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Research Article

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ABSTRACT The effect of 5,5′-diphenylhydantoin on thyroxine metabolism was examined in five normal volunteers. Intravenous injection of radiothyroxine was followed by a 10–12 day control and subsequent 9–14 day treatment periods. During oral administration of diphenylhydantoin, plasma thyroxine concentration decreased to about 80% of its pretreatment level and the plasma radiothyroxine disappearance rate increased a maximum of 20% over control estimates. These changes were a result of increases in both urinary and fecal excretion of radioisotope.

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These results indicate that thyroxine degradation can proceed at a normal rate in subjects receiving diphenylhydantoin despite decreases in plasma free thyroxine concentration. If free thyroxine is the only portion of the hormone available for cellular utilization, then free thyroxine clearance must be increased in these subjects. This increase in clearance could represent either a direct stimulation of peripheral thyroxine metabolism by diphenylhydantoin, or it could reflect the response of intrinsic regulatory systems to a diphenylhydantoin-mediated displacement of thyroxine from thyroxine-binding globulin. Whatever the mechanism for this effect, a decreased free thyroxine value in patients receiving diphenylhydantoin may not imply hypothyroidism.

INTRODUCTION

The association of a decreased serum protein-bound iodine (PBI) in euthyroid patients receiving 5,5′-diphenylhydantoin (DPH) has been widely recognized since the initial report of Oppenheimer, Fisher, Nelson, and Jailer in 1961 (1). As the effect of this drug on the PBI was identical whether given to hypothyroid patients receiving replacement therapy or to patients with normal thyroid function, its action was assumed to be extrathyroidal (1). In subsequent studies, Wolff, Standaert, and Rall (2) and Oppenheimer and Tavernetti (3) demonstrated that DPH competed with thyroxine (T_4) in vitro for binding sites on thyroxine-binding globulin (TBG). Similar competition in vivo is thought to explain the low PBI during DPH treatment. The thyroxine kinetics in these subjects would presumably resemble those found in patients deficient in TBG binding capacity. In these patients, the rapid turnover of a diminished thyroxine pool leads to the degradation of normal amounts of thyroxine and euthyroidism despite the low PBI (4, 5).

These assumptions have not, to our knowledge, been verified by direct examination of thyroxine kinetics in

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subjects before and during DPH administration. This question has become more intriguing since the recent demonstration by Chin and Schussler that some patients receiving DPH have decreased free thyroxine concentrations (6). If free thyroxine is rate-limiting in thyroxine degradation, a decrease would be expected to result in a decrease in thyroxine degradation and possibly to hypothyroidism. The following study was undertaken to examine the magnitude and time course of the effects of DPH on thyroxine metabolism and to correlate these effects with changes in the free thyroxine.

METHODS

The five normal volunteers used in this study were males, ages 35 through 61, hospitalized solely for the purpose of this study on the Clinical Research Unit of the University of Cincinnati Medical Center. There was no evidence of thyroid, renal, hepatic, or other disease known to affect thyroxine turnover in any volunteer by physical examination or laboratory study. Each received 100–200 mg of potassium iodide per day beginning several days before the intravenous administration of tracer T_4-^{131}I and throughout the study.

About 100–200 μCi of thyroxine-^{131}I (Abbott Laboratories, North Chicago, Ill., specific activity 30–70 μCi/μg) was administered using a calibrated syringe on day zero. The tracer for W. E., H. R., and C. S. had been dialyzed to remove contaminating $^{131}I^-$ as suggested by Nicoloff and Dowling (7). The residual $^{131}I^-$ was less than 0.2% as determined on the day of injection by paper electrophoresis in glycine-acetate buffer, pH 8.6. The undialyzed tracer for D. S. and J. H. contained 2.4% $^{131}I^-$ and 2.1% $^{131}I^-$, respectively.

DPH (Dilantin, Parke, Davis & Co., Detroit, Mich.) was administered orally on the following various schedules: W. E. and H. R. received 6.7 mg/kg and 4.7 mg/kg, respectively, on days 12 through 26; J. G., 2.4 mg/kg on days 10 through 17 and 4.9 mg/kg on days 17 through 24; D. S., 7.0 mg/kg on days 10 through 18; and C. S., 5.3 mg/kg on days 10 through 25. These dosages correspond to the usual therapeutic range of 300–400 mg/day. The subjects received no other medication during the period of study.

In subject D. S. the DPH was discontinued after 9 days because of the discovery of thrombocytopenia. He was subsequently found to have idiopathic thrombocytopenic purpura, which was thought to antedate this study.

Isotope measurements. After injection of T_4-^{131}I, blood samples were collected in heparinized syringes at 5, 10-, 15-min, 3-hr, and 24-hr intervals throughout the control and treatment periods.

In the initial studies with W. E. and H. R., early morning ad libitum activity was allowed before venipuncture. We subsequently became aware of the increase in plasma thyroxine associated with the upright posture (8), and all subsequent plasma samples were obtained after 6–8 hr bed rest. The plasma thyroxine values for W. E. and H. R. were in the upper range of normal (*vide infra*).

Plasma T_4-^{131}I concentration was estimated from the total radioactivity contained in 3-ml specimens counted in an automatic gamma well counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Its activity was compared with suitably diluted fractions of the administered dose prepared at the time of injection.

The total 24-hr urine and stool collections were homogenized and counted with standards of like volume made from aliquots of the injected T_4-^{131}I in a low-level, steel shield with a 4 inch × 4 inch NaI (T1) crystal and multichannel analyzer (Packard Model No. 115, Packard Instrument Co., Downers Grove, Ill.). Counting times were selected to obtain counting errors of less than 2%. Plasma, urine, and stool radioactivity were all expressed as a per cent of the injected dose.

In vivo counting. An estimate of total body radioactivity was obtained from a profile scan of the patient in a whole body counter equipped with a 4 inch × 6 inch NaI (T1) crystal. The patient was placed in a supine position and timed counts were obtained at nine standard locations 16 cm from the mid-coronal plane. The locations chosen were the head, neck, and shoulders, upper thorax, liver, mid-abdomen, pelvis, thigh, knees, and ankles. The placement of the crystal over these positions was constant from day to day. Each day the timed counts from each of the nine areas were totaled and this figure was used as an approximation of whole body activity. This was expressed as a percentage of the initial count obtained immediately after injection. These results were used to verify the accuracy of excretory collections. In all subjects there was good agreement between the profile scan results and the residual activity predicted from the urine and stool collections.

In addition, thyroid, liver, and heart radioactivity was also specifically monitored by either a dual 3 inch × 1 inch (thyroid, thigh) or 4 inch × 4 inch NaI (T1) crystal. The hepatic and cardiac areas were located by percussion, and the desired crystal position was marked with indelible pencil. Only relative changes in organ radioactivity were obtained by this technique. Both profile scan and organ counts were obtained 5–7 days/wk.

Laboratory determinations. Plasma thyroxine levels were determined by the method of Murphy (9) with minor modifications. The yield of T_4 in the initial ethanol extraction of plasma was 79.6 ±1.3% (mean ±SD) based on the extraction of T_4-^{131}I (free of contaminating $^{131}I^-$) from 21 different plasma samples at different times over the course of these studies. The presence of DPH had no effect on this extraction nor did it affect the subsequent displacement assay in amounts greatly in excess of those present in these samples. All reported thyroxine values were corrected for this incomplete yield. For these studies, duplicate 1-ml samples of plasma were extracted with ethanol and duplicate aliquots of each extraction were then assayed. Each sample group contained equal numbers of control and treatment samples to eliminate any error due to day to day variation in technique. A 1 ml sample from a single large serum pool was assayed with each determination for quality control. Over a 3 month period, the mean of 16 such determinations was 8.1 ±0.1 μg/100 ml (mean ±SE). The normal range of *uncorrected* values in our laboratory, 4–11 μg/100 ml as thyroxine, is similar to values reported by others (9, 10).

