result of both increased liver mass and increased binding per gram liver. The ensuing shift of thyroxine from extrahepatic hormonal stores to the liver results in an increased pool of exchangeable hepatic thyroxine. This serves to stimulate both hepatic deiodinative and excretory processes. Since no major alterations in enteric absorption of thyroxine occur, both fecal and deiodinative clearances increase. The fall in plasma and extrahepatic thyroxine activates a negative feedback system which results in increased thyroidal secretion. It seems possible that depletion of the hypothalamic or pituitary content of exchangeable thyroxine serves as a stimulus to augment pituitary secretion of thyroid-stimulating hormone (TSH) and to increase thyroidal activity. A new steady state is achieved in which plasma and extrahepatic thyroxine tissue stores have returned to near normal levels. The total body pool of exchangeable thyroxine has increased as has the net turnover of hormone. Although our previous studies have failed to demonstrate any statistically significant fall in serum PBI in the intact phenobarbital-treated rat (6),3 it is conceivable that a small reduction in PBI might not have been detectable with relatively crude chemical methods. A small reduction might serve to explain the perpetuation of increased thyroidal activity and thyroxine flux in the new steady state.

Although considerable attention has been given to the role of plasma proteins in regulating the peripheral turnover of thyroxine, the importance of cellular factors has also been recognized (13–16). Discrepancies between the turnover of thyroxine and the serum-free thyroxine concentration have been attributed to alterations in the cellular disposal of hormone (17–21). On the basis of experiments employing tissue slices, Freinkel, Ingbar, and Dowling have previously demonstrated the existence of an equilibrium state between thyroxine bound to cellular and extracellular sites (13) and have discussed the possible role of such binding sites in thyroxine metabolism. The nature of thyroxine binding by subcellular components has also been extensively investigated by Tata (16). The precise relationship between intracellular binding and thyroxine metabolism, however, was not experimentally defined in these studies.

Our analysis of intracellular binding differs from previous approaches inasmuch as binding is defined in terms of the in vivo tissue:plasma thyroxine concentration ratio (4, 5). A much larger fraction of total exchangeable thyroxine appears to be associated with cellular binding sites than was suggested by earlier studies. Our results also allow some tentative generalizations regarding the relationship of intracellular binding and metabolism. The direct correlation between hepatocellular binding, deiodination, and biliary transport suggests that the thyroxine–cellular binding complex might participate as a unit in intracellular metabolic reactions. If it were necessary for thyroxine to dissociate from the intracellular binding

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3 Additional studies have failed to reveal statistically significant decreases in the serum PBI after 1, 5, and 10 days of treatment with phenobarbital (100 mg/kg). Groups of five control and five experimental animals were used at each time interval.
complex into a free form before becoming available for deiodination or biliary excretion, increased hepatocellular binding, like increased plasma protein binding, might be expected to result in decreased rather than increased fractional disposition.

Our results raise the possibility that exchangeable intracellular thyroxine may play a pivotal role in determining the peripheral metabolism of thyroxine. One would anticipate that perturbation in plasma protein binding would lead to a new steady state characterized by a normal exchangeable intracellular pool and a normal hormonal turnover. The constancy of hormonal turnover with selective alterations in plasma binding is well recognized (22). Although comparatively few measurements of exchangeable intracellular thyroxine have been made, patients with an idopathic decrease in thyroxine-binding globulin show no systematic variation in cellular hormone (2, 4). With primary alterations in intracellular binding, however, one would expect changes in steady-state thyroxine flux under all circumstances in which the operation of the normal pituitary feedback mechanism remains unimpaired. It is quite likely that simultaneous alterations in cellular and extracellular binding may occur under specific physiological and pathological settings. The possibility should also be recognized that some factors may independently influence the rate constants describing the relationship between intracellular binding and subsequent metabolic transformations.

Changes in intracellular binding after administration of phenobarbital are confined to the liver. It would be important to uncover examples of primary changes in intracellular binding involving other organs and tissues. Lastly, it seems reasonable to suppose that the metabolic action of thyroxine as well as its rate of disposal is closely geared to the intracellular exchangeable thyroxine pool. This suggestion appears amenable to experimental verification.

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