A THREE-COMPARTMENT MODEL

OF

THYROID UPTAKE IN MAN

A Thesis submitted to the University of Glasgow for the degree of Doctor of Philosophy

By

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SUMMARY

There is sufficient evidence to show that the kinetics of thyroidal uptake of iodide in man can normally be described in terms of an open three-compartment binding model. The parameters of this model consist of a unidirectional clearance of iodide from the plasma compartment into the iodide compartment of the gland, an exit rate constant describing return of iodide to plasma, and a binding rate constant describing incorporation of iodide into the bound iodine compartment. In the event of there being complete absence of binding, the bound compartment and binding rate are ignored.

Published work with radioactive tracers has produced fairly consistent results as far as unidirectional clearance and exit rate are concerned, but there is considerable variance in the binding rate estimates for iodide. Values for the latter in the uninhibited gland have varied by almost two orders of magnitude; namely, from being similar in magnitude to the exit rate, to being much greater. A probable explanation for this discrepancy, however, is to be found in recent work where tracer levels in arterial plasma (which are greater at early times) were applied in the analysis rather than levels in venous blood. In these circumstances the net clearance of tracer into the gland was found to be constant with time, suggesting that the binding rate is normally much greater in magnitude than the exit rate.

A new "least sum of squares" analysis of kinetic uptake data, which incorporates a correction for arterio/venous differences in tracer levels, is described in this thesis. The method is based on the

open three-compartment binding model of the gland and assumes intravenous administration of the tracer; radioactivity levels in thyroid and plasma being monitored for a period of 60 minutes following administration of the tracer. No constraints are imposed on the parameters of the model but a further parameter, initial free uptake, is introduced to allow for uncertainty in plasma tracer levels immediately after injection. The use of the latter parameter also compensates for rapid early reduction in clearance due to protein binding of the tracer in plasma. A computer programme was written to provide estimates of the various parameters and the associated random errors. An analysis of the uptake changes after a blocking dose of perchlorate is also described which provides a means of verifying results from the uptake phase. (Empirical studies revealed also that an estimate of binding rate could be obtained from the fraction of uptake discharged by perchlorate).

When the new method of analysis was applied to radioiodide uptake data from normal and untreated thyrotoxic subjects, neither exit nor binding rate could be satisfactorily determined. The results were consistent with theoretical predictions as to the problems that might arise if the binding rate were much greater than the exit rate. Thus it was concluded that, when the binding function of the gland is not inhibited by drugs or disease, the binding rate of iodide is normally greater than the exit rate.

Further support for a relatively high binding rate was found in the results of perchlorate discharge studies in a number of untreated thyrotoxic subjects. Even at early times after administration of the radioiodide, there was only one subject in whom a significant discharge

of radioiodide was observed. (Other published work in normal and untreated thyrotoxic subjects also reveals a similar virtual absence of free radioiodide in the uninhibited gland).

The finding of a small discharge in one untreated thyrotoxic subject did, however, permit a lower limit of 0.150 $minute^{-1}$ to be estimated for the binding rate of iodide in the uninhibited gland. Studies in subjects given antithyroid drugs, or in subjects with binding defects, provided an estimated mean value ($\pm$sd) of 0.046 ± 0.031 $minute^{-1}$ for the exit rate. Consideration of the steady state equations of the model revealed that with these estimates, the effective clearance of iodide into the gland is normally 75-100% of the unidirectional clearance. Unidirectional clearance of iodide was found to be dependent upon thyroid status, the mean value ($\pm$sd), 160.7 ± 138.5 ml $minute^{-1}$, in thyrotoxic subjects being greater than the mean value, 23.1 ± 9.5 ml $minute^{-1}$, in normal subjects.

Both unidirectional clearance and exit rate of pertechnetate were found to be linearly related to the corresponding parameters for iodide. However, whilst the exit rate for pertechnetate was greater, the unidirectional clearance was less than that for iodide. A mean value ($\pm$sd) of 0.001 ± 0.003 $minute^{-1}$ was found for the binding rate of pertechnetate in normal and thyrotoxic subjects.

The new method of analysis was applied to serial uptake data of radioiodide and ^{99m}Tc-pertechnetate in thyrotoxic subjects being treated routinely with antithyroid drugs. No clear association was found between the parameters of the model and the final outcome of therapy. However it was demonstrated that, on occasions, lack of response to these drugs can be due to a lower than normal exit rate of iodide

rather than failure of the drug to effectively reduce binding rate. In these circumstances the effective clearance of iodide remains relatively high.

The occasional observation of uptake values of pertechnetate that exceed those of iodide, during drug therapy, was found to be due to there being greater intial free uptake of the former. Initial free uptake, which ranged from 0-18 % dose for iodide and 0-24 % dose for pertechnetate, was considered to reflect the inherent affinity of the gland for a specific anion, before any plasma binding has time to be completed. The results support published work on the fundamental properties of anions which suggests that the affinity for pertechnetate should be greater than that for iodide. Initial free uptake was found to increase as the symptoms of thyrotoxicosis were suppressed by the action of the antithyroid drug. This is what would be expected if initial free uptake were a measure of the fraction of the injected tracer bolus that is trapped by the thyroid, with the knowledge that cardiac output, but not thyroidal blood flow, is reduced by drug action.

Studies of untreated thyrotoxic subjects, and of subjects who had completed antithyroid drug therapy, revealed no increased incidence of defective binding in the latter group. This would suggest that antithyroid drugs have no long-term effect on iodide binding. In one treated thyrotoxic subject who did manifest defective binding after therapy, it was finally proven that antithyroid drugs were being taken surreptitiously. A small persistent defect was found in another treated thyrotoxic subject but no clear association between the defect

and previous drug treatment could be established.

The new method of analysis was found to be valuable in quantifying the effect of different dosages of antithyroid drug in the short-term. Untreated thyrotoxic subjects were placed on specific dosages of carbimazole in the range 5 mg d^{-1} to 40 mg d^{-1} for consecutive periods of one week. A radioiodide uptake study was performed at the end of each week's treatment with a given drug dosage. In most cases there was little to be gained, in terms of reduction in binding rate and effective clearance of iodide, by increasing drug dosage beyond 10 mg d^{-1}.

Subjects with iodination defects were also investigated with the object of quantifying the defect by kinetic analysis. This also provided an opportunity of checking the predictions of the analysis, against the observations after perchlorate, for a range of binding rates. The results indeed confirmed the reliability of the new method of analysis. Binding rate of iodide in the subjects ranged from 0.0-0.091 $minute^{-1}$ and effective clearance of iodide from 0-68% of the unidirectional clearance. When the effective clearance was greater than 60% there were no biochemical nor clinical signs of thyroidal disease. There was good correlation between the effective clearance of iodide in absolute terms (i.e. ml $minute^{-1}$) and the residual uptake after perchlorate. This might prove to be a simple method of assessing the degree of replacement therapy in subjects with defective binding.

CHAPTER ONE

MODELS AND ANALYSIS OF ANION UPTAKE BY THE THYROID GLAND IN MAN - A REVIEW OF PREVIOUS WORK

1.1 Introduction

The thyroid gland has the ability to concentrate several different anions of which iodide and pertechnetate have been studied widely in radioactive tracer form (1, 2). Included also are the thiocyanate and perchlorate ions but these are best known for their action, when administered in pharmacological doses, as inhibitors of thyroidal uptake (1). The common features linking these ions are physical rather than chemical, thyroidal concentration being determined by ionic size, shape and charge rather than by place in the Periodic table of elements (1). Iodide is unique, however, in that concentration of the ion is only the first stage in its utilisation for physiological purposes. Whilst there may be some metabolism and binding of the other ions trapped by the thyroid (1, 3-7) these have no known role in the natural function of the gland.

Thyroidal concentration of iodide (and other ions) involves initially an active transport mechanism (1, 8, 9). An ion pump transports iodide from the blood to the thyroid cells through the basal membrane, but subsequent movement of iodide from cell to lumen through the apical membrane is probably not the result of an active process but simply the result of an existing electrical gradient (2). At this stage the trapped ion is still freely exchangeable with iodide in plasma. However it is

soon oxidised and bound to thyroglobulin where it appears within minutes as monoiodotyrosine and diiodotyrosine, and later, by coupling of these two tyrosines, as triiodothyronine (T3) and thyroxine (T4) (9-11). After a variable period of storage the bonds to thyroglobulin are broken resulting in T3 and T4 being released as active hormones into the circulation. Some newly formed hormone may leave the gland within 2 hours (11-13), but the biological half period of intra-thyroidal hormone ranges from 7-164 days in normal subjects (11). Iodide produced by the peripheral degradation of T3 and T4 is available for recycling through the thyroid. There is also some intra-thyroidal deiodination of organic material producing an additional iodide pool, but this is functionally separate from that derived by active transport (1, 9, 14).

1.2 Models of the Thyroid Gland

Various models have been devised to describe the complete iodine cycle in humans. The simplest model has three compartments - an inorganic iodide pool which includes iodide within the thyroid, and two pools of organic iodine, one within the thyroid and the other comprising all extra-thyroidal organic iodine (15-16). There are additional compartments in more elaborate models which have been constructed to cover all aspects of thyroid function (17-20), including regulation of thyroid function by endogenous and exogenous agents (21-22). In practice the main application of these models has been in the analysis of tracer data over intervals extending to 50 days after administration. Generally there has been good agreement between the predictions of these models and experimental data, thus allowing the evaluation of parameters which are useful indices of thyroid function.

The present work, however, is not concerned with the complete iodine cycle, but only with the early stages. It covers the initial accumulation of inorganic iodide from the blood (trapping) and subsequent organification (binding) of trapped iodide within the thyroid, up to the time before any substantial release of organified iodine occurs. For the purposes of analysing the variation in early uptake of a radioactive tracer by the thyroid, it is customary to describe the interaction between thyroid and blood in terms of compartmental models. In the models described by Larsson (23), and Wollman and Reed (24-26), which are known as "open" compartment models, the size of the plasma iodide pool is not strictly defined. Transport of ion into the gland is expressed as the product of plasma iodide concentration and unidirectional clearance of the ion from plasma.

Alternatively the plasma pool may be strictly defined and hence the use of "closed" compartmental models such as those described by Rall et al (17) and Berman (20). The work of Berson and Yalow (12) was also essentially based on the "closed" compartment concept of the plasma iodide pool. Transport of iodide into the gland is the product of a transfer rate constant and the total iodide in the plasma compartment. In practice, evaluation of the latter parameter requires the additional measurment of plasma volume which makes the "closed" compartment models less convenient to apply. However, for the purpose of comparing results of various studies of tracer kinetics, it is a straightforward matter to reduce the parameters of the "closed" compartment models to the form of those in the "open" compartment type, using the expression:-

transfer rate constant x plasma volume = unidirectional clearance.

Wolff (1) and Robertson (27) have reviewed the application of "open" compartment models. There are four models in all (Figure 1.1), two

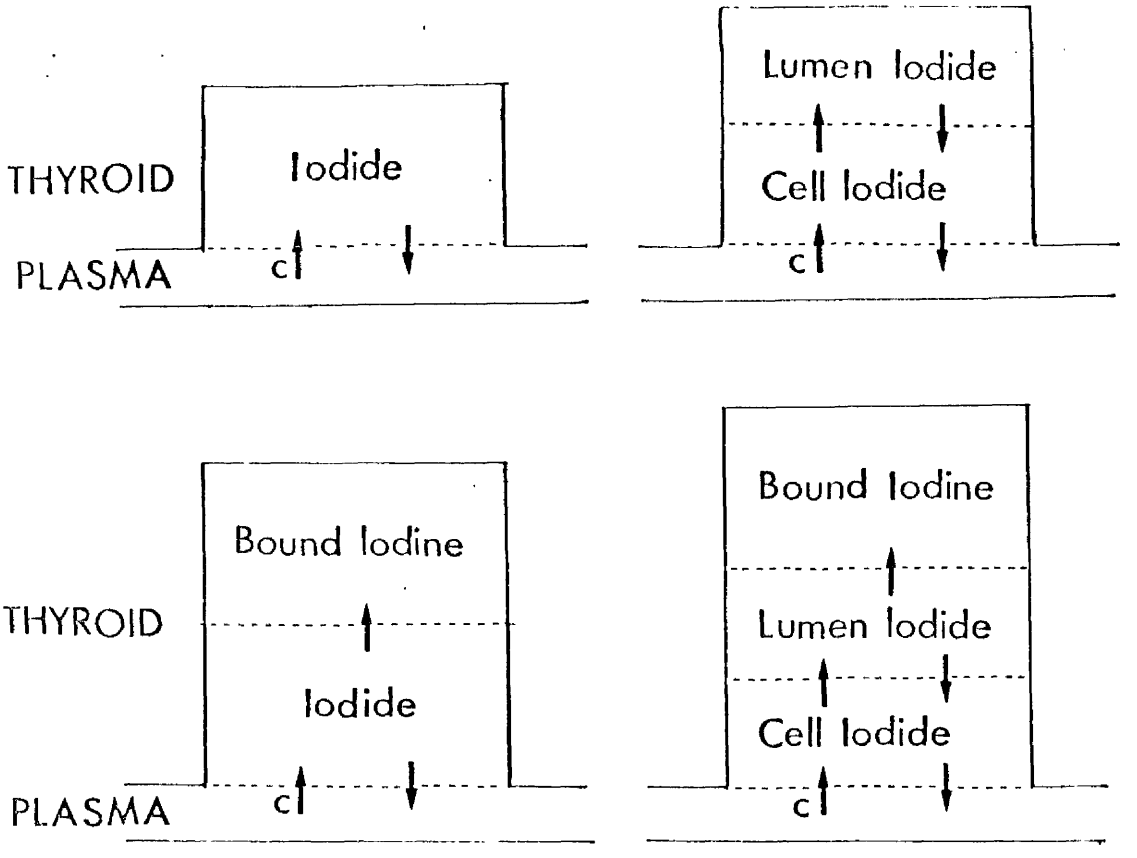


Figure 1.1 Compartmental models of iodide uptake by the thyroid gland. Except for the arrow labelled "C", representing unidirectional clearance of iodide from plasma, arrows represent rate constants of iodide transfer between the appropriate compartments.

for use when there is no intra-thyroidal binding and the other two for application when there is significant binding. All the models allow for return of unbound iodide to plasma. The basic difference within each pair is that one model has only a single iodide compartment within the thyroid whilst the other has two compartments, which represent separate iodide pools in the cells and lumen.

Robertson (27) has reviewed the more recent literature on the distribution of radioiodide within the thyroid and concluded that, except in the case of the hypothyroid gland, a concentration gradient between the lumen and cells is rapidly established. (From a review of the literature and from his own studies Gray (28) has shown that the same is true for the pertechnetate ion). This means that the rate constant of flow into the lumen is much greater than the exit rate. Thus the mathematical equations describing the models with two iodide compartments can be simplified to the form of those with a single intra-thyroidal iodide compartment (1) and, as far as external measurements of early thyroid uptake are concerned, it would be impossible to distinguish two pools of unbound tracer. As a consequence, published analyses of the early stage of ion input to the gland have not, usually, advanced beyond the single iodide compartment models of Figure 1.1. It must be noted that the sophisticated model of human iodine metabolism described by Degroot et al (19) has two iodide pools, but the additional pool is to account for degradation within the thyroid of previously organified iodine.

1.3 General Consideration of Methods

Because there are a number of suitable radioisotopes of iodine (^{123}I, ^{131}I and ^{132}I), in vivo studies of ion uptake by the human thyroid

gland may be performed conveniently using radioactive tracers (27).

Pertechnetate-^{99m}Tc has also been used in the study of ion transport and Wolff in his recent review (2) has discussed the reasons for this. In the first instance pertechnetate shares many of the transport properties of iodide, although binding of the ion is minimal. It is accumulated by all the organs known to accumulate iodide (the thyroid and salivary glands, the choroid plexus, the gastric mucosa, etc.). It shares the same active transport process as iodide and other ions, and is displaced by iodide. If the binding function of the gland is completely inhibited, the steady state concentrations of pertechnetate are of the same order of magnitude as those of iodide. Most of the pertechnetate accumulated by the gland is readily discharged by pharmacological doses of perchlorate and thus the ion behaves as iodide when binding is blocked. Secondly, the radioisotope ^{99m}Tc has favourable characteristics for in vivo studies. The photon energy is relatively low (140 KeV) and the radiation is readily collimated. Furthermore, the radiation dose to subjects is minimal (27) because of the short physical half-life (6 hours).

There have been several studies of the kinetic behaviour of ion uptake by the human thyroid gland using radioiodide (12, 16, 23, 29-40), ^{99m}Tc - pertechnetate (41-46) or both (47-51) as tracers. Dual tracer investigations, involving the use of ^{99m}Tc, facilitate study of the trapping function of the gland in the presence of binding. Studies have consisted of serial measurements of thyroid uptake following administration of the tracer using either an uptake counter, a radioisotope scanner or gamma camera (Chapter 4), combined with, in some cases serial measurements of plasma activity.

In some of the earliest work, analysis of the experimental data consisted of plotting thyroid uptake against the square root of time (29). This facilitated clearer separation of the uptake curves for various thyroid states. More recent analysis has ranged from straightforward calculations of clearance (rate of change of thyroidal uptake divided by tracer concentration in plasma), over various time intervals (16, 30, 32, 34, 36-38, 53), to analysis in terms of compartmental models using graphical or computing techniques (12, 17, 20, 27, 28, 31, 39, 41, 49-52).

1.4. Estimation of Clearance and its Variation with Time.

After intravenous administration of ^{99m}Tc - pertechnetate, or of radioiodide when binding is inhibited, the net clearance of tracer from blood to thyroid is found to fall rapidly with time and may even become negative within the first hour (34,41). This happens because, in the absence of binding, the tracer accumulated by the gland gradually returns to plasma, thus producing a falling uptake which eventually parallels the fall in plasma radioactivity.

When binding is allowed to continue as normal, the net clearance of radioiodide has again been found to decrease with time. Initially, however, Berson and Yalow (12) found the clearance of radioiodide to be relatively constant over the period 4-80 minutes after intravenous administration of the tracer but Larsson (23) reported a falling clearance between 5-65 minutes in a proportion of patients. When uptake at earlier times is included a definite decrease in clearance is observed. Koutras and Sfontouris (36) found that, in normal and thyrotoxic subjects, the 2-20 minute clearance was greater than the 20-120 minute clearance and

suggested that the former represented the unidirectional clearance of iodide into the thyroid gland. Yamamoto, Moriya and Horiuchi (38) measured clearance in normal subjects at various times during the period 0-120 minutes after tracer administration. The clearance from 0-4 minutes was found to be greater than that at later times and was taken to represent the unidirectional clearance. Degroot (16) reported the clearance of radioiodide to decrease over the period 2-30 minutes in subjects with relatively active glands. Thereafter the clearance remained virtually constant for several hours after administration of the tracer. These observations of changing clearance at early times would suggest that there is significant return of iodide from thyroid to plasma, even when binding is proceding normally.