Free thyroxine was calculated from the product of the total plasma thyroxine concentration and the free thyroxine fraction (FTF). Radioactive thyroxine of high specific activity (labeled with ^{125}I or ^{131}I) was obtained from Abbott Laboratories and dialyzed overnight against 4 liters of 0.15 M phosphate buffer after dilution (1:1) with human serum. As was first noted by Schussler and Plager (11), labeled T_4 preparations contain dialysable contaminants, which may give artifactually high values for FTF. After dialysis, the inorganic I^- concentration of the labeled T_4 was consistently less than 0.1% as determined by paper

electrophoresis and remained less than 1% over the 7–10 days it was used.

The purified tracer was added to 8–10 ml of plasma at a concentration of 2–3 μg of T_4 per 100 ml and incubated for 30 min in a shaking water bath at 37°C in stoppered 25-ml Erlenmeyer flasks. These flasks and subsequent containers were gassed with 5%/95%-CO_2/O_2 to maintain the pH at 7.6–8.0. The serum was then transferred to bags made of Visking No. 20 (Visking Co., Division of Union Carbide Corp.) dialysis tubing prepared as described by Sterling and Brenner (12). These bags were suspended in 50-ml polycarbonate test tubes (Ivan Sorvall) which were then placed in a Model CS International Centrifuge preheated to 37°C by a water-heated coil of Tygon tubing (U. S. Stoneware Co., Akron, Ohio).

The tubes were centrifuged at speeds sufficient to produce about 0.6–0.8 ml of ultrafiltrate per hr at a temperature of 36–38°C. It was determined that the first 0.1–0.2 ml of ultrafiltrate often contained a slightly higher (5–10%) concentration of T_4-^{131}I than the subsequent aliquot (or aliquots) of ultrafiltrate. Since this was observed for both plasma ultrafiltrate and in similar studies using T_4-^{131}I in protein-free 0.15 M PO_4 buffer (pH 7.4), it was thought to be artifactual. In the studies with phosphate buffer, the concentration of T_4-^{131}I in the 2nd, 3rd, and 4th aliquots of ultrafiltrate was identical to that inside the bag. The initial 0.2–0.3 ml of ultrafiltrate was, therefore, discarded and the bags transferred to fresh polycarbonate tubes. Sequential studies in our laboratory have shown the FTF to be constant (after the first 0.2 ml) during removal of as much as 15% of the initial serum volume. The quantity of ultrafiltrate was determined from the weight gained by the tube during the ultrafiltration process.

Quantitative transfer of the ultrafiltrate to plastic counting tubes was accomplished by two 1-ml washes of thyroxine (1 mg/ml) in 0.033 M NaOH as described by Sterling and Brenner (12). 2 ml of 0.15 M PO_4 buffer were added and the subsequent $MgCl_2$ precipitation and washing procedure were carried out as described by the above authors. This technique results in a 95% recovery of T_4, 86% recovery of T_3, and less than 0.2% coprecipitation of labeled I^- as determined by addition of known quantities of these materials to plasma ultrafiltrate. The same methods were followed for the determination of FT_3F except that the serum was enriched with 2 mμg/ml of ^{131}I-labeled triiodothyronine (Abbott Laboratories). All FT_3 fractions were corrected for the 86% yield in the $MgCl_2$ precipitation step.

In 14 successive analyses of a single serum pool from hospital patients, a value for FTF of 0.035 ±0.002% (mean ±SD) was obtained. In 25 separate determinations in normal subjects, the FTF was 0.026 ±0.003% (mean ±SD). The absolute free thyroxine (FT_4) in these subjects was 2.8 ±0.3 mμg/100 ml (mean ±SD) as thyroxine. By the same methods, the FT_3F in 12 separate determinations in a single normal serum pool was 0.36 ±0.02% (mean ±SD).

In determining FTF and FT_3F in this study, all determinations on a given volunteer were performed in the same week with the same labeled thyroxine or triiodothyronine. Each run of 2–3 treatment samples contained at least one control sample since on one occasion prolonged predialysis had not removed all dialyzable contaminants. Sufficient counts were collected to obtain less than 2% counting error.

The distribution of small amounts (2–3 μg/100 ml) of T_4-^{131}I on the serum thyroxine-binding proteins was performed by the reverse-flow paper electrophoresis method of Elzinga, Carr, and Beierwaltes (13). Thyroxine-binding globulin capacity was determined using the same techniques with unlabeled thyroxine added in a concentration of 75 μg/100 ml. Thyroxine-binding prealbumin (TBPA) capacity was determined by the method of Oppenheimer, Martinez, and Bernstein with concentrations of 600 μg/100 ml of unlabeled thyroxine (14). All enrichment of plasma for a given volunteer was carried out with the same stock solutions of labeled and unlabeled thyroxine and the specimens of each subject were run simultaneously in duplicate with a quality control sample. The distribution of the labeled thyroxine was determined by planimetry after scanning with a Packard Model 7201 radiochromatogram scanner.

The DPH levels were kindly measured by Dr. Emile E. Werk of the Metabolism Division of the University of Cincinnati. The method used requires extraction of diphenylhydantoin into chloroform, followed by thin-layer chromatography and ultraviolet absorption spectroscopy. A tracer amount of DPH-^{14}C is added to the inital sample to permit correction for losses of DPH during the extraction.

Analysis of kinetic data. Multicompartmental models were developed to permit the simultaneous analysis of T_4-^{131}I tracer studies and the observed changes in total plasma T_4 and DPH concentrations. Linear first-order differential equations were used to describe the transfer of material between model compartments such that

$$df_i(t)/dt = \sum^{n} (\lambda_{ij} - \lambda_{ji}); \; (i = 1, 2, —n); \; (j = 1, j \neq i)$$

where $f_i(t)$ is the amount of material in model or function-generator compartment i at time t and λ_{ij} is the fractional rate of transfer of material from compartment j to compartment i. Certain parameters and variables for the single-compartment model were assigned special symbols to conform to the usage of previous investigators (15). They are the following: T_4, the quantity of total thyroxine in its volume of extrathyroidal distribution; $[T_4]$, the plasma concentration of total thyroxine; λ_{OT}, the fractional turnover of total extrathyroidal thyroxine; λ_{OS}, the fractional turnover of total thyroxine due to fecal elimination; λ_{OU}, the fractional turnover of total thyroxine due to peripheral deiodination, as indicated by urinary excretion of $^{131}I^-$; V_T, the extrathyroidal distribution volume of thyroxine; ρ_S, the net quantity of thyroxine entering V_T

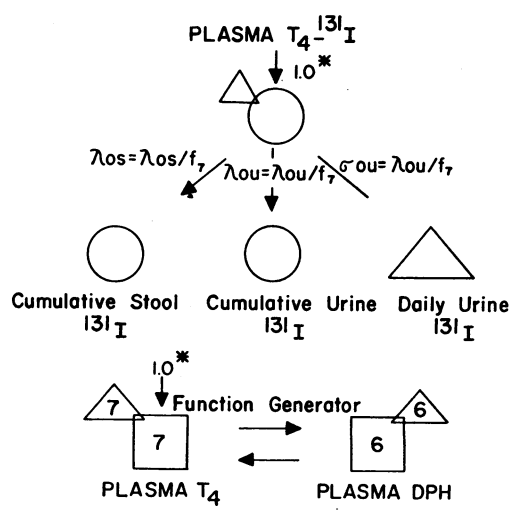

FIGURE 1 Single-compartment model for analysis of thyroxine kinetics.

per unit time by thyroid secretion; ρ_0, the net quantity of thyroxine leaving V_T per unit time by all routes, where $\rho_O = \lambda_{OT} \cdot T_4$.

In the schematic representation of the kinetic models (Figs. 1 and 4), the circles represent the compartments of T_4-^{131}I distribution and excretion. The arrows indicate assigned routes of transfer of T_4-^{131}I between these compartments. Triangles cutting into compartments represent observations that are directly proportional to the contents of the compartment. Other observations consisting of a linear combination of contributions from compartments are represented by free-standing triangles called summers by Berman, Weiss, and Shahn (16). For example, both plasma and interstitial fluid contribute to the activity that is shown as total body T_4-^{131}I in Fig. 4.

The interaction between DPH and plasma [T_4] is included in the models by means of the squares shown in the schematic drawings. An operational relationship between the plasma concentration of DPH and [T_4] is indicated by the interconnections between these compartments. As a result of preliminary testing, the function generated as f_7 was used to represent the change in T_4-^{131}I elimination that was observed during DPH administration. During the pretreatment period, f_7 was assigned a value of 1, so that in this period, λ_{OU}/f_7 and λ_{OS}/f_7 represented the control values of λ_{OU} and λ_{OS}. However, if DPH treatment resulted in a 20% decrease in plasma [T_4], the value of f_7 would then be 0.8, and the urinary and fecal elimination of T_4-^{131}I would be increased accordingly.

An analog computer (EAI TR-20) was used for the preliminary testing of the model and provided initial estimates of the parameter values. Further data fitting was accomplished with the Simulation, Analysis and Modeling (SAAM) computer program of Berman and Weiss implemented on a digital computer (IBM 360/50) at the University of Cincinnati (17). The computer automatically adjusted the initial estimates of the parameter values by an iterative process until a least squares fit to the experimental data was obtained. The values for the model parameters were used to generate theoretical curves based on the models, and these curves were then compared to the experimental data. Computations were repeated until no systematic deviation of the calculated curves from the experimental data was apparent and until further successive iterations improved the sum of the squared deviations by less than 2%.

The data that were simultaneously analyzed included plasma, daily urine, cumulative stool, whole body T_4-^{131}I activity (in one case), plasma total thyroxine, and DPH concentrations. When the single-compartment model was used, the criteria of Ingbar and Freinkel (18) were adapted for computer use to judge completeness of mixing of the injected radiothyroxine. Counting data obtained before complete mixing were not included in the kinetic analysis. Remaining data were given equal weight in the curve-fitting process save for urinary collections that were incomplete as determined from the 24 hr creatinine excretion. These data were not included in the computer analysis, but are shown as circled points in Figs. 2 and 3. The validity of this approach was tested in one case by including all mixing phase plasma, urine and fecal values, and incorporating profile scan data to estimate the parameters of a three-compartment model. The three-compartment model was chosen since it was the simplest one which could satisfy the distribution kinetics.

Daily urine T_4-^{131}I excretion data were used in preference to cumulative urine T_4-^{131}I excretion data because the latter are greatly affected by any losses of urine. The large variation in daily stool radioactivity, however, made the cumulative stool T_4-^{131}I excretion more suitable for analysis than daily stool T_4-^{131}I excretion. A 1–2 day transit time for T_4-^{131}I in stool was selected for each subject because of the time required for the initial appearance of fecal radioactivity, and whole body data were corrected accordingly.

Analysis of free thyroxine data. A statistical analysis was performed of the relationship between [T_4], free thyroxine fraction (FTF), and the absolute free thyroxine (FT_4) during the control and DPH treatment periods. The hypothesis first tested was that FT_4 would remain constant as [T_4] decreased. Since $FT_4 = FTF \cdot [T_4]$, a logarithmic transformation of this expression yields $\log FT_4 = \log FTF + \log [T_4]$. On rearranging, $\log FTF = -\log [T_4] + \log FT_4$. Thus if log FTF is plotted against log [T_4], a straight line with a slope of -1 would be expected if FT_4 remained constant. If, on the other hand, FTF were unchanged, the slope of the regression line would be zero. A regression analysis was performed using the logarithm [T_4] and FTF, and the slope of the regression line was determined for each patient (Fig. 6). The probability of chance differences between these calculated slopes and 0 or -1 was determined by a standard two-tailed t test (19).

RESULTS

Kinetic analyses. Theoretical curves were fitted to the experimental data according to the single-compartment model depicted in Fig. 1. The radiothyroxine kinetic data of one subject (H. R.) could not be used because of errors in the preparation of standards. In Figs. 2 and 3, the results of the analyses of W. E. and C. S. are depicted. The parameter estimates calculated from the single-compartment analyses for these and the other two subjects are presented in Table I. It can be seen that the observed data are generally within ±5% of the theoretical curves and that there are no systematic deviations of the expected from the actual data.

The thyroxine distribution volume, V_T, was calculated assuming a uniform distribution of the administered T_4-^{131}I within a single-compartment. This volume averaged 15% of body weight. The fractional turnover rate of thyroxine, λ_{OT}, is given for the control period. This parameter is the sum of the fractional urine and stool elimination rates with urinary excretion accounting for an average of 81% of total T_4-^{131}I elimination.

Both [T_4] and net thyroxine excretion, ρ_O, are expressed in Table I as total thyroxine rather than the more conventional thyroxine iodine. In addition, the [T_4] levels have been corrected for incomplete extraction into ethanol during the thyroxine determination (see Methods). The mean value for ρ_O in the control period was 103 μg of thyroxine per day or 67 μg of thyroxine iodine.

The changes in thyroxine metabolism which occur when DPH is administered can be seen in Figs. 2 and 3. In all subjects plasma DPH levels reached the therapeutic range for this agent (10–15 μg/ml) within 7 days. Over this period there was a gradual increase in the fractional turnover rate of T_4-^{131}I in all subjects. This

reached a maximum of 120% of control and is seen as an increase in the slope of the plasma decay curve. Both urine and fecal elimination of radioactivity are accelerated equally although the change in slope is difficult to appreciate visually. As a new steady state was reached, the daily urinary excretion curve again paralleled the plasma curve, but was shifted upward in relation to it, reflecting the increased fractional rate of thyroxine deiodination.

During DPH therapy the increase in T_4-^{131}I elimination was mirrored by the fall in [T_4] in each subject studied. In the initial days of DPH treatment, ρ_0 was increased over control values, but could be expected to return to pretreatment levels as the plasma [T_4] fell and a new steady state was reached. This new steady state appears to have been attained in subjects W. E. and C. S. (Figs. 2, 3) as the slope of the [T_4] curve in these subjects approaches zero in the latter part of the study. The agreement between the observed plasma [T_4] at the new steady state and the values predicted from the tracer kinetics suggests that the rate of thyroxine release into plasma, ρ_s, was not substantially altered during this period. In these two subjects, the increase in fractional turnover rate of thyroxine is balanced by the decrease in total T_4. Therefore, absolute thyroxine degradation is proceeding at the normal, pretreatment rate. The increase in T_4-^{131}I elimination and the decrease in [T_4] appear to be directly related to the rise in plasma DPH concentrations. The average molar ratio of plasma DPH to the decrement in plasma [T_4] is 2330:1. However, the drug level–response relationship, symbolized by the function generator shown in Fig. 1, is operational rather than necessarily indicative of a causal relationship (*vide infra*).