Another aspect of the phenomenon has been discussed by Shimmins et al (37) who showed that errors in measurement of uptake could seriously affect estimations of clearance. However, with more accurate evaluation of extra-thyroidal activity (the main source of error in early uptake determination), the clearance from 2-20 minutes was still found to be greater than that from 20-120 minutes.

In the most recent investigation of these changes in early clearance of radioiodide, Gray et al (53) argued that thyroid cells would be exposed to a plasma concentration of tracer that approximates to that in arterial blood. Venous and arterial plasma concentrations were measured from 1 minute onwards and it was found that arterial concentrations were significantly greater for the first 20 minutes after intravenous administration of the tracer (details are given in Section 3.2). When arterial plasma concentrations were applied to estimations of clearance, these arterial clearances were essentially constant from 2 minutes onwards. In effect these improved results suggest that, when binding is not inhibited, there is essentially no return of trapped iodide to blood perfusing the thyroid, i.e. the binding rate is much greater than the release

rate of unbound iodide. The measured clearance is in fact the unidirectional clearance of tracer into the thyroid.

1.5 Introduction to Compartmental Analysis

Whilst there are methodological differences amongst published analyses of early kinetic data using compartmental models, the results can usually be expressed in terms of the parameters of the "open" compartment models (Section 1.2). For ease of comparison these modifications will be made, whenever necessary, in the present review of published work. There is one exceptional method of analysis and that is the approach adopted by Esser et al (44) in their analysis of serial pertechnetate uptake data. Uptake curves were expressed as the sum of a constant and an exponential term. This form of equation was based on earlier work on tracer theory (54). The constants of these equations cannot be related readily to the parameters of the "open" compartment models.

Before a detailed examination of published work is undertaken, it is worthwhile to consider the mathematical equations describing the transport of ion in those "open" compartment models which have a single iodide compartment. General comments can then be made about methods of solution using either graphical or computing techniques. For present purposes it is necessary to consider only the "open" three-compartment binding model (Figure 1.2).

Let C be the unidirectional clearance of tracer into the thyroid and I_P the plasma concentration at any time t. Loss from the pool of unbound tracer U_F occurs by leakage back to plasma, represented by an exit rate constant K_{TP}, and by loss to the pool of bound tracer U_B,

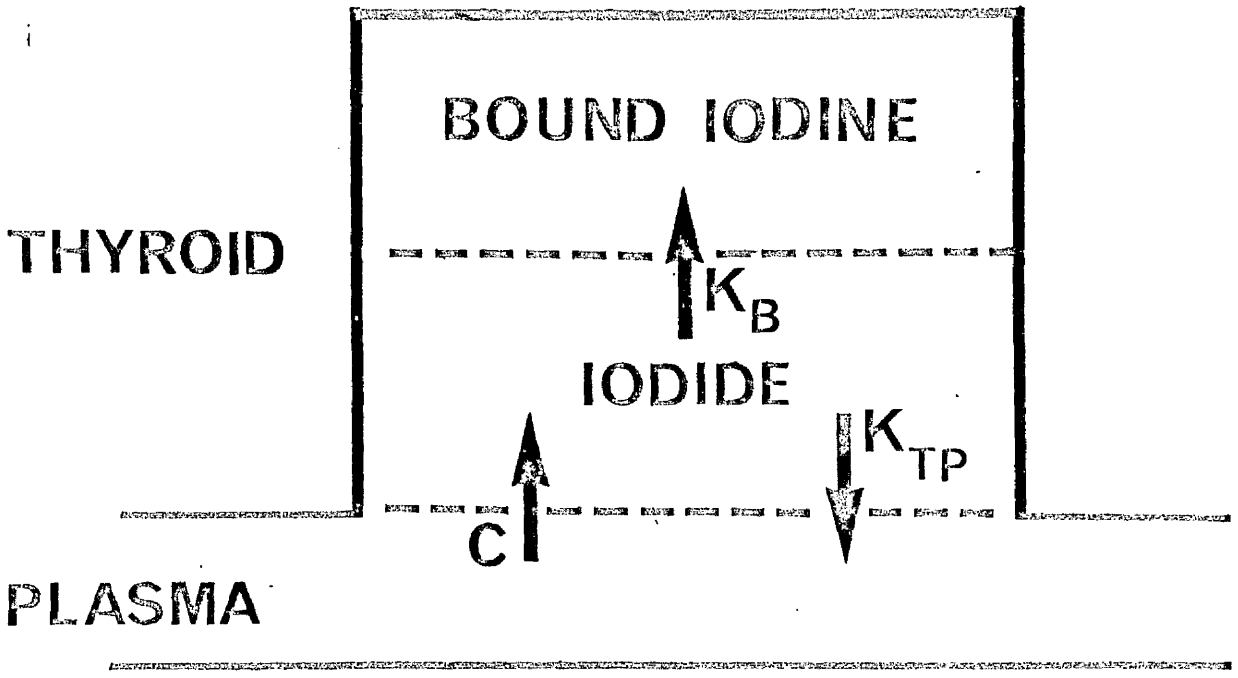


Figure 1.2 Open three-compartment binding model of iodide uptake by the thyroid gland. C is the unidirectional clearance of iodide into the gland from plasma, K_{TP} is the exit rate constant of free iodide and K_B is the binding rate constant of free iodide into the bound iodine compartment.

represented by a rate constant K_B. If binding is blocked, i.e. $K_B = 0$, then

$$dU_F/dt = C\ I_P - K_{TP}\ U_F \qquad (1)$$

A convenient way of solving for C and K_{TP} under this condition is to divide throughout by I_P and to plot $(1/I_P)\ (dU_F/dt)$ versus U_F/I_P. The intercept on the axis of the former is C and the slope of the straight line is K_{TP}. One of the problems of this method is determining, precisely, the gradient of the thyroid uptake curve, dU_F/dt, from the experimental data. A graphical approach may be used which consists of fitting a curve, visually, through the uptake points and then drawing tangents to obtain estimates of the gradient at various times.

An alternative method of determining C and K_{TP} with $K_B = 0$ is to solve Equation 1 in the first instance for U_F. If both sides of the equation are multiplied by $e^{K_{TP} \cdot t}$ then,

$$d\ (U_F \cdot\ e^{K_{TP} \cdot t})/dt = C\ I_P\ e^{\ K_{TP} \cdot t}$$

In order to avoid constraining the curve to pass through (0,0), thus allowing for experimental errors in determining tracer levels in plasma at very early times, another parameter may be introduced. Thus, as a boundary condition, let the computed curve pass through $U_F(T)$ at some time T (this can be taken as the time when precise determination of plasma levels becomes possible). Therefore by integration

$$U_F(t) = C\ e^{\ -K_{TP} \cdot t} \int_T^t I_P\ e^{K_{TP} \cdot t}\ dt + U_F(T)\ e^{-K_{TP}(t-T)} \qquad (2)$$

(This expression for uptake has been used by Newcomer (55)in animal studies and by Robertson (27) in human studies). The problem is thereby reduced to finding those values of the parameters C, K_{TP}, and $U_F(T)$ which produce a computed uptake curve that best fits the experimental data. This may be achieved using an analogue of digital computer.

It is interesting to note that the solution for $U_F(t)$ contains two components, the first representing the amount of tracer accumulating since time T, and the second, the amount of tracer still remaining due to the starting value $U_F(T)$.

If binding is not blocked, i.e. $K_B \neq 0$, then

$$dU_F/dt = C\, I_P - K_{TP}U_F - K_BU_F \qquad (3)$$

$$dU_B/dt = K_BU_F \qquad (4)$$

$$d(U_F+U_B)/dt = C\, I_P - K_{TP}U_F \qquad (5)$$

Equation 5 can be re-arranged to yield,

$$U_F = (CI_P - d(U_F + U_B)/\, dt)/K_{TP} \qquad (6)$$

If C and K_{TP} have been determined previously in a trapping study, i.e. with binding blocked by administration of an antithyroid drug, K_B may be estimated by graphical methods (12). To do this, the slope of the observed uptake curve at various points is determined by drawing tangents. These values and the corresponding concentrations of tracer in plasma, I_P, are substituted in equation 6 to provide estimates of U_F at various

times. The curve for organically bound tracer is obtained by subtracting these estimates of U_F from the observed uptakes. Estimates of the binding rate, K_B, may then be made from the slope of the bound tracer curve and the value for unbound tracer, U_F at any given time. This follows from Equation 4 which can be re-arranged to yield

$$K_B = (dU_B/dt)/U_F$$

An alternative approach, which lends itself to curve fitting techniques using a computer, is to solve Equations (3) and (4) for U_F and U_B. Applying the same boundary condition as before when binding was blocked, the solution for U_F is

$$U_F(t) = C\, e^{-(K_{TP}+K_B)t} \int_T^t I_P e^{(K_{TP}+K_B)t}\, dt + U_F(T)\, e^{-(K_{TP}+K_B)(t-T)} \qquad (7)$$

This expression for the quantity of tracer in the unbound pool at any time is essentially identical to that derived by Berman (20). It reduces to the earlier equation for $U_F(t)$ when $K_B = 0$ (equation 2). Again there are two components, the first representing the uptake since time T, and the second, the uptake remaining in the free compartment due to the starting value $U_F(T)$. If it were possible from previous knowledge of C and K_{TP} to obtain values of $U_F(t)$ from the experimental data as indicated previously, then the problem reduces to finding the value of K_B which produces a computed curve for $U_F(t)$ that best fits those calculated values.

It is easier, however, to solve for U_B and fit the combined expression $U_B + U_F$ to the observed uptakes. From Equation 4

$$U_B(t) = \int K_B U_F(t)dt + \text{constant} \qquad (8)$$

Whilst a general expression for $U_B(t)$, based on the boundary condition $U_B(t) = U_B(T)$ at time T, could be developed, it is more convenient in practice to make certain simplifications. The tracer concentration in plasma, I_P, can be expressed as a sum of exponential terms thus enabling the integration in the expression for U_F(Equation 7) to be performed. It is then possible to obtain an analytical expression for U_B, and hence, $U_F + U_B$ (as will be described later in Section 1.9). Solutions for C, K_{TP}, K_B, $U_F(T)$ and $U_B(T)$ may be found by fitting the computed $U_F + U_B$ curve to the experimental data.

The preceding discussion serves as an introduction to the following review of published work on compartmental analysis of ion uptake by the human thyroid gland. In some publications very few details are given and the present description of the analyses is necessarily brief.

1.6 Compartmental Analysis of Iodide Data - Berson & Yalow (12)

Methods and results. Berson and Yalow studied the uptake of ^{131}I - iodide in untreated subjects (binding studies) and in subjects who had been given antithyroid drugs to block organification of iodine (trapping studies). In one group of thyrotoxic subjects, binding and trapping studies were carried out on the same day. Investigations consisted of serial determinations of thyroid uptake (using an uptake counter) for at least 30 minutes following intravenous administration of the tracer. Measurements were also made of plasma radioactivity and of the urinary excretion of the tracer. Intravenous sodium thiocyanate was used to check for the presence of dischargeable ^{131}I - iodide.

The variation of uptake with time in the trapping study always had the same form - thyroidal radioactivity increased with time, reaching a peak value between 5 and 120 minutes, and then declined in equilibrium with plasma radioactivity. For analysis, thyroid uptake and plasma observations at any time were expressed in terms of the dose retained in the body (which was determined from the urinary excretion measurements). Let these be U_F' and I_P' respectively. Beginning with an equation identical to Equation 1, except that the clearance, C, is replaced by $K_{PT}V$ (where K_{PT} is the rate constant of transfer between plasma and thyroid, and V is the plasma volume) Berson and Yalow showed that, at any time,

$$K_{TP} = (dU_F'/dt)/(I_P'S - U_F')$$

and

$$K_{TP}S = K_{PT}\,V$$

S, with the dimension of volume, is the thyroidal iodide space at equilibrium (i.e. when thyroid uptake falls in parallel with plasma radioactivity) so that

$$S = \frac{U_F' \text{ (equilibrium)}}{I_P' \text{ (equilibrium)}}$$

Inherent in this derivation is the assumption (which was justified by the authors) that the rate constant of loss of tracer from the body is very small compared to K_{TP} and so could be neglected.

These expressions were used to evaluate the unknown parameters K_{TP} and $K_{PT}V$, the gradient dU_F'/dt at any given time (typically at 4-9 minutes) being obtained by drawing the tangent to the curve of U_F' versus time. The mean ($\pm$ s d) exit rate in 21 thyrotoxic subjects was 0.038 $\pm$ 0.024 minute $^{-1}$, while in 1 normal subject the exit rate was 0.047 minute^{-1}. Corresponding values for $K_{PT}V$ (equivalent to unidirectional clearance in the "open" compartment model) were 336.6 $\pm$ 256.7 and 40.0 ml minute^{-1} respectively.

In the binding studies an attempt was made to determine the binding rate by the graphical method outlined earlier (Section 1.5). This required that K_{TP} and $K_{PT}V$ (=C) be determined in a separate trapping study. Once these are known, the amount of trapped tracer can in theory be obtained using a modified version of Equation 5. Thus

$$U_F = (K_{PT}\ V\ I_P - d\ (U_F + U_B)/dt)/K_{TP}$$

where $d(U_F + U_B)/dt$ may be obtained from the observed thyroid uptake curve. Estimates of U_B, may be obtained by subtracting these calculated values of U_F from the observed uptakes. The binding rate follows from Equation 4,

$$K_B = (dU_B/dt)/U_F$$

When the results from 6 previously untreated thyrotoxic subjects were subjected to this form of analysis, it was found impossible to construct a curve for U_F, the unbound uptake. (The calculated values were scattered about zero within the limits of experimental error). These observations suggested that binding must have occurred almost instantaneously and the authors estimated a lower limit of 0.5-1.0 minute^{-1} for the binding rate.

Further corroboration for this was obtained by testing for the presence of dischargeable radioiodide in an group of 17 thyrotoxic and normal subjects. Sodium thoicyanate (1g) was given intravenously at times varying from 9-50 minutes after the tracer. In 16 cases there was no detectable discharge of radioactivity from the thyroid and only a small, if at all, discharge in the remaining subject.

Another feature of the binding studies was that the net clearance was virtually constant from 4 minutes onwards (see Section 1.4) and was equal in magnitude to the unidirectional clearance. This suggested that essentially all the trapped iodide was bound, in other words, that the binding rate was much greater than the exit rate. Successful analysis was, however, reported in one case-a patient treated with ^{131}I - iodide for thyrotoxicosis 5 months previously. Satisfactory curves for free and bound tracer were obtained, thus allowing calculation of the binding rate. The estimated values for clearance, exit rate and binding rate were 143.0 ml minute $^{-1}$, 0.050 minute $^{-1}$ and 0.112 minute $^{-1}$ respectively.

Discussion. The methods of Berson and Yalow suffer the disadvantage that separate trapping studies require to be performed in the estimation of binding rate. Furthermore, their method of measuring thyroid uptake is liable to error because of the problem of estimating extra-thyroidal activity (see Chapter 4). The authors themselves expressed reservations also about the implied assumption that there was no release of organified tracer during the period of investigation. They pointed out that, in patients with extremely active glands, detectable amounts of protein bound ^{131}I had been found at times as early as 20 minutes following administration of the tracer.

A further deficiency is the graphical approach to the analysis. It does not provide a measure of "goodness of fit" to the experimental

data (27). There is also the problem of errors due to difficulties in the graphical estimation of gradients.

1.7 Compartmental Analysis of Iodide Data - Ingbar (31)

Methods and Results. The experimental data collected by Ingbar consisted of serial measurements of thyroid uptake (using an uptake counter) from 1-3 hours following intravenous administration of ^{131}I-iodide. Plasma radioactivity was measured over the same period, during which time it was found to decrease in a single exponential manner. Both normal and thyrotoxic subjects were investigated. The methods employed did not require prior administration of antithyroid drugs.

Analysis of the data was approached from a different standpoint from that in the introductory discussion on compartmental analysis (Section 1.5). Ingbar assumed that there is immediate mixing of tracer between the plasma and intra-thyroidal iodide compartments. This results in an instantaneous uptake of free radioiodide which subsequently diminishes in parallel with the plasma radioactivity. (Some experimental justification for this concept was given by noting that measurable uptakes had been found at times less than 1-2 minutes. In one example, the uptake was 19% of the dose in less than 2 minutes). If it is further assumed that there is no release of organified tracer during the period of investigation, and that plasma radioactivity falls monoexponentially, an expression for the binding rate of intra-thyroidal iodide (K_B) can be derived. This is based on the selection of two points from each of the thyroid uptake and plasma radioactivity curves. Let these be U_1 and U_2, I_{P1} and I_{P2},

respectively, at the same two times in each curve. If K is the rate constant of the plasma curve and I_{PO} the intercept at time zero, then

$$K_B = K U_M/(SI_{PO})$$

where U_M, theoretically the maximum possible uptake by the thyroid (assuming no release of organified tracer at any time), is given by

$$U_M = (I_{P1} U_2 - I_{P2} U_1)/(I_{P1} - I_{P2})$$

and S, the thyroidal iodide space, is given by

$$S = (I_{P1} U_M - I_{PO} U_M + I_{PO} U_1)/(I_{P1} I_{PO})$$

Data from 28 normal and 20 untreated thyrotoxic subjects were analysed by this method. Mean binding rates (±sd) in the two groups were estimated to be 0.015±0.003 $minute^{-1}$ and 0.065±0.040 $minute^{-1}$ respectively. (Various other parameters were evaluated by Ingbar but these cannot be related, readily, to the parameters of the "open" compartmental models and are therefore excluded from this present review). These estimates of binding rate are at least one order of magnitude less than those of Berson and Yalow (Section 1.6) and suggest that binding is not instantaneous. It follows that a considerable proportion of radioiodide would remain unbound in the thyroid for some time. In support of this, Ingbar was able to demonstrate the presence of dischargeable radioactivity in 5 out of 6 thyrotoxic subjects who were given 1.5 g sodium thiocyanate, intravenously, 30-60 minutes after the tracer. Quantitative details

were not given except in one case where a discharge of 10-15% of the uptake was reported. These results again conflict with the work of Berson and Yalow (Section 1.6) who found no evidence of dischargeable tracer in most of their patients.