Because the single-compartment model does not permit a detailed analysis of thyroxine distribution kinetics and because the distribution pattern of thyroxine might be altered by DPH, a multi-compartmental model was

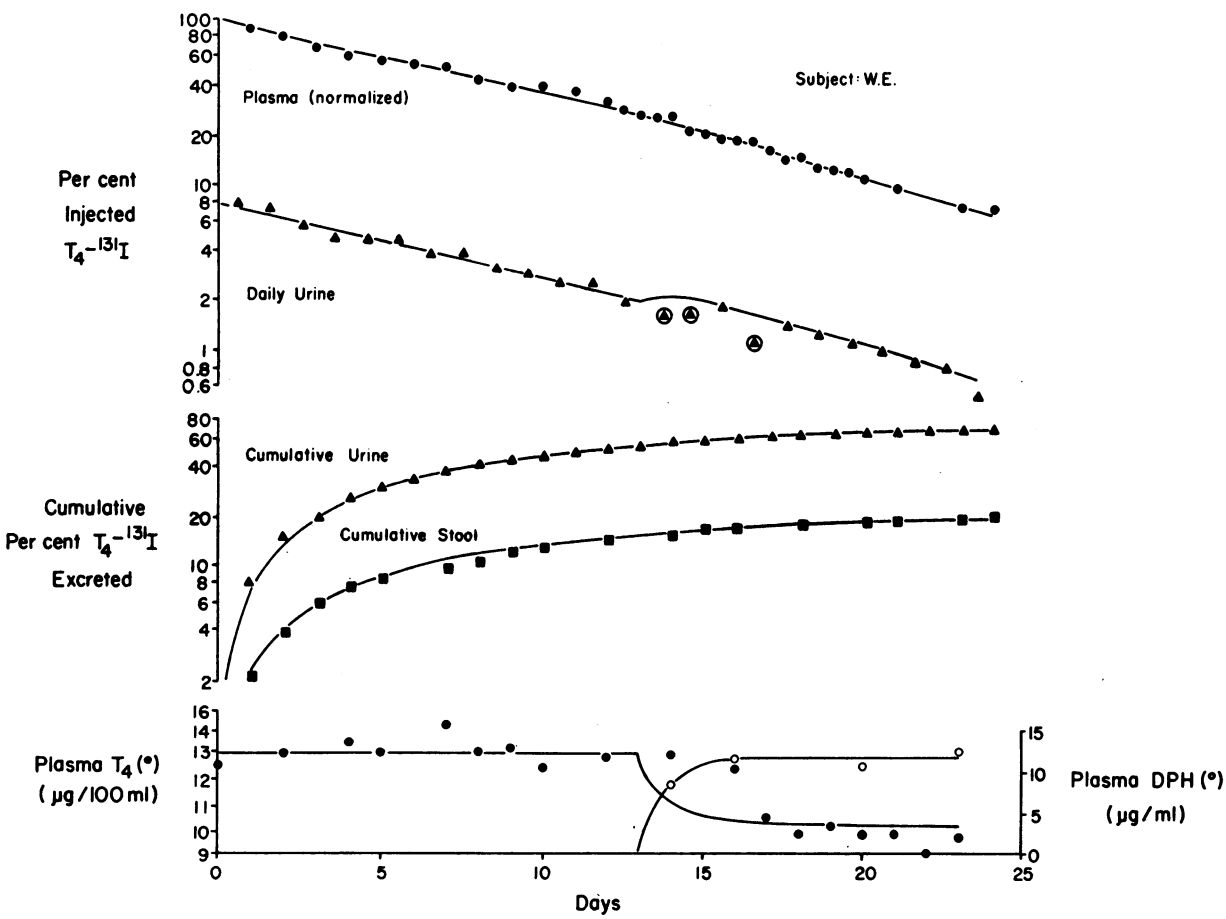

FIGURE 2 Kinetic analyses of thyroxine metabolism before and during diphenylhydantoin administration according to the single-compartment model. The lines represent the predicted values based on the simultaneous least squares analysis by digital computer and the discrete symbols, the observed values. Unweighted points are circled.

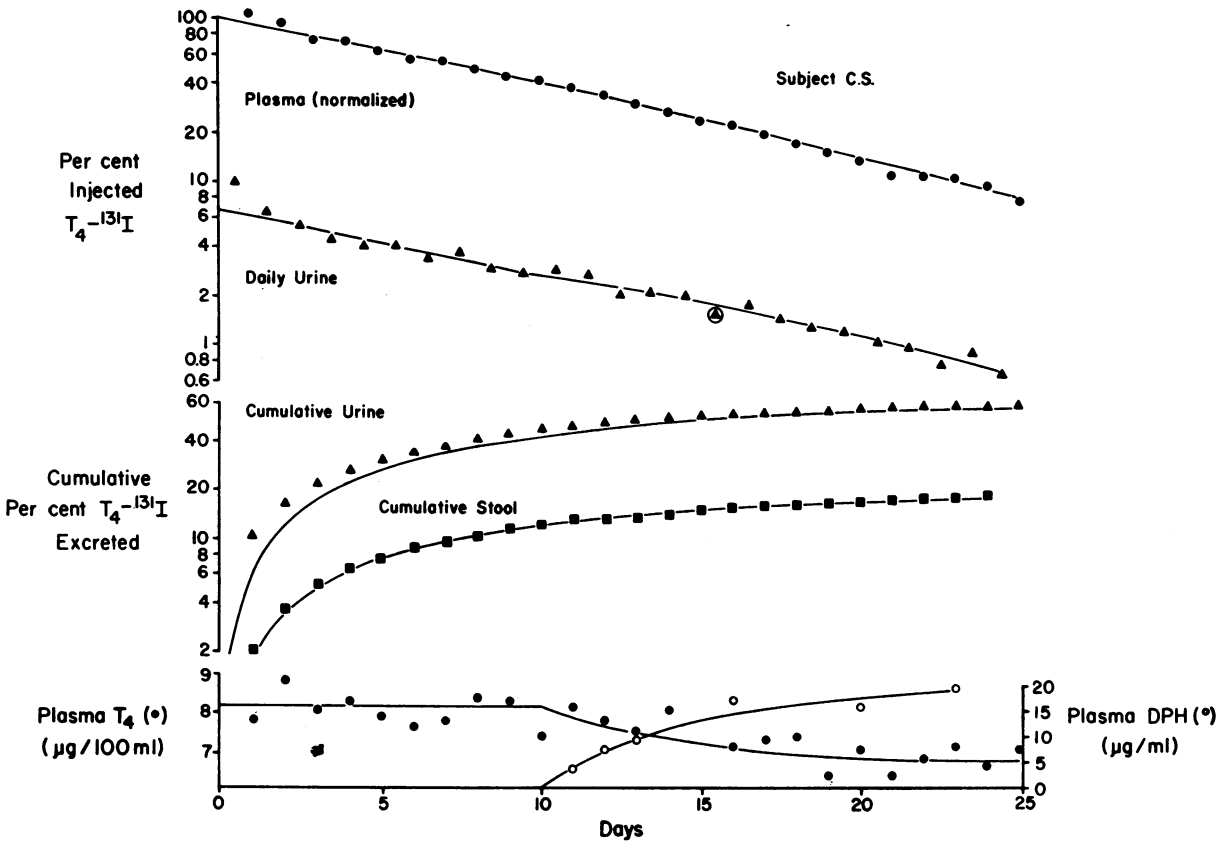

FIGURE 3 See Legend for Fig. 2.

used to analyze the data from D. S. (Figs. 4, 5). The shape of the plasma curve in the first 2 days after the injection of radiothyroxine indicated that at least three compartments are required. Similar observations have been made in studies of inulin distribution.[1] Since the calculated V_T approximated the extracellular fluid volume, the model shown in Fig. 4 subdivides the thyroxine space into plasma and rapidly and slowly equilibrating interstitial fluid compartments. The parameter estimates computed from the experimental data for subject D. S. are included in this figure. The total thyroxine distribution volume of 11.1 liters agrees closely with the value of 11.0 liters obtained in this subject with the single-compartment model.

The shape of the daily urine excretion curve (Fig. 5) suggests that thyroxine is deiodinated after entering cells from the rapidly equilibrating interstitial fluid. Although fecal T_4-^{131}I is shown arising from the plasma pool, the length and variability of the fecal excretory lag time make this a somewhat arbitrary assignment. Whole-body radioactivity was estimated independently from profile scans. Although the nonlinearity of the whole body curve shown in Fig. 5 indicates a larger contribution from the slowly equilibrating interstitial fluid compartment than would be expected if these were true whole-body counts, the profile scan does contain contributions from all three compartments in a fixed proportion. The results of this kinetic analysis suggest that there is no major shift of T_4 to the "slow" compartment during DPH therapy. In addition, serial collimated detector counts over the thigh, liver, and heart of each subject (not shown) demonstrated no change in the relative distribution of radioactivity during the DPH treatment period.

Free thyroxine. The estimates of [T_4], FTF, and the FT_4 of the five subjects are presented in Table II. Despite significant decreases in plasma [T_4], little change in FTF is observed. Therefore, there is a gradual decrease in the calculated FT_4 during treatment with DPH. Subject J. H. appears to be an exception to this, primarily because the [T_4] values obtained on the 2 days FTF was measured are the lowest of the seven control values and may not be representative.

Since the effect on [T_4] is progressive as DPH levels increase, the greatest changes in FT_4 were observed in

[1] A. J. Atkinson, Jr. Unpublished observations.

TABLE 1
Results of the Single-Compartment

Subject	Weight	V_T	Control	
			[T_4]	T_4
	kg	*liters*	*% weight*	*μg/100 ml*	*μg*
W. E.	59.8	9.1 ±0.2‖	15.2	13.2	1201
J. H.	82.3	10.8 ±0.3	13.1	10.2	1102
D. S.	71.4	11.0 ±0.4	15.4	9.0	990
C. S.	75.0	12.2 ±0.3	16.3	8.2	1000
Mean ±SD	72.1	10.8 ±1.3	15.0 ±1.4	10.2 ±2.2	1073 ±99

V_T, extrathyroidal distribution volume of thyroxine; [T_4], plasma thyroxine concentration (corrected for 79.6% extraction in Murphy-Pattee method); T_4, total extrathyroidal thyroxine; λ_{OT}, fractional turnover of total extrathyroidal thyroxine; λ_{OU}, fractional turnover of total extrathyroidal thyroxine due to peripheral deiodination as indicated by urinary excretion of $^{131}I^-$; λ_{OS}, fractional turnover of total extrathyroidal thyroxine due to fecal elimination; ρ_0, net quantity of thyroxine leaving V_T per day.
* Minimum plasma thyroxine during DPH administration.
‡ Molar ratio of plasma DPH to plasma thyroxine lost.
§ ρ_0 at new equilibrium plasma thyroxine concentration.
‖ Mean ±SD of parameter estimates.

the 2nd wk of therapy. It is conceivable that some of the values in subjects treated for shorter periods (D. S., J. H.) may not be maximally depressed, since a new steady-state [T_4] level was not attained. Nevertheless, if the FT_4 values obtained during the control period are compared with the last three treatment values (excluding J. H.), a mean decrease of 15% ($P < 0.01$) in FT_4 is observed. There is no overlap between the FT_4 levels during control and treatment periods in these subjects. It should also be noted that only in D. S. and C. S. do the absolute FT_4 values during the DPH period differ by more than 2 SD from the mean normal value in our laboratory (2.8 ±0.3 mμg/100 ml).

Fig. 6 shows the basis for the statistical analysis designed to test the hypothesis that FT_4 remained constant as [T_4] decreased (see Methods). The broken line labeled B corresponds to the relationship that would have been observed between FTF and [T_4] if this hypothesis were correct. In four of the five subjects studied, the slopes of the regression lines calculated from the experimental data and labeled A were significantly different from the slope of line B (C. S., $P < 0.005$; W. E., $P < 0.001$; D. S., $P < 0.025$; H. R., $P < 0.005$). On the other hand, these slopes were not significantly different from zero, the slope that would be observed if FTF remained unchanged. Thus, while there appears to be a slight increase in FTF in these patients, during the treatment period, the data do not warrant a firm conclusion in this regard. What can be concluded, however, is that under the experimental conditions, the decrease in plasma [T_4] observed during administration of DPH is accompanied by a definite fall in FT_4.

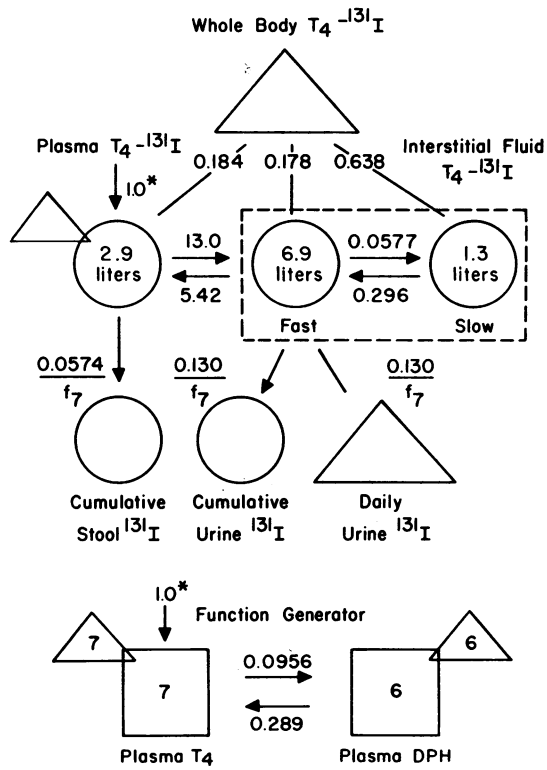

FIGURE 4 Three-compartment model for analysis of thyroxine distribution and metabolism. The fractional contribution of each of the three compartments to the whole body is indicated on the lines linking these compartments to the summer triangle. The compartment sizes shown are for subject D. S.