Discussion. The assumptions in Ingbar's analysis have been criticised by Berson and Yalow (12) and by other workers (23, 56). One of the main defects is the assumption that the injected radioiodide exchanges immediately with the intra-thyroidal iodide pool, in other words, that equilibrium is reached instantaneously. The author, aware of this problem, argued that the resulting errors were likely to be relatively small. However this is debatable. For instance, in some of Berson & Yalow's trapping studies (12) equilibrium was not reached until 1 hour or so after administration of the tracer.

With regard to the positive discharge results in thyrotoxic subjects, it could well be that the tracer released from the thyroid was organified, since thiocyanate is known to interfere with the binding process (1, 2, 26, 57). The dose of thiocyanate - greater than in the studies of Berson and Yalow (Section 1.6) - might have been sufficient to cause discharge of organified tracer.

1.8 Compartmental Analysis of Pertechnetate Data - Shimmins et al (41)

Methods and results. Shimmins et al used a radioisotope scanner in their studies of the early uptake of ^{99m}Tc - pertechnetate by the thyroid gland. This allowed more accurate estimation of extra-thyroidal activity (see Chapter 4) and hence the uptake data were probably more accurate than in previous studies (Sections 1.6 and 1.7). Both normal and thyrotoxic subjects were studied. Scanning commenced

1 minute after intravenous administration of the tracer and continued for a further 40-45 minutes. Each scan took 4 minutes and so the first uptake point was at 3 minutes. Plasma radioactivity was measured over the period 2-45 minutes.

The variation of thyroidal uptake of ^{99m}Tc - pertechnetate was found to be similar to that for radioiodide in Berson and Yalow's trapping studies (Section 1.6). Maximum uptake occurred before 40 minutes and in most cases the uptake curve had begun to decline in parallel with plasma radioactivity before the end of the study.

Analysis of the data was based on a "closed" compartment model with binding blocked (Section 1.2), but the final results were expressed in terms of the parameters of the corresponding "open" compartment model. The method was essentially the graphical one outlined in the introductory discussion (Section 1.5). Tangents to the thyroid uptake curve were drawn at various points (typically at 3, 10, 20, 30 and 35 minutes) to determine the gradients dU_F/dt. Plots were then made of $(1/I_P)(dU_F/dt)$ versus U_F/I_P, where I_P is the plasma concentration at these same times. The plotted values could be fitted satisfactorily by a straight line, which confirmed that there was virtually no binding of the tracer. Estimates of the unidirectional clearance (C) and exit rate (K_{TP}) were determined from the intercept on the appropriate axis and from the slope of the line, respectively.

The unidirectional clearance and exit rate (mean$\pm$sd) of pertechnetate in 9 normal subjects was 36.8 ± 23.7 ml minute $^{-1}$ and 0.083 ± 0.048 minute $^{-1}$ respectively. Corresponding values in 13 thyrotoxic subjects were 287.0 ± 226.0 ml minute^{-1} and 0.088 ± 0.031 minute^{-1}.

Discussion. The one main defect in the work of Shimmins et al is the graphical approach to analysis of the data. Graphical estimates of gradient are subject to error and there is no measure of "goodness of fit" to the experimental data (27). Very much depends on the gradients at early times. The points from later times on the straight line plot are of little value since these are found to be bunched together (which is a consequence of final equilibrium between thyroidal and plasma tracer concentration).

1.9 Compartmental Analysis of Iodide and Pertechnetate Data - Robertson et al (27, 39)

Methods and results. Robertson et al (39) reported kinetic studies of ^{131}I-iodide uptake by the thyroid, whilst Robertson (27) reported similar studies of both ^{131}I-iodide and ^{99m}Tc-pertechnetate. In each case, measurements of thyroid uptake were made using the same radioisotope scanning technique described in Section 1.8. Studies were continued for up to 150 minutes following intravenous administration of the tracer. The disappearance of radioactivity from plasma was expressed as a sum of two or three exponential terms. Both normal and thyrotoxic subjects were investigated, in some cases before and after the administration of antithyroid drugs. Data were analysed on the basis of the "open" three-compartment binding model (Section 1.5) with the binding rate set to zero when appropriate.

Robertson compared various methods of estimating parameters of the model. These consisted of the graphical method used by Shimmins et al (Section 1.8), a curve fitting method using an analogue computer and a "least sum of squares" curve fitting method using a

digital computer. It was noted that the first two have the disadvantage of having no inherent estimate of the "goodness of fit" to the experimental data. Furthermore the graphical method was liable to error owing to difficulties in the determination of gradients, and the analogue method was found to be tedious and time consuming. The method of choice was that using a digital computer. It provided an objective assessment of "goodness of fit" and it was faster than the other methods.

Only the digital method, which follows on from the introductory discussion (Section 1.5), will be considered here. The method of analysis for ^{99m}Tc-pertechnetate data and for ^{131}I-iodide data, when binding was completely inhibited, is described first. Equation 2, which gives the free uptake at any time in the absence of binding, is re-written as

$$U_F(t) = C\, f(t) + g(t) \qquad (9)$$

where

$$f(t) = e^{-K_{TP}t} \int_T^t I_P e^{K_{TP}t}\, dt$$

and

$$g(t) = U_F(T) e^{-K_{TP}(t-T)}$$

with I_P, the plasma radioactivity, expressed as a sum of three exponential terms, i.e.

$$I_p(t) = \sum_{i=1}^{3} A_i e^{-K_i t}$$

the expression for f(t) is integrated directly to yield

$$f(t) = \sum_{i=1}^{3} A_i \left(e^{-K_i t} - e^{-(K_{TP}(t-T)+K_i T)}\right)/(K_{TP}-K_i)$$

The free uptake at any time is thereby expressed as a function of the variables C, K_{TP}, $U_F(T)$ and T.

An iterative programme was written which searched for those parameter values that produced the least sum of differences squared between the observed uptake data and the computed values. Restrictions were placed, however, on the variables $U_F(T)$ and T because the computed curve was constrained to pass through the first observed uptake point. In other words, these two parameters were made equal to the first observed uptake, and the time of that uptake, respectively. The task was simplified further by the use of an analytical approximation for the optimum value of C for each value of K_{TP} chosen in the search procedure. Firstly, an estimate of C for each observed point, j, was obtained by re-arrangement of Equation 9, thus

$$C_j = (U_{Fj} - g_j)/f_j$$

The optimum value for C was then taken to be the mean value of C_j over all data points.

A similar approach was used in the treatment of ^{131}I-iodide data from the binding gland, but the analysis is complicated by the

addition of the extra parameter K_B. The thyroid uptake, U(t) at any given time includes an unbound component $U_F(t)$ and a bound component $U_B(t)$ (Equations 7 and 8). With the additional boundary condition $U_B(t) = U_B(T)$ at time T and with plasma radioactivity expressed again as a sum of three exponential terms, the following equations were derived;

$$U_F(t) = C\sum_{i=1}^{3} \frac{A_i e^{-K_i t} - A_i e^{-(K_i T + (K_{TP}+K_B)(t-T))}}{(K_{TP}+K_B-K_i)} + U_F(T)_e - (K_{TP}+K_B)(t-T)$$

and

$$U_B(t) = C\,K_B \sum_{i=1}^{3} \frac{A_i e^{-K_i T} - A_i e^{-K_i t}}{K_i(K_{TP}+K_B-K_i)} + C\,K_B \frac{(e^{-(K_{TP}+K_B)t} - e^{-(K_{TP}+K_B)T})}{(K_{TP}+K_B)} \sum_{i=1}^{3} \frac{A_i e^{(K_{TP}+K_B-K_i)T}}{(K_{TP}+K_B-K_i)} + U_F(T)K_B \frac{(1 - e^{-(K_{TP}+K_B)(t-T)})}{(K_{TP}+K_B)} + U_B(T)$$

For convenience the total thyroid uptake was expressed in terms of C and two functions that are independent of C, thus

$$U(t) = U_F(t) + U_B(t) = C\,F(t) + G(t) \qquad (10)$$

where

$$F(t) = \sum_{i=1}^{3} \frac{A_i e^{-K_i t} - A_i e^{-(K_i T + (K_{TP}+K_B)(t-T))}}{(K_{TP}+K_B-K_i)}$$

$$+ K_B \sum_{i=1}^{3} \frac{A_i e^{-K_i T} - A_i e^{-K_i t}}{K_i(K_{TP}+K_B-K_i)}$$

$$+ \frac{K_B(e^{-(K_{TP}+K_B)t} - e^{-(K_{TP}+K_B)T})}{(K_{TP}+K_B)} \sum_{i=1}^{3} \frac{A_i e^{(K_{TP}+K_B-K_i)T}}{(K_{TP}+K_B-K_i)}$$

and

$$G(t) = U_F(t)\ e^{-(K_{TP}+K_B)(t-T)}$$

$$+ \frac{K_B U_F(T)(1-e^{-(K_{TP}+K_B)(t-T)})}{(K_{TP}+K_B)}$$

$$+ U_B(T)$$

In the curve fitting procedure the computed uptake curve was again constrained to pass through the first observed uptake point. The boundary value $U_F(T)$ was taken to be 90% of that uptake and $U_B(T)$ was taken to be 10%. This assumed that 10% of the first uptake measurement (at 2-3 minutes after administration of the tracer) is organically bound. The procedure was further simplified, as before, by an approximation for the optimum value of C for each value of K_{TP}

and K_B in successive iterations. Firstly, an estimate of C for each observed point, j, was obtained by re-arrangement of Equation 10, thus

$$C_j = (U_j - G_j)/F_j$$

The optimum value for C was then taken to be the mean value of C_j over all data points. Thus the problem was reduced to finding, by iterative techniques, those values of K_{TP} and K_B that produced a "least sum of squares" fit to the observed uptake data.

Pertechnetate studies were performed in 36 thyrotoxic and 37 normal subjects. The mean values ($\pm$sd) for unidirectional clearance and exit rate in the toxic group were 138.0$\pm$144.0 ml minute^{-1} and 0.073$\pm$0.042 minute^{-1} respectively. Corresponding values in the normal group were 19.7$\pm$12.6 ml minute^{-1} and 0.075$\pm$0.036 minute^{-1}. These results are similar in magnitude to those of Shimmins et al (Section 1.8).

The kinetics of pertechnetate uptake before and after the administration of carbimazole (120 mg over 24 hours) where studied in 7 thyrotoxic and 11 normal subjects. Both unidirectional clearance and exit rate in the thyrotoxic group were found to be significantly increased by the drug, but no changes were detected in the normal group.

The uptake of ^{131}I-iodide after the same dosage of carbimazole was studied in 6 thyrotoxic and 9 normal subjects. Mean values ($\pm$sd) of 250.0$\pm$165.0 ml minute^{-1} and 0.036$\pm$0.020 minute^{-1} were found in the thyrotoxic group for unidirectional clearance and exit rate respectively. Corresponding values in the normal group were 40.2$\pm$29.4 ml minute^{-1} and 0.037$\pm$0.033 minute^{-1}. These results, when

compared with those for pertechnetate, suggest that, while the unidirectional clearance of iodide may be greater, the exit rate is less.. The results for iodide agree with those found by Berson and Yalow in their trapping studies (Section 1-6).

Binding studies with ^{131}I-iodide were performed in 15 untreated thyrotoxic and 7 normal subjects. Mean values ($\pm$sd) for unidirectional clearance in the two groups were 196.0$\pm$160.0 and 36.8$\pm$9.5 ml minute^{-1} respectively. The mean ($\pm$sd) exit rates were 0.022$\pm$0.011 and 0.030$\pm$0.016 minute $^{-1}$, and binding rates, 0.110$\pm$0.043 and 0.066$\pm$0.040 minute^{-1} respectively. Both unidirectional clearance and binding rate were significantly greater in the thyrotoxic group.

These binding rate results for iodide are similar in magnitude to those reported by Ingbar (Section 1.7) but considerably less than those reported by Berson & Yalow (Section 1.6). Some evidence that the values might be underestimates of the actual binding rate was found in the results of early potassium perchlorate discharge studies. No discharge was found in any of 11 untreated thyrotoxic and 6 normal subjects, who were given an oral dose of 500 mg potassium perchlorate 20 minutes after the tracer. However, if predictions based on the computed binding rates had been correct a few patients should have manifested significant discharge.

Discussion. The work of Robertson et al, while a major advance in the digital analysis of thyroid uptake data, is not without defects. One weakness is the choice of the first uptake point as a boundary condition. This constrains the computed curve to pass through that point. Further constraints are placed on the analysis, in the case of the binding gland, by the assumption that 10% of the first uptake is organically bound. It was argued that this assumption was justified

by the ensuing estimates for binding rate. Some limited investigation of the problem revealed that alteration of the bound fraction by a factor of three had little effect upon the parameter estimates. However, reported values of binding rate for iodide vary by as much as a factor of 67 (Section 1.12) and the constraint imposed upon the optimisation procedure would seem too restrictive.

One final point is that no errors were given for the parameter estimates. Unlike the previous graphical analyses (Sections 1.6-1.8), the method of "least sum of squares" does allow calculation of random errors due to variation in experimental data (58). However this facility was not exploited by Robertson et al.

1.10 Compartmental Analysis of Iodide and Pertechnetate Data - Gray (28)

Methods and results. Gray used a specially collimated uptake counter (59) that minimised the contribution from extra-thyroidal activity in his studies of thyroidal uptake. Both ^{131}I-iodide and ^{99m}Tc-pertechnetate were studied. The tracers were given together intravenously and neck radioactivity recorded continuously for a period of 20 minutes. Measurements were also made of venous plasma radioactivity. At the end of the uptake period sodium perchlorate was given intravenously and sufficient time was given for any discharge of thyroid radioactivity to occur. A second dose of ^{131}I and ^{99m}Tc was then given which allowed an estimate of extra-thyroidal activity to be made. Normal controls, untreated thyrotoxic subjects, and thyrotoxic subjects on carbimazole therapy, were investigated.

Before analysis a correction was made to the venous radioactivity curve to approximate the variation of tracer concentration in arterial plasma. This was introduced because of a previous observation (53)

that radioiodide levels were greater in arterial plasma at early times. The correction factors were based on further observations of arterio/venous differences in the first few minutes after the administration of ^{131}I and ^{99m}Tc (see Section 3.2). Application of these factors resulted in the modified plasma curves for the latter having a starting value at zero time of 0.03% dose ml^{-1}, and for ^{131}I a starting value of 0.02% dose ml^{-1}. By 6 minutes after administration of the tracer, the simulated arterial curve was virtually identical to the original venous curve.

The analysis procedure, when binding was absent, was essentially the graphical approach of Shimmins et al (Section 1.8). All ^{99m}Tc data and ^{131}I data from drug-treated thyrotoxic subjects were analysed in this manner. In some cases the data were also subjected to analogue computation. However, owing to difficulties in precise curve fitting with the analogue computer, correlation between the two methods was disappointing.

A simple approach - the estimation of net clearance - was used in the treatment of ^{131}I-iodide data from the normally binding gland. This was based on two pieces of evidence. Firstly, thyroid clearance was noted to be constant when arterial radioactivity levels, rather than the observed venous values, were used (Section 1.4). In other words, there is no return of free tracer to blood, the binding rate being much greater than the exit rate. The observed net clearance was therefore considered to be a good estimate of the unidirectional clearance. Secondly, no dischargeable radioactivity was found in 29 normal and 12 untreated thyrotoxic subjects who were given intravenous sodium perchlorate (200 mg) at the early time of 10 minutes after the tracer. It was deduced from these discharge results that binding is

virtually instantaneous and so there would be essentially no free radioiodide present in the gland at any time. The net clearance would, therefore, be constant and equal to the unidirectional clearance.

In such circumstances it is virtually impossible to obtain accurate estimates of the binding and exit rates (see Section 2.5). However, Gray gave an approximate value of 1.0 $minute^{-1}$ for the binding rate of iodide in normal and thyrotoxic glands. Estimates of exit rate were obtained from the trapping studies.

Gray also performed kinetic studies in subjects with binding defects such as occur in Hashimoto's thyroiditis, Pendred's syndrome and after radioiodide therapy. The object was to estimate the binding rate of iodide. This was achieved by an empirical method which was based on the equations of the open three-compartment binding model (Section 1.5), and which made use of the analogue computer. By generating a large series of uptake curves for different parameter values, Gray was able to show that binding rate was related to the fraction of uptake discharged by perchlorate at 10 minutes. The relationship was found to be virtually independent of unidirectional clearance and exit rate. Essential to these derivations, however, was the assumption that perchlorate reduces the binding rate to negligible levels in patients with defective organification. (The assumption was based on comparative observations of the exit rate of ^{131}I-iodide and ^{99m}Tc-pertechnetate before and after perchlorate. It was predicted that, if binding still continued after perchlorate, there would be a relative increase in the exit rate of iodide (Section 2.6). However, no such increase was observed).

The kinetics of ^{131}I-iodide and ^{99m}Tc-pertechnetate were studied in 7 normal and 13 untreated thyrotoxic subjects. A linear correlation was found between the unidirectional clearances of iodide and pertechnetate, the former being approximately 2-3 times greater. The mean ($\pm$sd) unidirectional clearance for iodide was 28.0$\pm$13.0 and 222.0$\pm$138.0 ml minute^{-1} in the normal and thyrotoxic group respectively. Corresponding values for pertechnetate were 19.0$\pm$8.0 and 93.0$\pm$53.0 ml minute^{-1} respectively. Mean exit rates for pertechnetate were 0.069$\pm$0.021 and 0.081$\pm$0.027 minute^{-1} in the normal and thyrotoxic group respectively. These results are similar to those in previous studies (Sections 1.6, 1.8, 1.9).

Comparative studies of the two anions were also made in 14 thyrotoxic subjects on carbimazole therapy. Again the unidirectional clearance of iodide was 2-3 times greater than that for pertechnetate. Mean values ($\pm$sd) were 167.6$\pm$57.7 for iodide and 76.8$\pm$24.2 ml minute^{-1} for pertechnetate. There was good correlation between the exit rates for the two ions, the mean value being 0.062$\pm$0.024 for iodide and 0.088$\pm$0.038 minute^{-1} for pertechnetate.

As indicated earlier, the binding rate for iodide in normal and untreated thyrotoxic subjects was estimated to be approximately 1.0 minute^{-1}. The estimated values in patients with binding defects were considerably less. In 15 patients with Hashimoto's disease, the mean ($\pm$sd) binding rate was 0.216$\pm$0.096 minute^{-1}; in 3 patients with Pendred's syndrome, the mean was 0.230$\pm$0.120 minute^{-1}; in 5 thyrotoxic subjects who had received radioiodide therapy, the mean was 0.210$\pm$0.070 minute^{-1}.

Discussion. The use of simulated arterial, rather than observed venous levels of tracer, in Gray's work was a significant advance in the analysis of kinetic uptake data. With this innovation, the clearance of radioiodide, in the nomally binding gland, was found to be constant from early times and thus it was deduced that binding rate is much greater than exit rate. The absence of dischargeable iodide at the early time of 10 minutes after the tracer was a further indication that the binding rate is relatively high. These findings lend support to the earlier conclusions of Berson and Yalow (Section 1.6) with regard to binding rate in the uninhibited gland.

One aspect of the work that could be criticised is the postulate that perchlorate completely inhibits organification in subjects with defects in that function of the gland (Section 3.8). It will be shown later (Section 6.3) that this can lead to the binding rate being considerably over-estimated. Gray referred to animal studies (57) that, possibly, gave support to his postulate. However the perchlorate levels required in those studies to produce binding inhibition were exceedingly high (Section 3.8).

Another criticism is that fact no rigorous estimates were made of errors in the final results due to variations in the experimental data. Errors were given for the results of the trapping studies but these referred only to uncertainty in fitting the final straight line in the graphical analysis (Section 1.8). No consideration was given, for example, to the problem of determining gradients from the observed uptake data.

1.11 Compartmental Analysis of Iodide and Pertechnetate Data - Various Authors

Larsson (23) used the method developed by Ingbar (Section 1.7) to determine binding rates for iodide before and after the administration of the antithyroid drug, thiouracil. Mean values ($\pm$sd) in 4 untreated thyrotoxic and 5 normal subjects were 0.097 ± 0.056 and 0.027 ± 0.014 minute^{-1} respectively. The effect of thiouracil was to reduce the binding rate to a mean value of 0.013 ± 0.003 minute^{-1} in 5 thyrotoxic subjects under treatment with the drug. In one normal subject given thiouracil (150 mg) the binding rate was estimated to be 0.003 minute^{-1}.

Berson (20) and Rall et al (17) gave values of 0.300-0.330 minute^{-1} for the exit rate of iodide and a value of 1.0 minute^{-1} for the binding rate. Complete details were not given as to how the values were derived. These were merely used for illustrative purposes in a theoretical consideration of the analysis of iodide kinetic data.

Comparative studies of iodide and pertechnetate kinetics were undertaken by Dige - Peterson (49) who used an uptake counter and continuous recording facility. The studies covered the first 30 minutes after intravenous administration of ^{132}I-iodide and ^{99m}Tc-pertechnetate. Frequent venous blood samples were taken from 1 minute onwards. A graphical analysis, similar to that applied to pertechnetate data by Shimmins et al (Section 1.8), was applied to both iodide and pertechnetate data when there was no inhibition of binding. (Justification for neglecting the binding of iodide was based on the deduction of Robertson et al (Section 1.9) that the bound component was only 10% of the total uptake in the first few minutes). Only the first 8 minutes of the radioiodide uptake curves were, in fact, included in the analysis.

In 3 untreated thyrotoxic subjects the mean (±sd) unidirectional clearances for iodide and pertechnetate were 679.0±256.0 and 373.0±19.7 ml minute^{-1} respectively. The mean exit rate for iodide was 0.049±0.041 minute^{-1} and for pertechnetate 0.126±0.058 minute^{-1}. As in previous studies (Sections 1.9 and 1.10), these results suggest that whilst the unidirectional clearance for pertechnetate may be less, the exit rate appears to be greater than that for iodide.

The uptake of ^{131}I-iodide and ^{99m}Tc-pertechnetate was studied by Brooke et al (50) using a gamma camera and computer. Uptake data were collected over successive 1 minute intervals throughout the first 60 minutes after intravenous administration of the tracers. The form of the plasma radioactivity curve was determined from the tracer level in the cardiac regions, the curve being normalised using the measured radioactivity in one or two blood samples. (Gray (28) has shown that the variation of radioactivity in the cardiac regions lies somewhat between that in arterial and that in venous blood).

Initially the analysis consisted of estimation of early and late clearance of the tracers (51). In the case of iodide, this approach permitted the level of binding inhibition, produced by varying doses of antithyroid drug, to be quantified as follows:-

$$\text{Binding inhibition } (\%) = 100\ (1 - C_E/C)$$

where C is the earliest measured clearance (taken to represent unidirectional clearance), and C_E is the equilibrium clearance, measured at that point in the uptake curve when clearance became constant. It was found that an oral dose of 0.2 mg methimazole, given 30 minutes before the tracer, caused 40% inhibition of binding;

doses greater than 10 mg caused 95% inhibition. When there was no inhibition of binding, there was no change of iodide clearance with time.

Latterly, a "least sum of squares" analysis of the data was carried out using the generalised model building programme (SAAM programme) developed by Berman et al (60-62). No details were given of the initial conditions and constraints imposed on the parameters, but the programme is certainly comprehensive enough to cope with any such requirements. The normal range for the unidirectional clearance of both iodide and pertechnetate was reported as 9.5-52.0 ml minute^{-1}. For the exit rate the range was 0.028-0.100 minute^{-1}; for the binding rate of iodide, a single value of 0.080 minute^{-1} was given. The antithyroid drug, methimazole, given intravenously 30 minutes before radioiodide, was found to markedly reduce the binding rate. A dose of 1 mg reduced the binding rate to 0.020 minute^{-1}, while a dose of 2mg reduced it to 0.008 minute^{-1}. There was no measurable binding of iodide with a dose of 40 mg methimazole.

These values reported by Brooke et al (50) for the unidirectional clearance and exit rate of the two anions are similar to the results discussed previously (Sections 1.6, 1.8-1.10). However the value given for the binding rate of iodide in the uninhibited gland lies at the lower end of the reported range (Section 1.12). Such a value, similar in magnitude to the estimated exit rate, is difficult to reconcile with the authors' observation of a constant iodide clearance under normal binding conditions. Before that can happen, the binding rate requires to be much greater than the exit rate (Section 2.5).

1.12 Discussion of Published Compartmental Analyses

All the results mentioned in Sections 1.6-1.11 are compared in Table 1. In normal subjects, reported values for the mean unidirectional clearance range from 28.0-36.8 ml $minute^{-1}$ for iodide and 19.0-36.8 ml $minute^{-1}$ for pertechnetate; in thyrotoxic subjects the ranges are 196.0-679.0 ml $minute^{-1}$ for iodide and 93.0-373.0 ml $minute^{-1}$ for pertechnetate. Exit rate is similar in both normal and thyrotoxic subjects, reported mean values ranging from 0.022-0.062 $minute^{-1}$ for iodide and 0.069-0.126 $minute^{-1}$ for pertechnetate. Overall, the results suggest that whilst the unidirectional clearance of pertechnetate is less than that of iodide, the exit rate may be greater.

In the case of iodide, there would appear to be reasonable agreement amongst the various studies as far as unidirectional clearance and exit rate are concerned. However, estimates of binding rate in the uninhibited gland vary by as much as a factor of 67 (i.e. from 0.015-1.0 $minute^{-1}$). Such differences seem unlikely to be due to biological variation.

Evidence that the problem might be technical in origin can be gleaned from the work of Gray et al (Section 1.10). These authors were the first to apply arterial blood levels of radioiodide, which would seem to be appropriate, in the calculation of clearance. As a consequence, clearance was found to be constant at all times, i.e. binding rate is very much greater than exit rate. In such circumstances it is difficult to quantify binding rate (Section 2.5). If venous blood levels are used, however, net clearance does vary with time (Section 1.4), which might explain those reported binding rates which are comparable in magnitude to the exit rate.

TABLE 1

RESULTS OF PUBLISHED COMPARTMENTAL ANALYSES OF THYROID KINETIC DATA

THYROID STATUS	IODIDE			PERTECHNETATE		AUTHOR
	Unidirectional clearance (mean ± sd) ml min^{-1}	exit rate (mean±sd) min^{-1}	binding rate (mean±sd) min^{-1}	Unidirectional clearance (mean ± sd) ml min^{-1}	exit rate (mean±sd) min^{-1}	
Normal	40.0	0.047	> 0.5 - 1.0	-	-	Berson and Yalow (12)
Thyrotoxic	336.6 ± 256.7	0.038 ± 0.024	> 0.5 - 1.0	-	-	
Treated thyrotoxic after radioiodide therapy	143.0	0.050	0.112	-	-	
Normal	-	-	0.015 ± 0.003	-	-	Ingbar (31)
Thyrotoxic	-	-	0.065 ± 0.040	-	-	
Normal	-	-	-	36.8 ± 23.7	0.083 ± 0.048	Shimmins et al (41)
Thyrotoxic	-	-	-	287.0 ± 226.0	0.088 ± 0.031	
Normal	36.8 ± 9.5	0.030 ± 0.016	0.066 ± 0.040	19.7 ± 12.6	0.075 ± 0.036	Robertson et al (27, 39)
Thyrotoxic	196.0 ± 160.0	0.022 ± 0.011	0.110 ± 0.043	138.0 ± 144.0	0.073 ± 0.042	
Normal	28.0 ± 13.0	-	1.0	19.0 ± 8.0	0.069 ± 0.021	Gray (28)
Thyrotoxic	222.0 ± 138.0	0.062 ± 0.024	1.0	93.0 ± 53.0	0.081 ± 0.027	
Hashimoto's thyroiditis	-	-	0.216 ± 0.096	-	-	
Pendred's syndrome	-	-	0.230 ± 0.120	-	-	
Treated thyrotoxic after radioiodide therapy	-	-	0.210 ± 0.070	-	-	
Normal	-	-	0.027 ± 0.014	-	-	Larrson (23)
Thyrotoxic	-	-	0.097 ± 0.056	-	-	
Normal on thiouracil	-	-	0.003	-	-	
Thyrotoxic on thiouracil	-	-	0.013 ± 0.003	-	-	
Normal	-	0.300 - 0.330	1.0	-	-	Berman (20) Rall et al (17)
Thyrotoxic	679.0 ± 256.0	0.049 ± 0.041	-	373.0 ± 19.7	0.126 ± 0.058	Dige-Peterson (49)
Normal	9.5 - 52.0	0.028 - 0.100	0.080	9.5 - 52.0	0.028 - 0.100	Brooke et al (50)
Subject given methimazole						
1 mg	-	-	0.020	-	-	
2 mg	-	-	0.008	-	-	
40 mg	-	-	0.000	-	-	

Further support for a relatively high binding rate lies in the absence of dischargeable radioiodide reported by Berson and Yalow (Section 1.6), by Robertson (Section 1.9) and by Gray (Section 1.10). The results, especially from the studies by Gray in which perchlorate was given 10 minutes after the tracer, were considered not to be consistent with the lower estimates of binding rate in Table 1.

There is a fair degree of uniformity amongst the various studies as far as the results for pertechnetate are concerned. There would appear to be no significant binding of the anion on the basis of graphical analysis (Section 1.8). Whether the small degree of pertechnetate binding, known to occur from other work (3-7), can be quantified by more sensitive compartmental analysis, is a question that remains to be answered.

None of the studies to date has included a rigorous investigation of the accuracy of the final results. One likely source of error is random variation in the observed data. Secondly, errors may arise if the observation period is not carefully selected. It seems unlikely, at this stage, that observation periods ranging from 8-150 minutes (Sections 1.6, 1.8-1.11) produce results of the same accuracy. Thirdly, curve fitting techniques can be particularly sensitive to inital conditions and constraints on the parameters (63-65).

The present work sets out to apply the open three-compartment binding model (Figure 1.2) to the analysis of thyroidal uptake data. Every effort will be made to minimise constraint on the parameters of the model. Random and systematic error will be evaluated. Arterial levels of tracer will be applied, rather than venous levels, in the hope that improved numerical analysis will confirm the existence of a binding rate for iodide that greatly exceeds exit rate.

CHAPTER TWO

A NEW METHOD OF ANALYSIS USING THE OPEN THREE-COMPARTMENT BINDING MODEL AND DIGITAL COMPUTING TECHNIQUES

2.1 Introduction

Although the thyroid gland may contain at least two iodide pools (1), the studies reviewed in Chapter 1 provide some validation for use of those models (Figure 1.1) in kinetic analysis which have a single intra-thyroidal iodide compartment (2). Thus the conventional approach of choosing the simplest model to simulate the system under investigation (63) was adopted in the present work. No attempt was made to apply more complex thyroid models with two or more iodide compartments.

Since there is now sufficient evidence to show that some organification of pertechnetate occurs (3-7) it seemed appropriate to allow for this in any simulation studies.

The model of choice was therefore the open three-compartment binding model (Figure 1.2) and this was used throughout in the analysis of both iodide and pertechnetate kinetic data. Inherent in the use of this model is the assumption that any release of organified tracer during the period of investigation is small and may be neglected. Experimental evidence in support of this is reviewed in Chapter 3.

Having selected a suitable model to describe the trapping and binding functions of the thyroid gland, it is important to determine how well the predictions of the model fit experimental observations.

Various methods have been devised to quantify the degree of fit between simulated and experimental data but the method of "least sum of squares" is the one most widely used (58, 64). It has already been used in analysis of thyroid kinetic data (Sections 1.9 and 1.11), the best fit to experimental data occurring when the sum of squared differences between the simulated and experimental data is at a minimum. "Least sum of squares" analysis provides the best estimates of the unknown parameters if the differences between simulated and experimental data are uncorrelated and are normally distributed about zero. The variances of the experimental data also require to be equal. A modification is the method of "weighted least sum of squares" which is the method of choice when the last condition is not met. The initial intention in the present work was to make use of the method of "weighted least sum of squares" which is applicable in a wider variety of situations. It was expected that weighting factors could be estimated, when necessary, from the experimental data.

In practice, the optimisation procedure consists of searching for minima in the sum of squares function which can be thought of as a surface in the parameter space of the model. Bevington (58) has produced a useful review of several search procedures which make use of digital computing techniques. His guidelines were followed closely in this thesis.

An alternative would have been to use the generalised model building programme developed by Berman et al (60-62) or a "least sum of squares" fitting programme such as produced by the Nottingham Algorithms Group (66). However, it was possible to reduce the present problem by analytical methods to a simplified calculation.

A less sophisticated programme, specifically for analysis of thyroid kinetic data, was therefore written.

It is necessary when assessing the performance of any model to determine the effect of variation in the experimental data on the precision of the computed parameters (65). Attention was therefore paid to the estimation of errors in the present work. Some attention was given also to sensitivity analysis (67-70) which is directed towards identifying those parameters that have the greatest effect on performance of the system under investigation.

One advantageous feature of the thyroid trapping and binding system is that administration of a blocking agent, such as perchlorate, allows the quantities of free and bound tracer to be determined at any time. This permits certain aspects of the model to be independently checked and verified, which is a desirable step in any simulation analysis (63). A method of analysing the perchlorate discharge phase of a kinetic study was, therefore, developed.

Details of the present analysis of thyroidal uptake and discharge are given in this chapter. An outline is also given of the computer programmes and methods used to determine random errors in the parameter estimates.

2.2 Mathematical Derivations for the Uptake Phase

The present analysis follows on from the earlier introduction (Section 1.5) and makes use of certain derivations in the work of Robertson et al (Section 1.9). They expressed the total uptake of tracer in the binding gland, at any time, as

$$U(t) = CF(t)+G(t)$$

where C is the unidirectional clearance and where F(t) and G(t) are functions of K_{TP}, the exit rate, and K_B, the binding rate. Included also in the function G(t) are the parameters $U_F(T)$ and $U_B(T)$, the free and bound uptakes, respectively, at time T.

It is convenient to define T as the time after which the plasma tracer level is known precisely, so $U_F(T)$ and $U_B(T)$ become initial values for the free and bound components in the simulated uptake curve. These result from the thyroidal clearance of an unknown plasma concentration of tracer up to time T. Experimental data points at times less than T, cannot be included in the "least sum of squares" analysis. Simulation of these is impossible due to lack of knowledge about plasma radioactivity. To avoid imposing any constraints in the following analysis, $U_F(T)$ and $U_B(T)$ are treated as unknown parameters. The optimisation procedure is designed to yield, if possible, realistic values for these variables.

As a first step, the function G(t) is simplified thus,

$$G(t) = U_F(T)\ H(t) + U_B(T)$$

where

$$H(t) = e^{-(K_{TP}+K_B)(t-T)} + K_B \frac{(1-e^{-(K_{TP}+K_B)(t-T)})}{(K_{TP}+K_B)}$$

Thus

$$U(t) = CF(t) + U_F(T)\ H(t) + U_B(T)$$

Denoting the observed uptakes as $U_O(t_j)$, with absolute variance V_j, the weighted sum of squared differences, S, is given by

$$S = \sum_j (U_O(t_j) - U(t_j))^2 / V_j \qquad (11)$$

summed over all points included in the "least sum of squares" analysis (58). The function S depends on the parameters C, $U_F(T)$, $U_B(T)$, K_{TP} and K_B. Thus minima will occur when

$$\partial S/\partial C = \partial S/\partial U_F(T) = \partial S/\partial U_B(T) = \partial S/\partial K_{TP} = \partial S/\partial K_B = 0$$

(The same set of equations would hold at local maxima of the function but these were never observed in practice). Adopting the method of Widman and Powsner (71) for red-cell survival analysis, the first three equations are solved to yield optimum values of the linear parameters C, $U_F(T)$ and $U_B(T)$ for any K_{TP} and K_B. Substituting for $U(t_j)$ in Equation 11 and equating the partial derivatives to zero,

$$C\sum_j F^2(t_j)/V_j + U_F(T)\sum_j F(t_j)\,H(t_j)/V_j + U_B(T)\sum_j F(t_j)/V_j$$

$$= \sum_j U_O(t_j)F(t_j)/V_j \qquad (12)$$

$$C\sum_j F(t_j)H(t_j)/V_j + U_F(T)\sum_j H^2(t_j)/V_j + U_B(T)\sum_j H(t_j)/V_j$$

$$= \sum_j U_O(t_j)H(t_j)/V_j \qquad (13)$$

$$C \sum_j F(t_j)/V_j + U_F(T) \sum_j H(t_j)/V_j + U_B(T) \sum_j 1/V_j$$

$$= \sum_j U_0(t_j)/V_j \qquad (14)$$

Thus in any iterative search for the "least sum of squares" only the parameters K_{TP} and K_B need be considered. At each iteration, Equations 12-14 can be solved to yield optimum values for the remaining parameters.