Control				Diphenylhydantoin		
λ_{OT}	λ_{OU}	λ_{OS}	ρ_0	$[T_4]$*	ΔT_4‡	ρ_0§
%/day	*%/day*	*%/day*	*μg T_4/day*	*% control*	*M/M*	*μg T_4/day*
10.1 ±0.2‖	7.9 ±0.2‖	2.2 ±0.1‖	121	79	1270	121
9.2 ±0.2	7.6 ±0.2	1.6 ±0.1	101	79	1810	—
9.8 ±0.2	8.2 ±0.2	1.6 ±0.1	97	77	2160	—
9.5 ±0.2	7.4 ±0.2	2.1 ±0.1	95	83	4090	95
9.7 ±0.4	7.8 ±0.4	1.9 ±0.3	103 ±12	79.5 ±2.5	2330	—

Free triiodothyronine. Since triiodothyronine (T_3) is also bound to TBG, it might be expected that DPH would increase the free T_3 fraction (FT_3F) in vitro. A mean increase of 10 ±2% (mean ±SD) in FT_3F was observed in five normal serum pools at an added DPH concentration of 15 μg/ml. The FT_3F was measured during DPH administration in C. S.; the mean FT_3F in the control period was 0.37% compared with 0.38% during DPH treatment. Since total T_3 levels are not available in this subject, absolute free T_3 concentration cannot be determined. Nevertheless, the absence of a change in the FT_3F in vivo is similar to the lack of DPH effect on FTF.

Thyroxine-binding protein. Frequent examination of the plasma TBG and TBPA capacities did not show significant alterations during DPH administration (Table III). Furthermore, we were unable to demonstrate significant DPH-induced alterations in the distribution of small quantities (2–3 μg/100 ml) of T_4-^{131}I among the binding proteins.

DISCUSSION

The estimates of the parameters of thyroxine metabolism obtained using the single-compartment model are in general agreement with those obtained by previous investigators. The volume of distribution of thyroxine, 15% of body weight, is identical to that reported by Ingbar and Freinkel (20) and slightly greater than the 13.4% reported by Rall, Robbins, and Lewallen in their review of the literature (15). Control estimates for λ_{OT} and ρ_0 are also similar to those described previously, and the percentage of injected T_4-^{131}I excreted in the stool (16–22%) is similar to the values observed by Van Middlesworth (21).

Previous investigators have noted no alterations in 24 hr thyroidal ^{131}I uptake, thyroidal iodide clearance, cholesterol, or basal metabolic rate (BMR) in subjects receiving DPH (1). The suggestion that thyroxine secretion was unchanged during DPH therapy is in keeping with these reports as is the normal absolute rate of thyroxine degradation in the two subjects whose plasma thyroxine levels reached a new steady state.

Displacement of thyroxine from TBG by DPH has been considered the cause of the low PBI of patients receiving DPH therapy. The correlation between the observed decrease in plasma radiothyroxine and the observed increase in urinary and fecal excretion indicates that DPH treatment caused increased thyroxine elimination and is in agreement with this hypothesis. No significant redistribution of thyroxine during DPH therapy could be detected by the multicompartmental analysis of the data obtained from D. S. This is especially important since it eliminates the possibility of a shift of thyroxine from the fast compartments, adequately sampled as plasma, to the slowly equilibrating one. While we were unable to demonstrate significant shifts of thyroxine between these three compartments, intracompartmental redistribution might occur. The interstitial fluid compartments are defined by their kinetic behavior and may be anatomically heterogeneous. It is unlikely that any of the compartments of our model represents intracellular thyroxine. First of all, the slow interstitial fluid compartment is too small to account for the interstitial fluid

space by itself; and secondly, intracellular deiodination parallels thyroxine distribution within the fast rather than the slow compartment. Nevertheless, a certain amount of thyroxine must presumably be present within cells before deiodination. However, the fraction of the total hormone bound to extracellular protein (and hence restricted to the extracellular fluid space) is so large that the analysis of the experimental data lacks the resolving power to define an intracellular thyroxine compartment. We were, therefore, unable to demonstrate significant intracellular sequestration of thyroxine during DPH administration such as described during acute infusion studies by Oppenheimer, Bernstein, and Hasen (22). The data indicate that elimination of thyroxine, rather than redistribution, is the major fate of the thyroxine lost from the plasma during more prolonged DPH therapy.

When DPH was added to plasma in vitro we found that FT_4 increased by 10% at DPH concentrations of 10 μg/ml and 22% at concentrations of 20 μg/ml similar to the previous report of Chin and Schussler (6). Thus we anticipated that the administration of DPH to our subjects would result in an initial rise in FT_4 followed by a return to pretreatment levels as a new euthyroid steady state was established. On the contrary, we encountered a gradual fall in FT_4 after DPH administration that was similar in magnitude to the decrease of 13% reported after 2–3 wk of DPH treatment by the above investigators (6).