2.3 Location of the Minimum of the Sum of Squares Function

An ICL 1903A digital computer was used to locate the minimum of the sum of squares function and so provide estimates of the various parameters. The computer programme was written in Fortran.

The search procedure is begun by constructing a coarse plot of the function S against K_{TP} and K_B, optimum values for the parameters C, $U_F(T)$ and $U_B(T)$ at each point being determined from Equations 12-14. Suitable starting values for the two variables, i.e. values at the minimum of the coarse plot, are then chosen and the minimum located to any required degree of precision by the gradient search method (58). This method was selected because the gradients of the function S were determined analytically (Appendix 1) thus avoiding problems due to approximations. Furthermore, the method is ideally suited for approaching the minimum from distant starting values (58). (The latter feature, after some preliminary experience, obviated the need for a coarse map in most anlyses).

Both K_{TP} and K_B are incremented simultaneously, with the magnitude of the increments adjusted so that the resultant direction of travel in the parameter space is along the direction of greatest change of S. As in the coarse plot, optimum values for C, $U_F(T)$ and $U_B(T)$ are calculated for each new set of K_{TP} and K_B values. Following the procedure given by Bevington (58), the appropriate increments for each parameter, at any point in the search, are given by,

$$I(K_{TP}) = \frac{-(\partial S/\partial K_{TP}).\ DK_{TP}^2}{\sqrt{((\partial S/\partial K_{TP}).\ DK_{TP})^2+((\partial S/\partial K_B).\ DK_B)^2}}$$

$$I(K_B) = \frac{-(\partial S/\partial K_B).\ DK_B^2}{\sqrt{((\partial S/\partial K_{TP}).\ DK_{TP})^2 + ((\partial S/\partial K_B).\ DK_B)^2}}$$

where DK_{TP} and DK_B are chosen at the outset as step sizes for the parameters K_{TP} and K_B.

Instead of calculating the gradients, $\partial S/\partial K_{TP}$ and $\partial S/\partial K_B$, after each iteration, the search is continued along the original direction until the value of S begins to increase. At that point the gradients are re-calculated and the search begun in the new direction. Whenever the search straddles a minimum, parabolic interpolation of S improves the accuracy of locating that minimum. The procedure is terminated when the reduction in S, after a change in direction of the search, becomes less than 0.1%.

2.4 Calculation of Random Errors

Bevington (58) has shown how errors in the computed parameters, which result from random variations in experimental data, may be calculated. His methods were adopted.

The first step is the construction of the curvature matrix which is related to the rate of change of gradient of the function S in parameter space. Elements of that matrix, R_{kl} are given by

$$R_{kl} = (\partial^2 S/\partial A_k \partial A_l)/2$$

where the A represent the parameters K_{TP}, K_B, C, $U_F(T)$ and $U_B(T)$. A first order approximation, i.e. neglecting the second order partial derivatives of the fitting function U(t), is made of these elements. Thus,

$$R_{kl} = \sum_j (\partial U(t_j)/\partial A_k)(\partial U(t_j)/\partial A_l)/V_j$$

The error matrix, E, is then obtained by inverting the curvature matrix, thus

$$E = R^{-1}$$

Errors, DA_k, for each parameter are given by

$$(DA_k)^2 = E_{kk}$$

where the E_{kk} are the diagonal elements of the error matrix.

In the event of an unweighted analysis being performed (Section 2.1), the same procedure is followed but with all $V_j=1$. An estimate of the sample variance, V, is obtained from the value of S at its minimum. Thus

$$V = S_{min}/(N-5)$$

where the denominator is the number of degrees of freedom in fitting N data points with 5 parameters. Errors for each parameter are calculated from

$$(DA_k)^2 = V E'_{kk}$$

where E' is the error matrix calculated with all $V_j=1$

Expressions for the first order derivatives of the fitting function, U(t), with respect to each parameter are given in Appendix 1.

2.5 Sensitivity Analysis - Some Elementary Observations

There is considerable evidence to suggest that the binding rate of iodide, in the uninhibited gland, is much greater than the exit rate (Section 1.12). It is of interest, therefore, to determine the effect of neglecting K_{TP}, compared to K_B, on the preceding analysis.

When this is done, it can be shown that the function H(t) (Section 2.2) equals unity at all times and that F(t) (Section 2.2) is a function independent of K_B. Equations 13 and 14 are therefore

identical. Only the unidirectional clearance, C, has a unique solution, which is independent of K_{TP} and K_B, given by

$$C = \frac{\sum_j U_O(t_j)F(t_j)/V_j \sum_j 1/V_j - \sum_j F(t_j)/V_j \sum_j U_O(t_j)/V_j}{\sum_j F^2(t_j)/V_j \sum_j 1/V_j - \sum_j F(t_j)/V_j \cdot \sum_j F(t_j)/V_j}$$

Any values of $U_F(T)$ and $U_B(T)$ can be shown to be permissible provided,

$$U_F(T) + U_B(T) = \frac{\sum_j U_O(t_j)/V_j - C\sum_j F(t_j)/V_j}{\sum_j 1/V_j}$$

It can be shown also that the sum of squares function, S (Equation 11), is independent of exit and binding rate when $K_B >> K_{TP}$. Thus "least sum of squares" analysis provides a unique value for only unidirectional clearance.

With $K_B >> K_{TP}$, there is no significant loss of unbound tracer and the net clearance at all times is virtually equal to the unidirectional clearance. Because of the latter's independence of binding and exit rate, the shape of the uptake curve is dependent only upon unidirectional clearance and plasma tracer level. In other words, the observed variable, i.e. thyroid uptake, is sensitive only to the system parameter C, for the special case where binding rate is much greater than exit rate.

2.6 Simulation of the Perchlorate Discharge Phase

If it is assumed that perchlorate completely blocks further thyroidal uptake of tracer (Section 3.8), the rate of change of the free and bound components of uptake are given by

$$dU_F/dt = -K'_{TP}\, U_F - K'_B\, U_F \qquad (15)$$

$$dU_B/dt = K'_B\, U_F \qquad (16)$$

where K_{TP}' and K_B' are the exit and binding rates, respectively, in the post-perchlorate phase. If $U_F(T_p)$ and $U_B(T_p)$ are the free and bound components at the time of perchlorate administration, Equations 15-16 may be solved (28) to yield the future time course of these components. Thus,

$$U_F(t) = U_F(T_p)\, e^{-(K'_{TP}+K'_B)t}$$

$$U_B(t) = U_B(T_p) + K'_B\, U_F(T_p)\, \left(\frac{1-e^{-(K'_{TP}+K'_B)t}}{(K_{TP}' + K_B')}\right)$$

where t is the time after perchlorate. The total thyroid uptake $(U_F(t) + U_B(t))$ is given by

$$U(t) = U_B(T_p) + \frac{K'_B U_F(T_p)}{(K_{TP}'+K_B')}$$

$$+\left(U_F(T_p) - \frac{K_B'\, U_F(T_p)}{(K'_{TP}+K'_B)}\right) e^{-(K'_{TP}+K'_B)t}$$

The expression for U(t) may be simplified in terms of a final residual uptake, R, and the total uptake, $U(T_p)$ ($=U_F(T_p)+U_B(T_p)$), at the time perchlorate was given. Thus,

$$U(t) = R + (U(T_p)-R)e^{-Kt}$$

where

$$R = U_B(T_p) + \frac{K'_B U_F(T_p)}{(K'_{TP}+K'_B)} \qquad (17)$$

and

$$K = K'_{TP} + K'_B$$

In practice, values for R and K may be determined by fitting the function U(t) to the experimental uptake points, knowledge of $U(T_p)$ being assumed. If the study is continued long enough for the exponential term to vanish, R is simply determined from the flat tail of the uptake curve. A curve stripping approach can then be applied to estimate the exponential component. To avoid prolonging discharge studies in the present work, a "least sum of squares" analysis was developed to estimate the parameters. The approach is basically similar to that used in analysis of the uptake phase (Section 2.2).

If $U_0(t_j)$, with variance V_j, is the observed uptake at time t_j after perchlorate, then S, the sum of squares function, is given by

$$S = \sum_j (U_0(t_j) - U(t_j))^2/V_j$$

For any given value of K, the optimum value of the linear parameter, R, may be found by equating $\partial S/\partial R$ to zero. Thus substituting for $U(t_j)$, and differentiating S with repect to R, the optimum value is given by,

$$R = \frac{\sum_j U_0(t_j)/V_j - U(Tp)\sum_j e^{-Kt_j}/V_j}{\sum_j 1/V_j - \sum_j e^{-Kt_j}/V_j}$$

In this way the problem is reduced essentially to finding that value of K which minimises the sum of squares function.

A simple iterative programme was written which allowed the estimation of R and K to be performed on a programmeable calculator (Hewlett-Packard Model 9810A). To begin computation, a suitable starting value is chosen for K and that value incremented until the minimum of S is found. During the search, the optimum value of R is computed for each new value of K. Minima are found to any required degree of accuracy by reducing the increments of K as the minimum is approached. Random errors in the parameter estimates are determined from the error matrix just as in analysis of the uptake phase (Section 2.4).

Analysis of the discharge phase in this manner serves as a check on the results derived from the preceding uptake phase. Successful analysis in the latter case will have provided estimates of the free and bound uptake at any time. The observed residual, R, is simply compared with the value calculated from knowledge of the free and bound uptake at the time of perchlorate administration (Equation 17). This of course requires use of the estimated value of K and knowledge

of K_B', the binding rate in the discharge phase.

For reasons which are discussed later (Section 3.8), it may be taken that perchlorate, in normal dosage, does not affect binding. Thus K_B' is given the value estimated for binding rate in the uptake phase.

2.7 Steady State Equations

It is informative to consider the steady state equations of the open three-compartment binding model of the thyroid gland (Figure 1.2).

At equilibrium, input of iodine to any of the compartments of the gland is equal to output. Thus, if SI_p is the stable iodide concentration in plasma and SI_T is the quantity of free iodide in the iodide compartment of the gland, then, at equilibrium,

$$C \quad SI_p = (K_{TP}+K_B)SI_T$$

Solving for SI_T yields,

$$SI_T = C \quad SI_p/(K_{TP}+K_B)$$

The rate of transfer of iodide into the bound compartment is given by,

$$SI_T \; K_B = C \quad SI_p \quad K_B/(K_{TP}+K_B)$$

Thus the rate at which stable iodide in plasma is organified may be expressed as the product of the plasma iodide concentration, SI_p, and an effective clearance, $C\ K_B/(K_{TP}+K_B)$. Inspection of the latter expression reveals that when the binding rate, K_B, is much greater than the exit rate, K_{TP}, the effective clearance is equal to the unidirectional clearance, C. The relationship between effective clearance, exit rate and binding rate is discussed further in Section 5.5.

CHAPTER THREE

APPLICATION OF THE METHOD - PRACTICAL LIMITATIONS AND SYSTEMATIC ERRORS

3.1 Introduction

A number of factors are considered in this chapter that have a bearing on the application of the foregoing method of kinetic analysis (Chapter 2). It is important to ascertain whether these impose serious limitations on the usefulness of the open three-compartment binding model, and to evaluate any systematic errors introduced into the final results.

Foremost is the problem of estimating the tracer level in plasma perfusing the thyroid. In most previous analyses, tracer levels in peripheral venous blood have been taken to represent those in the thyroidal blood supply. However, Gray (Section 1.10) used arterial plasma levels which were considered to be more realistic. In fact these were approximated in the routine situation from measurements in venous blood. A similar approximation was devised for use in the present work and the resulting systematic errors investigated.

A particularly difficult period to evaluate in a kinetic study is that immediately following intravenous administration of the tracer. During this period of mixing, it is virtually impossible to determine the levels of tracer presented to the thyroid. The situation is further complicated by the fact that iodide and, to a greater extent, pertechnetate are bound to plasma protein (1, 72, 73). Any such binding will compete with the thyroidal uptake process. As a consequence the effective unidirectional clearance of tracer is

likely to diminish as plasma binding reaches saturation level (1). Because of these problems, the method of analysis is designed so that the early part of an uptake study can be ignored (Section 2.2). However, as will be explained, this proved to be an inappropriate step to take in the practical situation.

An important assumption in the analysis is that any release of organified tracer is small enough to be neglected (Section 2.1). Some tentative calculations, based on relevant published work, were made to determine whether this assumption could be justified. One possible way of minimising the effect of any loss of organified tracer is to limit the duration of the study. However, this in itself leads to difficulties for there has been no previous work to determine the time span necessary for satisfactory results. The effect of varying the duration of study was therefore investigated.

A practical aspect of the "weighted least sum of squares" method is the calculation of suitable weighting factors, which are normally taken to be the inverse of the variance (Section 2.2). However a number of sources may contribute to variance in experimental data and it may not always be possible to evaluate the combined effect. Thus some effort was made to determine whether there is any need, in practice, for weighting factors.

Some consideration has already been given (Section 2.5) to the likely effect on the analysis of the binding rate being much greater than the exit rate. Under such circumstances, it was shown that only the unidirectional clearance would have a unique solution. When the new method of analysis was applied to radioiodide data from the binding gland, there were indeed problems in the computation. These are summarised in this chapter.

Another feature of the previous chapter is the method proposed for checking the analysis, based on the ability of perchlorate to discharge free tracer (Section 2.6). However two assumptions were made, one being that the administered dose of perchlorate completely blocks further uptake and the other being that the perchlorate does not affect binding. Some justification for these two assumptions had to be found.

3.2 Correction for Arterio/Venous Differences

Methods The present method of approximating arterial tracer levels from observations in venous blood is based on the work of Gray (28, 74). His studies of normal and thyrotoxic subjects revealed that tracer levels for both iodide and pertechnetate were greater in arterial blood during the period 0-20 minutes after intravenous administration. The mean arterio/venous ratios (±sd) determined at various times, in 10 subjects, are given in Table 2; observed ratios for iodide and pertechnetate being combined to produce the tabulated values.

These results form the basis of the approximation. All computation was performed on a programmeable calculator (Hewlett - Packard Model 9810A). An outline of the programme follows.

The first step is to fit a curve consisting of two exponential components to the observed tracer levels in venous plasma. A curve stripping method is used, with the points from 30 minutes onwards being used to determine the longer-lived component. Those points in the venous curve which relate to the times given in Table 2 are then increased by the appropriate arterio/venous ratios. Finally, a curve

consisting of three exponential components is fitted to the simulated arterial data points. The original venous component from 30 minutes onwards is preserved but two new components for the earlier part of the curve are found by curve stripping. Simulated arterial curves for iodide and pertechnetate are compared with the original venous curves in Figure 3.1.

To check the accuracy of the present approximation, the mean simulated level in a representative group of subjects was compared with the observed arterial levels reported by Gray (28). In 6 subjects studied with ^{131}I-iodide, he found a mean ($\pm$sd) arterial concentration at 1 minute of $2.0\pm0.6 \times 10^{-2}$ % dose ml^{-1}. The corresponding value in 10 subjects studied with ^{99m}Tc-pertechnetate was found to be $2.8\pm0.8 \times 10^{-2}$ % dose ml^{-1}. In the present study, the mean ($\pm$sd) simulated concentration in 15 subjects was $2.2\pm0.5 \times 10^{-2}$ % dose ml^{-1} for iodide and $3.0\pm0.8 \times 10^{-2}$ % dose ml^{-1} for pertechnetate, both at 1 minute after intravenous administration of the tracer. The difference between these simulated and observed values is not significant ($p > 0.4$).

Systematic errors. Since the original studies of arterio/venous differences (28, 74) revealed considerable variation from subject to subject, there is likely to be variable error in using the present approximation. It was possible, however, to quantify the error from this source by noting the effect on the analysis of changes in magnitude of the arterio/venous correction. A number of data sets were analysed before and after using extreme values of the observed arterio/venous ratios (Table 2) in the arterial approximation programme. The resulting mean differences in the estimates of unidirectional clearance, exit rate and binding rate are summarised in Table 3. Differences are expressed as a percentage of the result with the normal arterio/venous

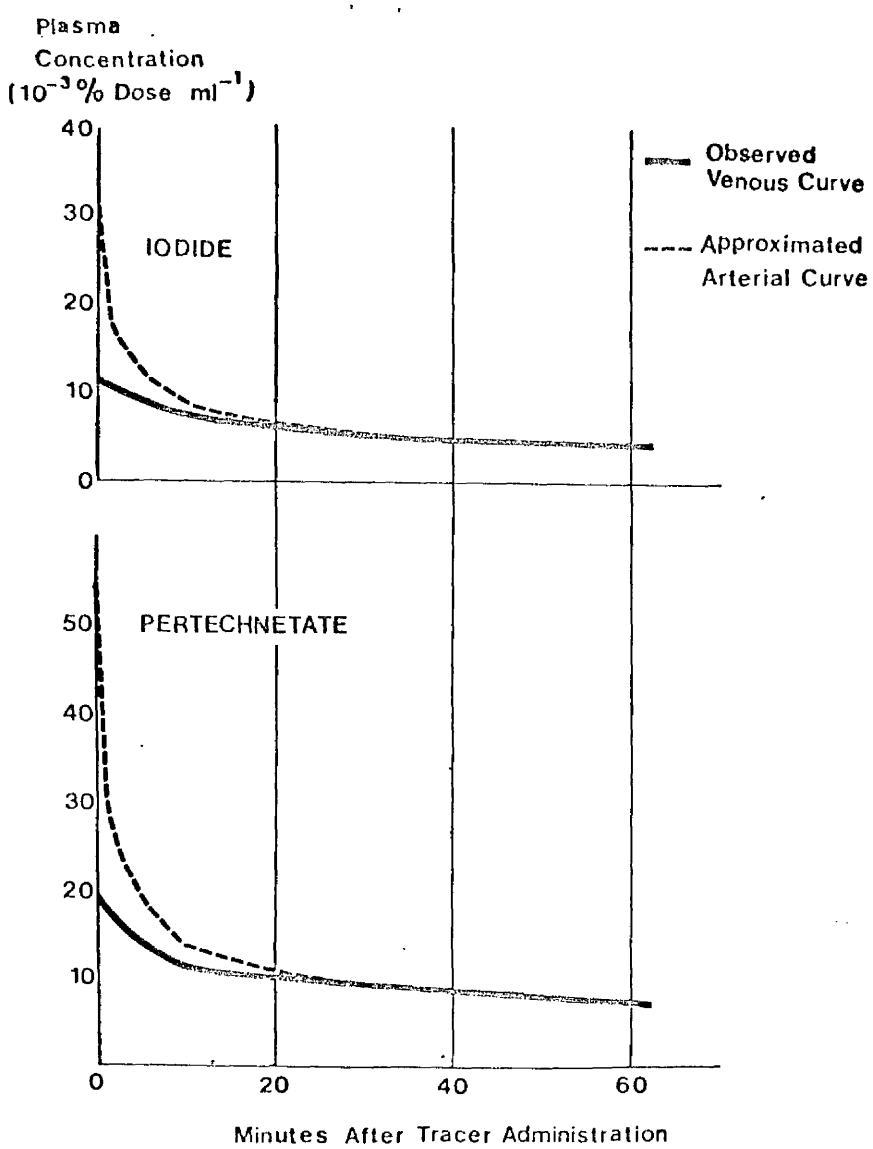


Figure 3.1 Application of the arterial approximation to the curve derived from observed tracer levels in venous plasma.