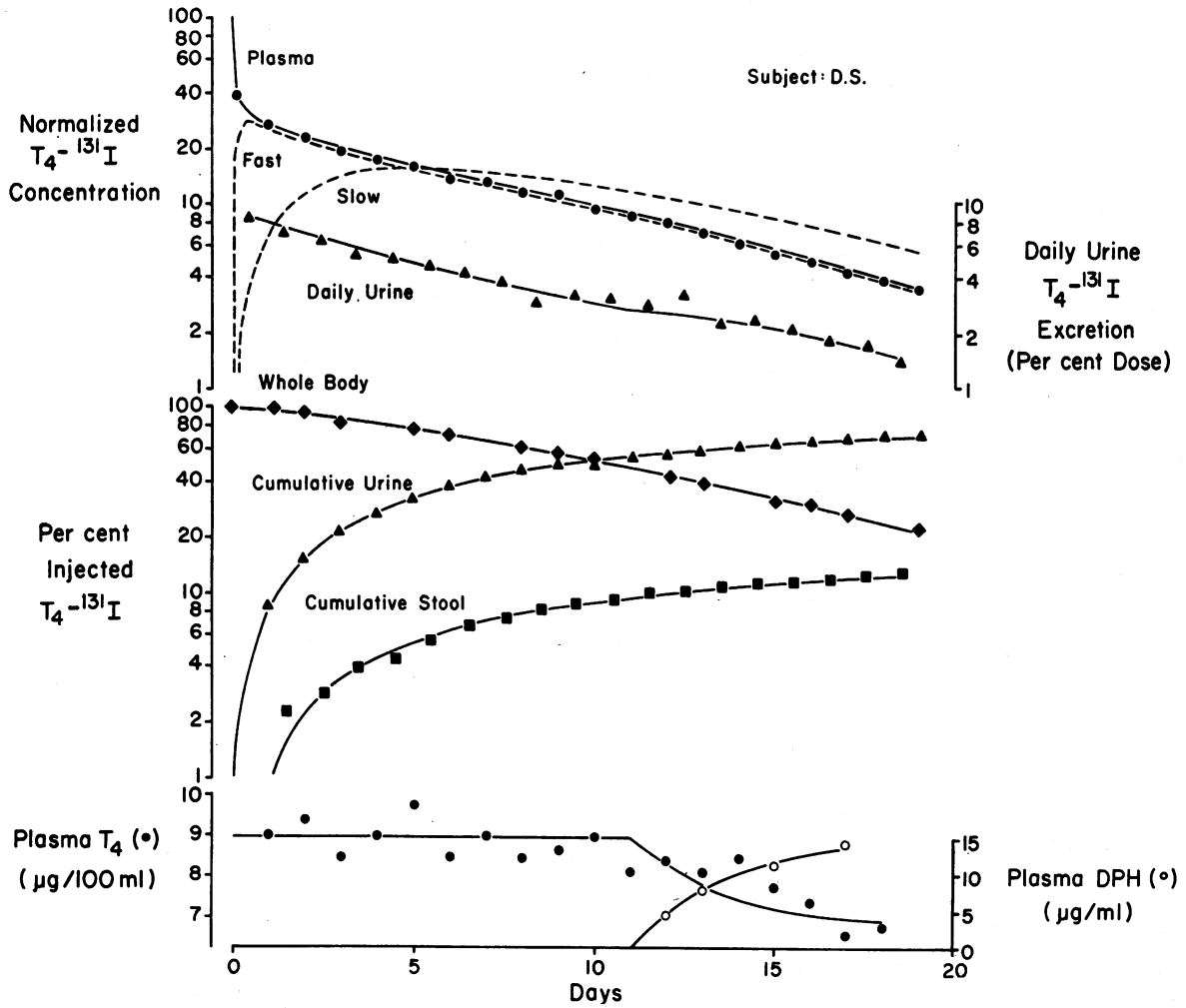

FIGURE 5 Kinetic analysis of thyroxine distribution and metabolism for subject D. S. according to the three-compartment model. The lines represent the predicted values based on the least squares analysis by digital computer and the discrete symbols, the observed values. The broken lines represent the predicted course of radiothyroxine accumulation and excretion in the hypothetical fast and slow interstitial compartments. All data points were given equal weight.

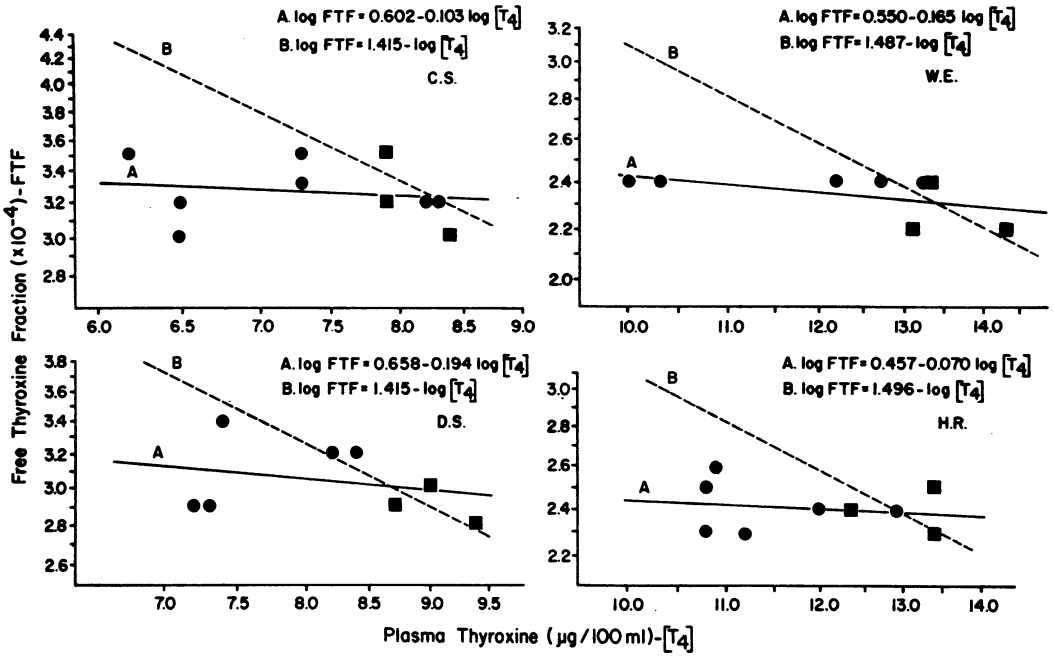

FIGURE 6 Regression lines relating free thyroxine fraction, FTF, to plasma thyroxine, [T_4], before and during diphenylhydantoin administration. Lines A are constructed according to their respective formulae as determined by least squares regression analysis. The broken lines, B, depict the expected relationship if free thyroxine were to remain constant as plasma thyroxine decreased.

Although neither these workers nor we were able to measure a significant rise in FTF during DPH administration, this drug can cause an increase in FTF under special circumstances. We have observed this phenomenon in a patient who ingested 5–8 g of DPH in an attempted suicide on 4/8/69. The DPH, T_4, and FT_4 values measured during his hospitalization are presented in Table IV. The effect of this high concentration of DPH on the distribution of thyroxine bound to TBG, TBPA, and albumin in this patient's serum is shown in Fig. 7. Initially, when plasma DPH was highest and the binding of thyroxine to TBG was reduced by 15%, there was a substantial increase in FTF which returned to normal on 4/14/69 when the plasma concentration of DPH was lower. The FTF values of 4/9 and 4/11 are more than 3 SD above the normal level of 2.6×10^{-4} for

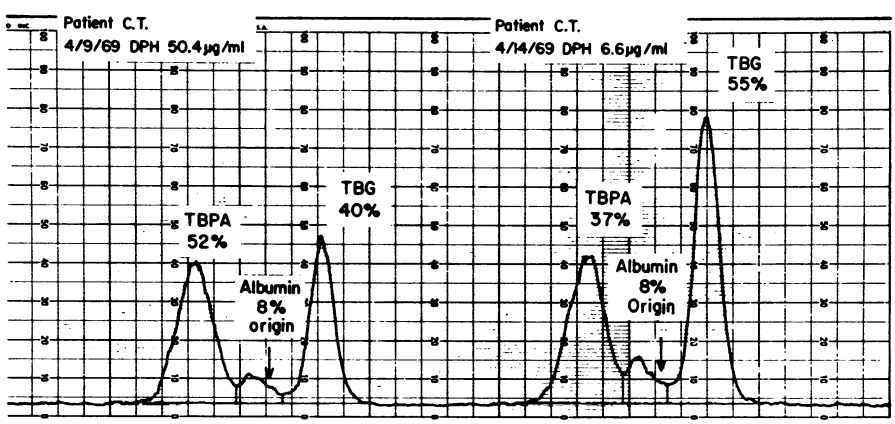

FIGURE 7 Distribution of T_4-^{131}I during the acute phase and after recovery from diphenylhydantoin intoxication. The plasma was enriched with 2.6 µg/100 ml of T_4-^{131}I and reverse-flow electrophoresis performed in glycine-acetate buffer at pH 8.6.

TABLE II

Free Thyroxine before and during

Subject..... W. E.					J. H.			H. R.	
Day	T_4*	FTF‡	FT_4§	Day	T_4	FTF	FT_4	Day	T_4
	μg/100 ml	*×10⁻⁴*	*mμg/100 ml*		*μg/100 ml*	*×10⁻⁴*	*mμg/100 ml*		*μg/100 ml*
Control									
2	13.3	2.4	3.2	2	10.0	2.4	2.4	2	12.4
7	14.3	2.2	3.1	7	9.2	2.5	2.3	7	13.4
12	13.1	2.2	2.9	10	8.9	3.3	2.9	12	13.4
Diphenylhydantoin									
14	13.2	2.4	3.2	12	9.3	2.6	2.4	14	12.0
16	12.7	2.4	3.0	14	9.2	3.2	2.9	16	12.9
19	10.3	2.4	2.5	17	8.8	2.7	2.4	19	10.9
21	10.0	2.4	2.4	19	9.0	2.6	2.3	21	11.2
24	12.2	2.4	2.9	21	9.4	2.7	2.5	24	10.8
								26	10.8

* Corrected for 79.6% yield in the estimation of plasma thyroxine (see Methods).