TABLE 2

OBSERVED ARTERIO/VENOUS RATIOS OF TRACER CONCENTRATION IN PLASMA AT VARIOUS TIMES AFTER INTRAVENOUS ADMINISTRATION OF EITHER ^{131}I - IODIDE OR ^{99m}Tc-PERTECHNETATE

Time after administration of tracer (min)	Mean ($\pm$ sd) arterio/venous ratio (10 subjects)
1	2.13 $\pm$ 0.65
2	1.77 $\pm$ 0.39
6	1.32 $\pm$ 0.20
10	1.23 $\pm$ 0.17
20	1.14 $\pm$ 0.13

TABLE 3

EFFECT ON PARAMETER ESTIMATES OF USING EXTREME ARTERIO/VENOUS RATIOS IN THE ARTERIAL APPROXIMATION. DIFFERENCES ARE EXPRESSED AS PERCENTAGES OF THE ESTIMATED VALUES WITH THE NORMAL ARTERIAL CORRECTION

	Difference in unidirectional clearance (%)	Difference in exit rate (%)	Difference in binding rate (%)
Upper limits of arterio/venous ratios applied	-18	-3	+35
Lower limits of arterio/venous ratios applied	+48	+23	-46

correction. Results for iodide and pertechnetate have been combined.

The results in Table 3 show the effect of either overestimating or underestimating the true arterial level by the widest possible margin. When the true arterial level is badly overestimated, then both unidirectional clearance and exit rate will be underestimated, by 48% and 23% respectively. The binding rate will be overestimated by 46%. In the case of the true arterial level being badly underestimated by the approximation, then both unidirectional clearance and exit rate will be overestimated, by 18% and 3% respectively. The binding rate will be underestimated by 35%.

These errors are relatively large when compared to the estimated random errors (Chapters 5-7) but must be accepted for the practical convenience of avoiding arterial sampling. It is unfortunate that radioactivity levels in the cardiac area are found not to reflect arterial tracer concentration (28). However, many of the investigations reported here are studies of the changes in tracer kinetics in individual subjects, so the effect of systematic variations is reduced. Furthermore, provided a sufficient number of cases is involved mean estimated values for the various parameters in a given thyroid state should be reasonably accurate.

3.3 Initial Conditions

An integral feature of the theoretical analysis (Section 2.2) is the facility whereby allowance can be made for uncertainty about plasma radioactivity levels immediatly after the tracer is given. This entails

setting starting values for the free and bound components of the simulated uptake curve, not at time zero, but after a time T. In theory, T could be set to 20 minutes which would obviate the need for the arterio/venous approximation described in the previous section.

Initial experience soon revealed, however, that, no matter the value given to T, realistic values for the initial free and bound components of uptake, $U_F(T)$ and $U_B(T)$, respectively, would not evolve from the analysis. It was apparent that the problem lay not in the value given to T, but in giving freedom to both $U_F(T)$ and $U_B(T)$. Thus some constraint had to be placed on one (or both) of these parameters.

Various manoeuvres were investigated but only one was found satisfactory. That consisted of constraining $U_B(T)$ to be zero at T=0. No restriction is placed on $U_F(0)$, the initial free uptake. The "least sum ofsquares" analysis provides an estimate of that parameter.

Implied in this procedure is the hypothesis that phenomena may occur immediately at the start of the thyroidal uptake process, which cannot be accounted for by the approximated arterial tracer levels. For example there may well be "instantaneous" uptake due to the passing of an initial bolus of tracer through the thyroid. The occurrence of very rapid uptake (within the first minute) has already been reported (28, 31, 46). There may also be a brief period of diminishing unidirectional clearance as plasma binding of the tracer (Section 3.1) is completed. The net effect would be an apparent "instantaneous" uptake, too large to be explained by the final unidirectional clearance. Any such "instantaneous" uptake is taken to be entirely free, there being no bound component.

Care has to be taken not to include the point (0,0) in the "least sum of squares" analysis. Inclusion of that point would contradict the implied hypothesis. Indeed, satisfactory fits to observed data were not achieved in the present work when the point (0,0) was included. Similar problems have been encountered in studies where an anologue computer was used for the purpose of curve fitting (28).

3.4 Release of Organified Tracer

Reservations have been expressed about the validity of neglecting the release of organified radioiodine from the thyroid, even at early times after administration of the tracer (16, 23). Indeed, it has been suggested that there is no practical period during which some labelled hormone is not released (16). Experimental evidence on the subject has revealed that labelled hormone begins to leave the gland within 2 hours in normal subjects (11), and within 1 hour, even as early as 15 minutes, in thyrotoxic subjects (12, 13). However, whether any steps should be taken in practice to allow for this, depends on the hormone release rate and concentration of labelled hormone in plasma.

There have been a number of studies of the release of organified radioiodine from the thyroid, with the results expressed in terms of the fractional release rate or biological half-life of iodine in the gland (11, 15, 16, 75-77). One problem in experimental determination of either parameter is that recycling of the tracer has to be prevented by administration of antithyroid drugs, which in themselves

may increase the release rate from the thyroid (15, 16, 78, 79). Thus the values given in the literature for the release rate of organified iodine may be overestimates. This means that the following argument could well be weighted against the case for neglecting the loss of organified tracer.

The most rapid turnover of intra-thyroidal hormone occurs in thyrotoxic subjects and, when results are expressed in terms of a fractional loss rate, the range of reported values in thyrotoxicosis is 8.5×10^{-6} - 1.6×10^{-4} $minute^{-1}$ (11, 15, 16, 75-77). Thus even the maximum reported value is at least 2 orders of magnitude less than any of the reported values for the exit rate of iodide (Table 1). Consequently, whenever the bulk of thyroidal uptake is in unbound form, loss of organified tracer from the gland will be negligible compared to the loss of free tracer.

In the normally binding gland, however, radioiodide is likely to be rapidly organified (Section 1.12), leaving a negligible proportion in free form. Under these circumstances loss of organified tracer may well be the greater. Whether this loss will seriously affect thyroidal uptake of tracer, depends on its relationship to the input of tracer. To clarify the issue, a simple calculation was performed on kinetic data from an extremely thyrotoxic subject, applying the maximum reported value for the fractional loss rate of thyroid hormone. The estimated loss of organified tracer tracer was only 4.5% of the input rate of tracer at 60 minutes, increasing to 27% at 150 minutes.

The matter was further investigated by empirical measurement of the organified tracer content of plasma. These studies revealed that, even in thyrotoxic subjects, the proportion of organified tracer

to total tracer concentration in plasma is unlikely to exceed 4% at 60 minutes.

Thus theoretical and expirical investigation has revealed that for studies of 60 minutes duration, or less, the release of organified tracer may be ignored. Any effect of this process on the shape of the thyroidal uptake curve, and on plasma radioactivity concentration is likely to be negligible. However, for longer studies, the loss of organified tracer in extreme cases may have a significant effect during the latter part of the study.

3.5 Optimum Time Interval for Data Collection

An empirical approach was employed to determine whether computed parameter values are dependent upon the duration of the thyroidal uptake study. Use was made of several data sets which were acquired over the periods 0-60 and 0-150 minutes after intravenous administration of the tracer. Analysis was performed in each case using the complete data set and again with later data points removed. In this way it was possible to compare three different time intervals, namely, 0-30, 0-60 and 0-150 minutes. Data in the 0-150 minute studies were too sparse to permit comparison of these three time intervals within the same data sets.

Results for analysis of pertechnetate data from the intervals 0-60 and 0-150 minutes are compared in Table 4. Values are given in the table for unidirectional clearance, exit rate, binding rate and initial free uptake (Section 3.3). A similar comparison for iodide data from the normally binding gland is presented in Table 5. In this

TABLE 4

ANALYSIS OF PERTECHNETATE UPTAKE DATA FROM THE INTERVALS 0-60 AND 0-150 MINUTES AFTER INTRAVENOUS ADMINISTRATION OF THE TRACER

SUBJECT	Unidirectional clearance ml min^{-1}		Exit rate min^{-1}		Binding rate min^{-1}		Initial free uptake % dose	
	0-60 min	0-150 min	0-60 min	0-150 min	0-60 min	0-150 min	0-60 min	0-150 min
M.R.	46.1	47.6	0.060	0.063	0.0015	0.0017	7.93	7.91
S.S.	46.1	46.0	0.027	0.027	-0.0006	-0.0009	1.36	1.33
H.L.	112.5	118.2	0.075	0.078	0.0011	0.0009	-1.04	-1.68
D.McM.	46.6	50.3	0.055	0.066	-0.0033	-0.0004	-0.57	-0.66
A.McK.	107.3	97.8	0.049	0.042	0.0021	0.0010	5.44	6.21
W.C.	135.8	121.4	0.030	0.026	0.0016	0.0007	13.16	13.85
D.N.	25.3	22.9	0.046	0.037	0.0052	0.0018	-0.45	-0.13
M.McL	19.0	18.7	0.031	0.030	0.0007	0.0008	4.36	4.36

TABLE 5

ANALYSIS OF IODIDE UPTAKE DATA (BINDING UNINHIBITED) FROM THE INTERVALS 0-60 AND 0-150 MINUTES AFTER INTRAVENOUS ADMINISTRATION OF THE TRACER

SUBJECT	UNIDIRECTIONAL CLEARANCE ml min^{-1}		INITIAL FREE UPTAKE % dose	
	0-60 min	0-150 min	0-60 min	0-150 min
C.H.	18.8	21.6	-0.33	-1.02
E.McP.	17.7	14.5	0.04	1.11
A.W.	30.7	34.3	-0.07	-0.84
S.S.	85.7	81.8	0.89	1.80
E.L.	160.6	164.2	1.93	1.33
M.R.	123.5	120.1	2.52	3.30
R.D.	409.4	395.7	9.87	11.52
W.C.	471.3	503.1	2.52	-0.32

case, however, values are given only for unidirectional clearance and initial free uptake (Section 3.7). Results for pertechnetate data acquired over the intervals 0-30 and 0-60 minutes are compared in Table 6. Similar results for iodide data are compared in Table 7.

Statistical analysis of these results was carried out using the Wilcoxon rank test for pair differences (80). There is no significant difference ($p > 0.1$), in the case of either ion, between the results for the intervals 0-60 and 0-150 minutes. The same is true of all parameters, except the exit rate of pertechnetate ($p < 0.05$), for the time intervals 0-30 and 0-60 minutes. Estimates of the exit rate of pertechnetate (Table 6) can be seen to be greater, by approximately 15% on average, for data acquired over the shorter time interval.

These results suggest that a detectable systematic error is likely to be introduced only when the study period is restricted to 30 minutes or less. Studies of 60 minutes duration, and greater, are likely to produce virtually identical results. It would appear that, for present purposes, there is no need to extend study of thyroidal uptake beyond 60 minutes after the tracer is given.

3.6 Assessment of Weighting Factors

"Weighted least sum of squares" analysis requires each data point to be weighted by a factor which is usually taken to be the inverse of the absolute variance (Section 2.2). In the present work, the data to be analysed consist of estimates of thyroidal uptake which are subject to random error from a number of sources (Chapter 4). These include factors such as patient movement and variation in

TABLE 6

ANALYSIS OF PERTECHNETATE UPTAKE DATA FROM THE INTERVALS 0-30 AND 0-60 MINUTES AFTER INTRAVENOUS ADMINISTRATION OF THE TRACER

SUBJECT	Unidirectional clearance ml min^{-1}		Exit rate min^{-1}		Binding rate min^{-1}		Initial free uptake % dose	
	0-30 min	0-60 min	0-30 min	0-60 min	0-30 min	0-60 min	0-30 min	0-60 min
M.A.	61.2	62.1	0.062	0.062	0.0079	0.0053	2.06	2.00
D.K.	72.7	79.9	0.044	0.052	-0.0013	0.0020	5.59	5.36
D.W.	440.7	387.6	0.110	0.093	0.0059	0.0040	22.60	23.90
M.C.	271.0	256.7	0.120	0.110	0.0050	0.0030	9.98	10.38
C.McD.	24.2	22.8	0.079	0.068	0.0097	0.0035	0.45	0.54
D.W.	112.4	105.5	0.052	0.043	0.0120	0.0050	0.26	0.68
S.D.	109.7	90.3	0.180	0.130	0.0160	0.0069	0.01	0.73
L.McL.	951.9	857.8	0.270	0.233	0.0062	0.0041	-0.59	1.04

TABLE 7

ANALYSIS OF IODIDE UPTAKE DATA (BINDING UNINHIBITED) FROM THE INTERVALS 0-30 AND 0-60 MINUTES AFTER INTRAVENOUS ADMINISTRATION OF THE TRACER

SUBJECT	UNIDIRECTIONAL CLEARANCE ml min^{-1}		INITIAL FREE UPTAKE % dose	
	0-30 min	0-60 min	0-30 min	0-60 min
J.P.	69.7	67.6	1.03	1.48
M.C.	100.8	109.1	0.84	-0.47
D.W.	27.9	29.5	2.85	2.49
A.McG.	319.1	324.4	2.02	1.57
D.K.	170.7	177.0	-1.54	-2.24
M.C.	218.9	218.1	2.42	2.49
J.J.	82.7	86.0	1.05	0.51
J.H.	49.7	51.4	-0.34	-0.60

in physiological activity during the course of the study.

The major source of error, however, is likely to be statistical variation in thyroidal and background radioactivity. Unlike other sources of error, the variance arising from this source can be determined readily (Sections 4.2 and 4.3). It was decided therefore to perform some preliminary analyses before and after applying "count rate" variances. The outcome would provide some indication as to whether weighting factors would have a significant effect on the estimated parameter values.

These exploratory studies revealed that application of "count rate" variances had little effect on the results of the "least sum of squares" analysis. Two typical examples are presented in Table 8. The first demonstrates the minimal effect of weighting in a set of pertechnetate data, while the second shows a similarly small effect in iodide data from a patient with defective binding. Variances differed by factors of five and two, respectively, within the two sets of data.

Since other sources of error are unlikely to contribute markedly to the overall variance, these findings suggest that there is no practical necessity for weighting in the present studies.

3.7 Practical Effect of High Binding Rate on Data Analysis

In the earlier theoretical discussion (Section 2.5) it was shown that if the binding rate is much greater than the exit rate, then only the unidirectional clearance and sum of the intial free and bound uptake have unique solutions. The latter is modified when, as a practical necessity, the initial bound uptake is constrained to have a

TABLE 8

EFFECT OF WEIGHTING ON THE OUTCOME OF LEAST SUM OF SQUARES ANALYSIS

		Unidirectional clearance (±se) ml min^{-1}	Exit rate (± se) min^{-1}	Binding rate (±se) min^{-1}	Initial free uptake (±se) % dose
TcO_4^-	Unweighted	30.6 ± 1.1	0.153 ± 0.006	0.0057 ± 0.0004	0.35 ± 0.09
	Weighted	29.4 ± 1.0	0.147 ± 0.005	0.0056 ± 0.0005	0.44 ± 0.08
I^-	Unweighted	84.9 ± 4.0	0.040 ± 0.005	0.052 ± 0.003	1.04 ± 0.23
	Weighted	86.9 ± 4.1	0.043 ± 0.006	0.053 ± 0.004	0.94 ± 0.24

value zero (Section 3.3). In these circumstances the initial free uptake has a unique solution. These consequences of a relatively high binding rate are manifested by the sum of squares function having the same minimum value for all exit and binding rates satisfying the condition $K_B \gg K_{TP}$.

When the new method of analysis was applied to iodide data from the binding gland, computational problems were immediately encountered. Full details are given later (Chapter 5). However it suffices to state at this stage that the surface near the minimum of the sum of squares, in exit/binding rate space, was very flat. In quantitative terms, the ratio of the minimum sum of squares, when the exit rate is zero, to that at the true minimum was never greater than 3.07 in all cases investigated. As a consequence, the estimated parameter values at the true minimum had large random errors.

These findings confirm that the binding rate of iodide is normally much greater than the exit rate (Section 1.12). Thus only unidirectional clearance and initial free uptake may be evaluated. A lower limit of 0.150 $minute^{-1}$ for the binding rate of iodide in the uninhibited gland is estimated in Section 5.5.

3.8 Effect of Intravenous Sodium Perchlorate

Intravenous sodium perchlorate was used to block thyroidal uptake in the discharge studies undertaken in the present work. This meant that the problems of incomplete gastro-intestinal absorption and delay in effect (81, 82), associated with the use of oral perchlorate, were avoided.

Various workers have used intravenous sodium perchlorate to block uptake of iodide or pertechnetate by the thyroid gland and other tissues (28, 56, 81-85). Doses have ranged from 50-450mg, the largest dose being that found necessary, in brain scanning, to completely block uptake of pertechnetate by the choroid plexus (82).

Blocking of thyroidal uptake. Observations early in the present work threw some light on the question of appropriate dosage for effective thyroidal blocking. Initially, a dose of 300 mg was selected for use in studies to determine the extra-thyroidal neck activity seen by an uptake counter (Section 4.2). In the first nine subjects, who were all thyrotoxic, neck radioactivity decreased continually throughout the observation period in all cases except one. This was true for both iodide and pertechnetate. Thus in eight cases, 300 mg sodium perchlorate effectively blocked thyroidal uptake. In the subject who did not demonstrate a falling neck count (who in fact had the highest uptake previous to the extra-thyroidal activity study), it was estimated that the unidirectional clearance had been reduced by a factor of 13 by the perchlorate. The study was repeated giving a dose of 600 mg sodium perchlorate, and the usual pattern of falling neck activity was observed.

Further information on the efficacy of intravenous perchlorate was obtained from discharge studies in four subjects who were given two doses of perchlorate. All four subjects were thyrotoxic, two were on antithyroid drug therapy and the remaining two were untreated. The first dose of perchlorate (300 mg) was given 60 minutes after the simultaneous administration of radioiodide and ^{99m}Tc-pertechnetate. A second dose of 600 mg sodium perchlorate was given 30 minutes later. Analysis of the discharge phase was performed as described previously (Section 2.6).