‡ Free thyroxine fraction.

§ Absolute free thyroxine.

this method in our laboratory. The observations in this patient suggest that in order to mimic the in vitro conditions, plasma DPH concentrations must be increased rapidly. The gradual increases associated with the conventional DPH dosage employed in this study may allow time for excretion of the displaced thyroxine. Thus the thyroxine concentration in vivo can decrease while it remains constant in the in vitro studies. The molar ratio of plasma DPH to plasma thyroxine displaced is a further example of the differences between the in vivo and in vitro circumstances. The value calculated for this ratio by Oppenheimer and Tavernetti using as in vitro method was 18,000 (3). However, in our subjects the calculated ratio averaged 2300, indicating that DPH was almost 10 times more effective in lowering plasma thyroxine than would have been expected on the basis of the in vitro study.

These observations do not explain the presence of a decreased absolute FT_4 during DPH treatment. It has been postulated from repeated observations that FT_4 is the only portion of this hormone available for cellular uptake (15). Free thyroxine is also thought to control the release of thyrotropin (TSH) through its feedback regulation of TSH-releasing factor (TRF). A low FT_4 concentration, by decreasing thyroxine uptake in the hypothalamus, would allow secretion of TRF which would mean an increase in TSH and thyroid gland stimulation. In two of these subjects, plasma FT_4 concentrations decreased while absolute thyroxine degradation was unchanged. This indicates that the peripheral clearance of free thyroxine has increased.

The mechanism by which DPH increases the peripheral clearance of FT_4 is not known. Since administration of this drug to mice causes increases in the smooth endoplasmic reticulum of the liver, DPH could conceivably increase the hepatic clearance of thyroxine through enzyme induction (23). Other agents which induce microsomal enzymes, such as phenobarbital, have been associated with increased rates of thyroxine metabolism in rats though the effect on free thyroxine clearance was not examined (24). The studies of Mendoza, Flock, Owen, and Paris have shown that pretreatment of rats with 60–80 mg/kg of DPH (more than 10 times the amount used in these studies) increases the excretion of thyroxine glucuronide in bile by about 30% (25). However, the increase in free thyroxine clearance observed in our subjects cannot be attributed entirely to an increase in the biliary excretion of thyroxine since the kinetic studies indicate that peripheral deiodination increases to an equal extent. Furthermore, in order to explain the lack of TSH release and the persistent decrease in FT_4, the hypothalamus would presumably be required to participate in this increased clearance of thyroxine.

It is premature to conclude that the observed increase in free thyroxine clearance is mediated by DPH itself. Conceivably, it might reflect an increased cellular uptake of thyroxine due to decreased availability of TBG. If this were true, some similarities might be expected between DPH-treated patients and those with a congenital deficiency in TBG-binding capacity. These patients do resemble our subjects in having both accelerated urinary and fecal radiothyroxine clearances, a decreased

Diphenylhydantoin Administration

H. R.		D. S.				C. S.			
FTF	FT_4	Day	T_4	FTF	FT_4	Day	T_4	FTF	FT_4
$X10^{-4}$	mμg/100 ml		μg/100 ml	$X10^{-4}$	mμg/100 ml		μg/100 ml	$X10^{-4}$	mμg/100 ml
2.4	3.0	2	9.4	2.8	2.6	1	7.9	3.5	2.8
2.3	3.1	7	9.0	3.0	2.7	4	8.4	3.0	2.5
2.5	3.3	9	8.7	2.9	2.5	7	7.9	3.2	2.5
2.4	2.9	11	8.2	3.2	2.6	11	8.3	3.2	2.7
2.4	3.1	12	8.4	3.2	2.7	14	8.2	3.2	2.6
2.6	2.8	16	7.4	3.4	2.5	16	7.3	3.3	2.4
2.3	2.6	19	7.3	2.9	2.1	19	6.5	3.2	2.1
2.3	2.5	20	7.2	2.9	2.1	23	7.3	3.5	2.5
2.5	2.7					27	6.2	3.5	2.2
						30	6.5	3.0	2.0

total body thyroxine pool, and an approximately normal absolute thyroxine degradation rate (4, 5). Unfortunately, FT_4 measurements using predialyzed thyroxine tracer have not been generally employed in these subjects. A subnormal value of 0.9 mμg/100 ml can be calculated for one such patient from data in a recent study reported by Woeber and Ingbar (26). Additional information is needed to determine whether or not this low value is typical of this condition and, if so, whether thyroxine degradation rates are nevertheless maintained at normal levels.

Regardless of the mechanism by which DPH therapy increases free thyroxine clearance, it appears that a low plasma free thyroxine does not imply hypothyroidism in

TABLE III

Thyroxine-Binding Globulin and Thyroxine-Binding Prealbumin Capacities before and during Diphenylhydantoin Administration

Subject	Control			Diphenylhydantoin		
	Mean	Range	Endogenous T_4*	Mean	Range	Endogenous T_4
	μg/100 ml	μg/100 ml	%	μg/100 ml	μg/100 ml	%
Thyroxine-binding globulin						
W. E.	23.5	23.0, 23.9	57 (2)‡	22.8	21.3–24.9 (4)	57 (3)
J. H.	24.4	23.3–25.9 (3)	59 (2)	23.9	22.0–25.3 (4)	60 (3)
H. R.	20.2	18.7–21.2 (3)	57 (2)	18.9	18.1–19.4 (3)	57 (2)
D. S.	17.6	17.4–18.0 (3)	63 (3)	19.3	18.6–20.1 (3)	61 (3)
C. S.	19.6	19.3–20.2 (3)	62 (3)	20.4	19.8–21.0 (4)	61 (4)
Thyroxine-binding prealbumin						
W. E.	277	254, 300		273	268–298 (4)	
J. H.	327	306–356 (3)		317	302–335 (4)	
H. R.	265	262–269 (3)		273	232–298 (4)	
D. S.	253	245–262 (3)		265	257–273 (4)	
C. S.	263	255–272 (3)		257	249–264 (4)	

* Mean percentage T_4-^{131}I bound to thyroxine-binding globulin with 2–3 μg/100 ml of T_4-^{131}I enrichment.

‡ Figures in parenthesis are the number of individual duplicate determinations.

TABLE IV

Free Thyroxine and Thyroxine Distribution in a Patient with Diphenylhydantoin Intoxication

Date	DPH	T_4*	FTF‡ $\times 10^{-4}$	FT_4§	T_4-^{131}I‖ bound to TBG
	μg/ml	*μg/ 100 ml*		*mμg/ 100 ml*	%
4/9/69	50.4	6.3	3.8	2.4	40
4/11/69	30.2	—	3.7	—	—
4/14/69	6.6	7.3	3.0	2.2	55

* Corrected for 79.6% yield in the estimation of plasma thyroxine (see Methods).

‡ Free thyroxine fraction.

§ Absolute free thyroxine.

‖ Reverse-flow electrophoresis after enrichment with 2.6 μg/100 ml of T_4-^{131}I.

a patient receiving this drug. Evidently, other factors are involved in determining the euthyroid state. It is possible that feedback control mechanisms act primarily to maintain a normal thyroxine degradation rate. This can occur, at least in some circumstances, independently of changes in free thyroxine concentration.

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