As can be seen from Table 9, the residual uptakes after the first dose of perchlorate were similar to those after the second dose. The observed differences for each anion are not significant ($p > 0.2$) on the basis of the Student t test for pair differences (80). Thus the first dose of 300 mg seems to have effectively discharged most of the available tracer from the gland.

When all these results are considered, it would appear that, in most cases, 300 mg sodium perchlorate will completely block thyroidal uptake. Only in exceptional circumstances will the dose require to be increased to 600 mg.

In the present work, the smaller dose (300mg) was used in discharge studies. This was considered necessary because many of the investigations were repeated at frequent intervals. It seemed prudent to restrict the dosage of perchlorate to minimise the possible risk of any cumulative effect on gland function. The larger dose (600 mg) was used in all future estimations of extra-thyroidal activity.

Binding rate after perchlorate. Up till recently, it has been customary to assume that in such doses perchlorate has little effect on the binding function of the thyroid gland (1, 86). (In much greater doses perchlorate may well affect binding if extrapolation is made from the studies of Greer et al (57) in rats. They found that with the lowest dosage tested - 25mg sodium perchlorate to a rat weighing 100 g - binding was inhibited by approximately 40%). However Gray (28) made the original postulate that perchlorate in normal doses reduces the binding rate to negligible levels in subjects with binding defects, but has no significant effect when binding is normal.

TABLE 9

DISCHARGE STUDIES WITH TWO DOSES OF INTRAVENOUS SODIUM PERCHLORATE

THYROID STATUS	IODIDE Residual (±se) % dose		PERTECHNETATE Residual(±se) % dose	
	After 300 mg $NaClO_4$	After a further 600 mg $NaClO_4$	After 300 mg $NaClO_4$	After a further 600 mg $NaClO_4$
Thyrotoxic on antithyroid drugs	8.21 ± 0.54	7.12 ± 0.40	2.30 ± 0.15	1.97 ± 0.10
Thyrotoxic on antithyroid drugs	4.71 ± 0.52	4.35 ± 0.23	3.30 ± 0.43	2.62 ± 0.18
Untreated thyrotoxic	No discharge		0.28 ± 0.54	0.74 ± 0.20
Untreated thyrotoxic	No discharge		6.98 ± 0.32	6.21 ± 0.31

Experimental evidence for this was reputed to have been found in comparative measurements of the discharge rate for iodide and pertechnetate in the post-perchlorate phase. It can be shown theoretically (Section 2.6) that the discharge rate for iodide should be greater owing to the presence of a binding rate factor. No such relative increase was found and it was concluded that the binding rate in the perchlorate discharge phase was negligible.

The present work shows (Chapter 7), however, that typical binding rates for iodide in patients with binding defects are small compared to the total discharge rate in the perchlorate phase. These would not be expected to produce any significant difference in the relative discharge rates between iodide and pertechnetate. In this present work, therefore, binding rate is assumed not to be affected by perchlorate.

3.9 Summary

As a consequence of these considerations and exploratory studies, a working procedure was finally adopted which provided a reliable and useful means of investigating the early uptake kinetics of iodide and pertechnetate. The essential details are as follows.

(1) For both anions, thyroid uptake data are collected over the time interval 0-60 minutes after intravenous administration of the tracer. Longer studies would require, where appropriate, correction for loss of organified tracer (Section 3.4). On the other hand, shorter studies could lead to systematic error in the

parameter estimates (Section 3.5).

(2) Venous blood samples are taken at 2, 10, 20, 30, 40, 50 and 60 minutes, and estimations made of the tracer concentration in plasma. A curve consisting of two exponential components is fitted to the plasma data. This enables an approximate arterial plasma curve, consisting of three exponential components, to be constructed (Section 3.2).

(3) The uptake data, with no weighting factors applied (Section 3.6), and the approximated arterial data are analysed by the methods described in Chapter 2. However, two modifications are made - both the delay time, T, and the initial bound uptake, $U_B(T)$, are constrained to have the value zero (Section 3.3). The point (0,0) is not included in the analysis (Section 3.3).

(4) When analysis of radioiodide data is complete, the minimised sum of squares with the exit rate, K_{TP}, equal to zero is compared with the true minimum value. If the ratio is ≤ 3.07, the parameter estimates at the minimum are rejected (Section 3.7). The optimum unidirectional clearance and initial free uptake with $K_{TP}=0$ are accepted instead. In these circumstances the analysis indicates that the binding rate is much greater than the exit rate.

A lower limit of 0.150 $minute^{-1}$ is assumed for the binding rate of iodide in normal and untreated thyrotoxic subjects (Section 5.5).

(6) In discharge studies, sodium perchlorate is given intravenously in a dose of 300 mg (Section 3.8), 60 minutes after the tracer. Observations of thyroidal radioactivity are continued thereafter for 30 minutes. Binding is is assumed to remain unchanged. The discharge phase is analysed as described previously (Section 2.6).

CHAPTER FOUR

THE MEASUREMENT OF THYROIDAL UPTAKE

4.1 Introduction

A number of factors contribute to errors in the measurement of early thyroidal uptake of radioactive iodide or pertechnetate (87-90). These include statistical variations in source count rate and the problem of simulating thyroid position and anatomy for calibration purposes. One major problem is that of estimating the contribution from radioactivity in the blood vessels of the gland, and in other tissues, to the output of the detecting device.

In measurements with an uptake counter, different assumptions have been made with regard to extra-thyroidal activity (91). For example, the uptake over the thigh has been used in the estimation of extra-thyroidal activity (31, 42, 92, 93). Alternatively, it has been assumed that neck activity, at 2 minutes after injection of the tracer, is entirely extra-thyroidal and thereafter varies as blood activity (36, 94), or remains a constant fraction of total body radioactivity outwith the thyroid and kidneys (95).

The extra-thyroidal activity in the field of view of an uptake counter has been measured directly, by blocking thyroidal uptake by administration of Lugol's iodine (96), and indirectly, using a radioisotope scanner (88, 97, 98). These studies provided a set of mean values for routine use as approximations to the extra-thyroidal activity in individual subjects. Partial measurement of extra-thyroidal activity may be achieved by placing a lead block over the

thyroid to attenuate radiation arising from the organ (23, 99, 100).

Individual precise determination of extra-thyroidal activity is, however, important in kinetic studies (37), which has lead to the use of improved techniques for measurement of thyroid uptake. One approach, using an uptake counter, is to perform two consecutive studies, once before and again after blocking thyroidal uptake by the administration of perchlorate (49). Inaccuracies in this method can be reduced further if the field of view of the counter is restricted by special collimation so as to minimise the contribution from extra-thyroidal activity (28, 59). A different approach is to use an imaging device such as a profile (101-102) or rectilinear scanner (39, 41, 103-105), or a gamma camera (7, 40, 43-46, 48, 51, 85, 106-113). Areas adjacent to the thyroid may be used to estimate the contribution from extra-thyroidal activity to counts within the thyroid region.

Two methods of measuring thyroid uptake were used in the present work. Firstly, for sequential studies of the kinetic behaviour of iodide and pertechnetate in antithyroid drug therapy (Chapter 6) use was made of a sodium iodide scintillation counter fitted with an IAEA standard collimator (114). No attempt was made to modify the collimater since the studies were intended as a follow-up to previously reported work with the same counter (115, 116). Both ^{132}I-iodide (670 KeV) and ^{99m}Tc-pertechnetate (140 KeV) were studied simultaneously. The former radioisotope was used rather than ^{131}I, because the radiation dose to the thyroid is lower by a factor of 100 (117), and rather than ^{123}I (159 KeV), because the photon energy is clearly separated from that of ^{99m}Tc. Secondly, for studies with only one tracer, for example the investigation of iodine binding defects with ^{123}I-iodide (Chapter 7),

use was made of a gamma camera (Ohio-Nuclear Series 100) linked to a computer (Varian 620/L-100).

Details of these two methods and the experimental procedure for kinetic studies are given in this chapter.

4.2 The Uptake Counter and Results of Extra-Thyroidal Activity Studies

Method. The counter is placed at 8.5 cm from the neck with the patient in the supine position. Care is taken to exclude the salivary glands from the field of view. 50 uCi ^{132}I-iodide and 250 uCi ^{99m}Tc-pertechnetate in 2.5 ml isotonic saline are injected into the antecubital vein and a series of counts are taken, each with a preset time of 40 seconds. The first count is taken at 30 seconds, the second at 2 minutes and succeeding counts at 4 minute intervals until the end of the study. Each study is continued for 60 minutes or, if a subsequent perchlorate discharge test is required, a further 30 minutes (Section 3.9). Counts are recorded simultaneously in two energy intervals, 120-160 KeV for ^{99m}Tc, and 400-800 KeV for ^{132}I. An aliquot of the dose solution is placed in a neck phantom (88) and counted under the same conditions. A sample of pure ^{132}I is counted likewise to obtain the contribution from that radioisotope in the ^{99m}Tc energy interval. The neck counts for each tracer are then expressed in terms of percent administered dose, after appropriate correction for radioactive decay.

For determination of the true thyroidal uptake, a separate study is performed to estimate the extra-thyroidal contribution to these calculated neck uptakes. The same procedure is followed except that 600 mg sodium perchlorate are given intravenously (Section 3.8) 5 minutes before the tracers. With thyroidal uptake now completely

blocked, the resulting neck count (% dose) represents extra-thyroidal activity seen by the uptake counter (ETA).

To calculate thyroidal uptake in the original study without perchlorate, allowance has to be made for the fact that the extra-thyroidal activity will be less than ETA because of the presence of thyroidal uptake. It may be shown that thyroidal uptake (% dose) is related to neck uptake (% dose), in the absence of perchlorate, and ETA by

$$\text{Thyroidal uptake} = 100\ (\text{Neck uptake} - \text{ETA})/(100 - \text{ETA})$$

Inherent in this formula is the assumption that any changes in tracer kinetics within extra-thyroidal tissues, between the two studies, has no further effect upon the parameter ETA. It is well known that such changes do occur, since perchlorate inhibits uptake in those tissues where iodide and pertechnetate are normally concentrated, such as the salivary glands (1). The effect on plasma concentration may be variable (84, 118). However, with the salivary glands excluded from the direct field of view of the counter, the overall effect of these changes in tracer kinetics on ETA were considered to be minimal. (Collimator penetration, even for ^{132}I, was not a problem. When a thick lead block was placed over the open end of the collimator, the observed count was less than 1% dose).

Extra-thyroidal activity. It was considered worthwhile to combine all the results from extra-thyroidal activity studies with a view to providing an average curve of the variation of ETA with time, for each tracer. These would have a longer time span than curves for the period 0-30 minutes, previously published for the same uptake

counter (88, 97) and could be used in any future measurements of thyroidal uptake where high precision was not essential.

Extra-thyroidal activity of ^{132}I-iodide, in 9 thyrotoxic subjects, and of ^{99m}Tc-pertechnetate, in 8 of the same group of subjects, is plotted against time in Figures 4.1 and 4.2 respectively. Second order polynomial curves were fitted to these two sets of data by the method of "least sum of squares". Thus for ^{132}I-iodide,

$$ETA = 7.92 - 9.15 \times 10^{-2}t + 9.03 \times 10^{-4} t^2$$

and for ^{99m}Tc-pertechnetate,

$$ETA = 8.81 - 9.14 \times 10^{-2}t + 9.03 \times 10^{-4} t^2$$

where ETA (% dose) is the mean extra-thyroidal activity at any time, t (minutes), in the absence of uptake. The difference between these curves reflects the fact that in most cases the extra-thyroidal activity for ^{99m}Tc was greater by 0.5-1.0 % dose.

Venous blood samples were taken in 4 of the subjects to compare the fall in plasma radioactivity with that in extra-thyroidal activity. There was considerable discrepancy between the two curves during the first 30 minutes, the fall in plasma radioactivity being the greater. From 30 minutes onwards, however, both plasma and extra-thyroidal activity fell at approximately the same rate. This fact may be used in the calculation of extra-thyroidal activity at times greater than 60 minutes.

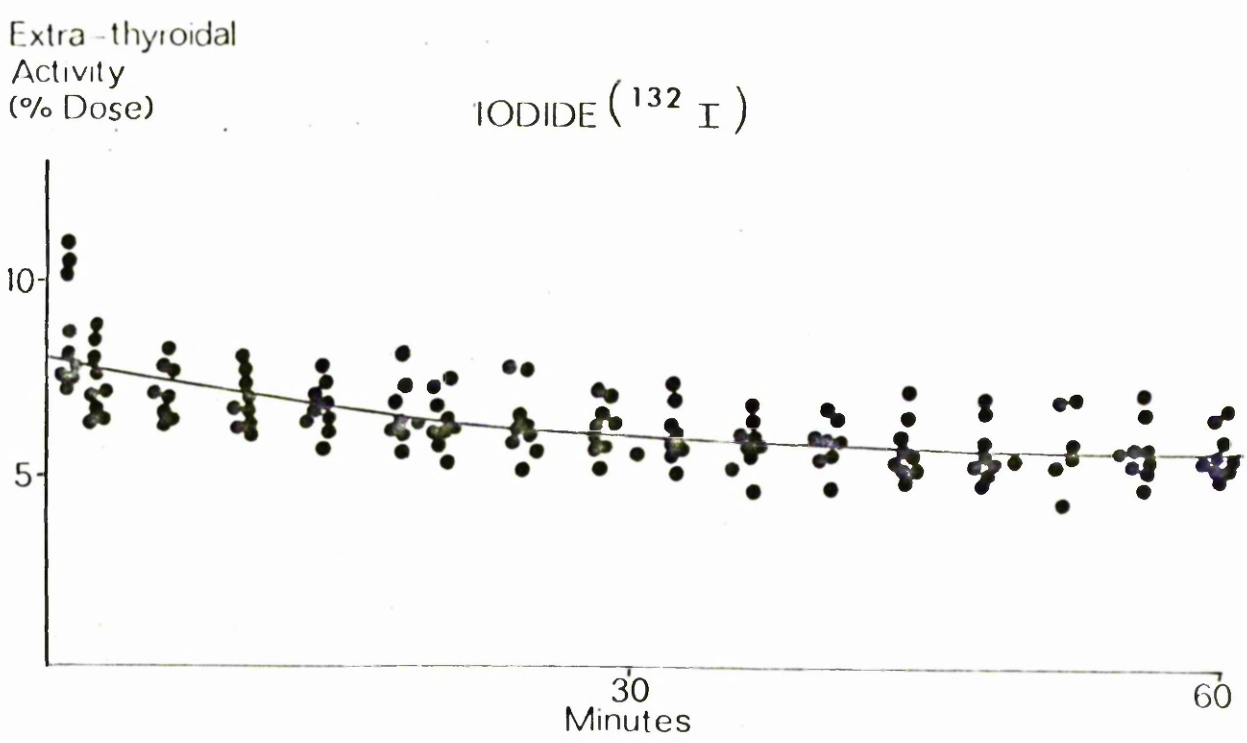


Figure 4.1 Extra-thyroidal activity of ^{132}I seen by the uptake counter in 9 thyrotoxic subjects given a blocking dose of perchlorate.

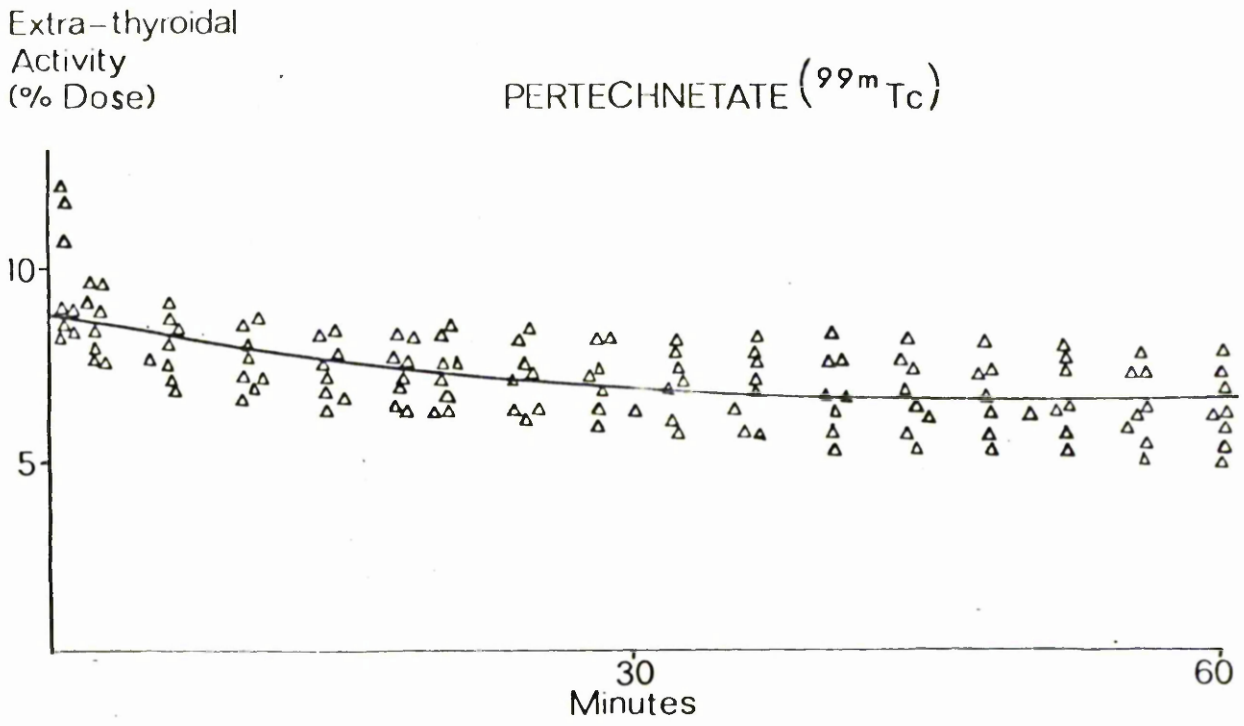


Figure 4.2 Extra-thyroidal activity of ^{99m}Tc seen by the uptake counter in 8 thyrotoxic subjects given a blocking dose of perchlorate.

The plasma levels for pertechnetate were consistently greater than those for iodide, which may explain the observation that the extra-thyroidal for the former was usually greater. That would be the case if much of the radioactivity in the field of view of the uptake counter were in blood rather than in extra-vascular space.

Counting errors. Unlike random errors arising from other sources, such as patient movement and physiological variation, those errors arising from statistical variation in radioactive decay may be calculated readily. These counting errors, in the case of ^{132}I, were estimated to be less than 4% for uptakes greater than 5% dose. In the case of ^{99m}Tc, the estimated error, over the same uptake range, was less than 2.5%. Subjects with thyroidal uptakes less than 5% dose were, as a rule, not studied with the uptake counter because such uptakes are small compared to the magnitude of extra-thyroidal activity.

The fact that the total random error was not estimated is of no practical disadvantage. A facility of the present method of analysis is that sample variance can be calculated from the observed data (Section 2.4). This enables parameter errors to be determined in the absence of variance estimates for each data point. (Individual variances were not required as weighting factors, because weighting was considered not to be necessary (Section 3.6)).

4.3 The Gamma Camera/Computer System

Use of ^{123}I-iodide. Because of the relatively low energy (159 KeV) of its principal emission and relatively short half-life

(13.3 hours) ^{123}I-iodide has favourable characteristics for in vivo imaging studies (40, 119-124). However, the material, as available, contains a variable proportion of impurities (125). That from the MRC Cyclotron Unit (Hammersmith Hospital), which was used in the present work, contained comparatively high proportions of impurities at the time of production; 3% ^{124}I($T_{\frac{1}{2}}$ 4.2 days), 0.52% ^{125}I ($T_{\frac{1}{2}}$ 51.4 days) and 0.24% ^{126}I($T_{\frac{1}{2}}$ 13 days) (126). Because of transport difficulties, the material could not be used until the day following production, by which time the proportions of ^{124}I, ^{125}I and ^{126}I increase to 11%, 2.4% and 1.2% respectively. These impurities increase the radiation dose to the thyroid by a factor of 10, but even so the dose from impure ^{123}I-Iodide is still much less than that from ^{131}I-iodide (150 mrem/uCi compared to 1300 mrem/uCi) (117). However because the radiation dose is not inconsiderable, the amount of ^{123}I-iodide given to each subject was restricted to 150 uCi.

The presence of impurities - in particular ^{124}I which emits photons of energy 510 and 600 KeV - caused some further inconvenience as far as imaging with the gamma camera was concerned. Exploratory studies of a thyroid phantom revealed that, even with a high energy (400 KeV) collimator, significant counts were found outwith the thyroid image, as defined by the principal radiation of ^{123}I. This is the result of penetration of the collimator by the high energy photons from the ^{124}I contaminant. Other workers (124, 127-128) have reported similar degradation of image quality.

In practice, the net effect was that the region chosen for calculation of room background and extra-thyroidal activity, in the patient study, contained a contribution from activity in the thyroidal

area. Likewise in the calibration study, when a standard was assayed within the thyroid phantom, the area chosen for calculation of room background contained similar additional counts. Studies of the phantom revealed that these additional counts amounted to approximately 6% of the thyroidal counts. However, it was considered that if the background regions are similarly placed with respect to the thyroidal area, the spill-over fractions in patient and phantom are likely to be equal. Any systematic error in the calculation of thyroid uptake, resulting from collimator penetration of the photons from the ^{124}I contaminant, may thereby be minimised.

Choice of background area. Essential to the measurement of thyroidal uptake using a gamma camera, is the selection of a suitable background area in the scintigraph from which extra-thyroidal activity in the thyroidal area may be estimated. Some workers have chosen an area immediately inferior to the gland (85, 106, 108-111), while others have chosen areas both inferior and superior to the gland (40, 46, 51). As a rule, the choice of background area in these studies was based on studies of athyreotic subjects, or of subjects given perchlorate to block thyroid uptake.

Recently, Armstrong et al (46) have suggested that, in thyrotoxic subjects, the gland may be much more vascular than those adjacent tissues normally used in the estimation of extra-thyroidal activity. They considered that activity of ^{99m}Tc-pertechnetate in the thyroidal area, which was not true uptake, could as a result be seriously underestimated; in other words thyroid uptake could be overestimated. It is not clear, however, whether any background studies had been undertaken in thyrotoxic subjects who had received a blocking dose of

perchlorate. In view of this, the present work included some further studies of tissue radioactivity in the neck region, when thyroidal uptake was absent, in a group of subjects of varying thyroid status.

Five subjects were investigated, 2 were thyrotoxic, 1 was euthyroid and the remaining 2 were athyreotic. The first 3 were given ^{99m}Tc-pertechnetate intravenously, a few minutes after an intravenous blocking dose of 600 mg sodium perchlorate (Section 3.8); the last 2 received ^{123}I-iodide but did not require perchlorate. Radioactivity in the neck region was monitored continuously on the gamma camera/computer system for at least 15 minutes. Data were integrated over successive periods of either 30 seconds, in the case of ^{99m}Tc, or 120 seconds, in the case of ^{123}I, and recorded on magnetic tape. At analysis, several background areas were compared with the thyroidal area, after correcting for non-uniform camera response.

The most satisfactory background area was one of approximately one third of the size of the thyroidal area. It lay just inferior to the gland and was slightly displaced relative to the mid-line, away from the site of administration of the tracer. The eccentric position of this background area meant that very little of the image of the injection bolus, as it passed through the appropriate subclavian vein (129), was included. This area gave the best correspondence with the thyroidal area, especially in the period immediately after administration of the tracer.

The similarity between counts in the thyroidal and background areas is shown by the results presented in Table 10. All counts have been normalised to the same area (that of a normal thyroid image) and

TABLE 10

COMPARISON OF NORMALISED COUNTS WITHIN THE THYROIDAL AND BACKGROUND AREAS IN SCINTIGRAPHS OF THE NECK REGION; thyroidal uptake either blocked by perchlorate or absent due to thyroidal malfunction.

Time after tracer administration (minutes)	^{99m}Tc Counts (% dose)						^{123}I Counts (% dose)			
	Thyroid	Background	Thyroid	Background	Thyroid	Background	Thyroid	Background	Thyroid	Background
0.25	3.05	4.45	2.86	3.25	1.48	2.55				
0.75	2.44	2.82	2.32	2.09	1.90	2.25				
1	2.29	2.58	2.09	2.01	1.83	2.20	2.36	3.24	3.91	3.60
3	2.07	2.41	1.99	1.86	1.81	1.93	2.30	2.42	3.50	3.13
5	2.04	2.23	1.94	1.77	1.74	1.87	2.20	2.28	3.29	3.11
10	1.97	2.19	1.85	1.67	1.70	1.78	2.09	2.06	3.21	2.71
15	1.93	2.09	1.82	1.61	1.68	1.72	2.07	2.02	3.06	2.69
20					1.66	1.70	2.03	1.97	3.01	2.56
30					1.63	1.66	1.92	1.88	2.97	2.50
40					1.57	1.63	1.82	1.71		
50					1.55	1.62	1.75	1.65		
	Thyrotoxic		Thyrotoxic		Euthyroid		Athyreotic		Athyreotic	

expressed as a percentage of the administered dose. As can be seen, the background area provides at all times, except the earliest, an estimate of the activity of the thyroidal area to within 0.5% dose. Observed differences at any time, in the data as a whole, are not significant ($p>0.05$) by the Student t test for pair differences (80).

It is of interest to note that, even in the thyrotoxic subjects, the background was satisfactorily estimated. This casts doubt upon the deductions of Armstrong et al (46) with regard to the relative vascularities of the thyroid gland and adjacent tissues.

Method. Use is made of two different parallel hole collimators, a low energy, high resolution one for ^{99m}Tc studies and a high energy (400 KeV) collimator for ^{123}I studies. Energy intervals of 110-170 KeV and 130-190 KeV are chosen for ^{99m}Tc and ^{123}I respectively. The subject is placed in the supine position with the gamma camera at 12 cm from the neck. A dose of 1 mCi ^{99m}Tc-pertechnetate, or 150 uCi ^{123}I-iodide, in a volume of 5 ml isotonic saline, is injected into the antecubital vein and the changes in neck radioactivity monitored using the gamma camera/computer system. The study is continued for either 60 minutes, or 90 minutes, if a perchlorate discharge test is required (Section 3.9). At the end of the study, an aliquot of the administered dose is placed in a neck phantom (Section 4.2) and data recorded for a few minutes.

For ^{99m}Tc, data are integrated over successive periods (frames) of 30 seconds, starting at the time the tracer is given. However, because of restriction on the administered dose, the frame time for ^{123}I is 120 seconds. Recording of the data in the case of ^{123}I is delayed

by 30 seconds to avoid possible problems in estimating extra-thyroidal activity in the first frame. (Exploratory studies revealed that, even with the background area placed eccentrically, there could be a significant contribution from the injection bolus).

The first step in the analysis programme is correction of each data frame for non-uniform response of the camera. This makes use of the image of a uniform flat source of ^{99m}Tc-pertechnetate. Counts in each frame, attributable to thyroid uptake, are then determined from the thyroidal area, after subtracting the normalised extra-thyroidal counts. Thyroid uptake, in terms of percent administered dose, is calculated using the net standard counts from the phantom study, with appropriate correction for radioactive decay. The time of each measurement is considered to be the time from administration of the tracer to the mid-point of the data frame.

Counting errors. As in the case of measurements with the uptake counter only counting errors due to statistical variation in source count rate were calculated. These errors were estimated to be less than 4% and less than 1.5%, respectively, for ^{123}I-iodide and ^{99m}Tc-pertechnetate uptakes of greater than 2% dose. Because of the lower contribution from extra-thyroidal activity, the gamma camera was the instrument of choice when thyroidal uptake was low. Thus errors were calculated for a wider range of uptakes than in the case of the uptake counter.

CHAPTER FIVE

COMPARTMENTAL ANALYSIS OF IODIDE AND PERTECHNETATE UPTAKE

5.1 Introduction

The review of published work in Chapter 1 has revealed shortcomings in previous compartmental analysis of iodide or pertechnetate uptake by the thyroid gland. Of considerable importance is the wide variation reported for the binding rate of iodide in the uninhibited gland (Table 1). Another feature is the virtual absence of information about the precision to which the various parameters were estimated.

The studies of Gray (Section 1.10) have gone some way towards explaining the wide range of binding rate estimates for iodide. His innovation was to use arterial radioactivity levels, rather than venous levels, in the calculation of clearance (Section 1.4). With that modification, the clearance of radioiode in the uninhibited gland was found to be constant with time, thus suggesting that the binding rate is much greater than the exit rate. Further indication that the binding rate is relatively high, i.e. at the upper limit of the range of reported values, emerges from his failure to detect dischargeable radioiodide at the early time of 10 minutes after administration of the tracer. However, the analysis employed in Gray's studies relied on graphical techniques and was subject to errors of human judgment. It would seem appropriate, therefore, to determine whether the findings are supported by improved digital analysis.

The method of analysis developed in the present work includes a correction for arterio/venous differences in plasma radioactivity levels

(Section 3.2) and also provision for the estimation of random errors (Section 2.4). Reported in this chapter is the outcome of applying the method to the results of simultaneous studies of iodide and pertechnetate uptake. A principal objective was to test whether "least sum of squares" analysis would produce results which were consistent with the findings of Gray concerning the binding rate of iodide. Furthermore it was hoped that the technique would be sufficiently sensitive to quantify, in terms of binding rate, the small extent to which pertechnetate is organified (3-7). The analysis would also allow comparison, in fundamental terms, of the kinetic behaviour of iodide and pertechnetate.

Many of the experimental data used in this exploratory study were those obtained and analysed previously by Robertson (27, 130). The present author has simply applied an improved kinetic analysis to those data which, because of the relatively long observation period (up to 2.5 hours), are extremely valuable.

5.2 Patients and Methods

Kinetic studies of iodide were performed in 15 untreated thyrotoxic and 8 normal subjects. The experimental data were acquired and first analysed by Robertson (27,130). He used a radioisotope scanning technique to measure thyroid uptake after an intravenous dose of 25 uCi ^{131}I-iodide. Scanning was started 30 seconds after injection of the tracer, with the duration of each scan being approximately 4 minutes, and continued for up to 150 minutes. Venous blood samples were taken at 2, 8, 18, 35, 105 and 150 minutes. The net thyroidal count in each scan was obtained by subtracting an estimate of the

tissue and room background which was based on an adjacent extra-thyroidal area. Comparison of this net count with that from the scan of a standard, placed within a thyroid phantom, allowed thyroid uptake to be estimated.

Fourteen of the thyrotoxic subjects and all the normal subjects received 1 mCi ^{99m}Tc-pertechnetate at the same time as the radioiodide. Thyroidal uptake of pertechnetate was measured in exactly the same way as of the latter, except that correction had to be made for the contribution from ^{131}I to the count within the ^{99m}Tc energy interval.

Studies of the short-term effect of the antithyroid drug, carbimazole (120 mg in divided oral doses over the 24 hours before the study), on thyroid uptake kinetics were also undertaken. Seven thyrotoxic subjects and 1 normal subject were investigated for drug-induced changes in iodide kinetics, and 7 thyrotoxic and 3 normal subjects for possible changes in pertechnetate kinetics. Investigations in all subjects, except 2, were carried out by Robertson (27, 130) using the radioisotope scanning method just described. Thyroid uptake of radioiodide in the remaining 2 subjects was determined by the present author using the gamma camera method described earlier (Section 4.3).

5.3 Analysis

As a first step in analysis of the data, an approximated curve of tracer concentration in arterial plasma was obtained from the measurements in venous plasma (Section 3.2). This entailed fitting a single exponential term to the venous plasma measurements from 30

minutes onwards and then, by the method of curve stripping, determining a further exponential term to complete description of the earlier part of the plasma curve. The approximated arterial curve, consisting of 3 exponential terms, could then by derived making use of the factors derived previously.

In most of the cases where the study extended to 150 minutes, the levels of plasma radioiodide from 30 to 150 minutes lay on the same straight line on log-linear graph paper. However there were 4 exceptions (all thyrotoxic subjects) where the radioiodide concentration at 150 minutes lay above the line through earlier points. This was considered to be the result of early discharge of labelled thyroid hormone into the circulation (Section 3.4). Thus, to minimise error in analysis of these cases, the measured plasma radioactivity at 150 minutes was excluded in estimation of the final exponential component of the venous radioiodide curve.

The observed thyroid uptake data were then combined with this approximated arterial curve and analysed on the basis of the open three-compartment binding model following the procedure outlined earlier (Section 3.9). For iodide data obtained from normal and and untreated thyrotoxic subjects, the minimised sum of squares with exit rate equal to zero was compared to the least sum of squares obtained without constraint on the exit rate.

Statistical analysis of the results was performed, whenever possible, using the non-parametric Wilcoxon rank test or the Wilcoxon rank test for pair differences (80). Relationships between parameters were tested by linear regression using the "t" statistic (80) to assess significance.

5.4 Results

The variation with time of thyroidal uptake radioiodide, before and after carbimazole, and of ^{99m}Tc-pertechnetate is illustrated by the example in Figure 5.1. A close similarity was noted in all cases between the uptake curves for pertechnetate, where binding is minimal, and for iodide in the presence of antithyroid drug.

Problems were immediately encountered in analysis of radioiodide data from the uninhibited gland. The parameter estimates at the true minimum of the sum of squares function had large errors and the values from case to case varied markedly (Table 11), apparently due to lack of curvature, in exit/binding rate space, of the minimised sum of squares function (Figure 5.2). This lack of curvature is quantified in Table 12, where the minimised value, i.e. with optimum unidirectional clearance and initial free uptake, when the exit rate is zero, is compared to the true minimum value of the function. As can be seen, the ratio of those values in 24 untreated thyrotoxic subjects (the present 15 and a further 9 from studies reported in Chapter 6) and 8 normal subjects, did not exceed 3.07. These results imply that the binding rate is much greater than the exit rate, and so neither can be estimated reliably. It follows that the analysis can provide estimates only for unidirectional clearance and initial free uptake (Section 3.7). In contrast, the sum of squares ratio for pertechnetate data, and for iodide data when binding was inhibited, was always greater than 3.07.

Results of the compartmental analysis, for both iodide and pertechnetate, in the 15 untreated thyrotoxic and 8 normal subjects are presented in Tables 13 and 14 respectively. The mean ($\pm$sd)

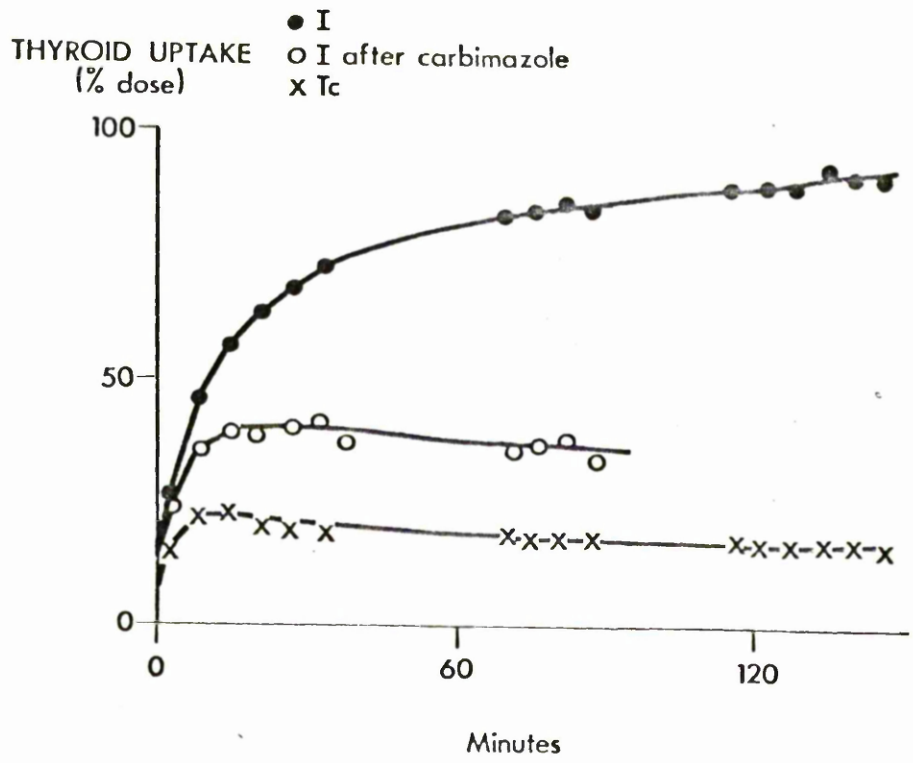


Figure 5.1 Thyroid uptake curves for ^{131}I, before and after carbimazole, and for ^{99m}Tc in a thyrotoxic subject.

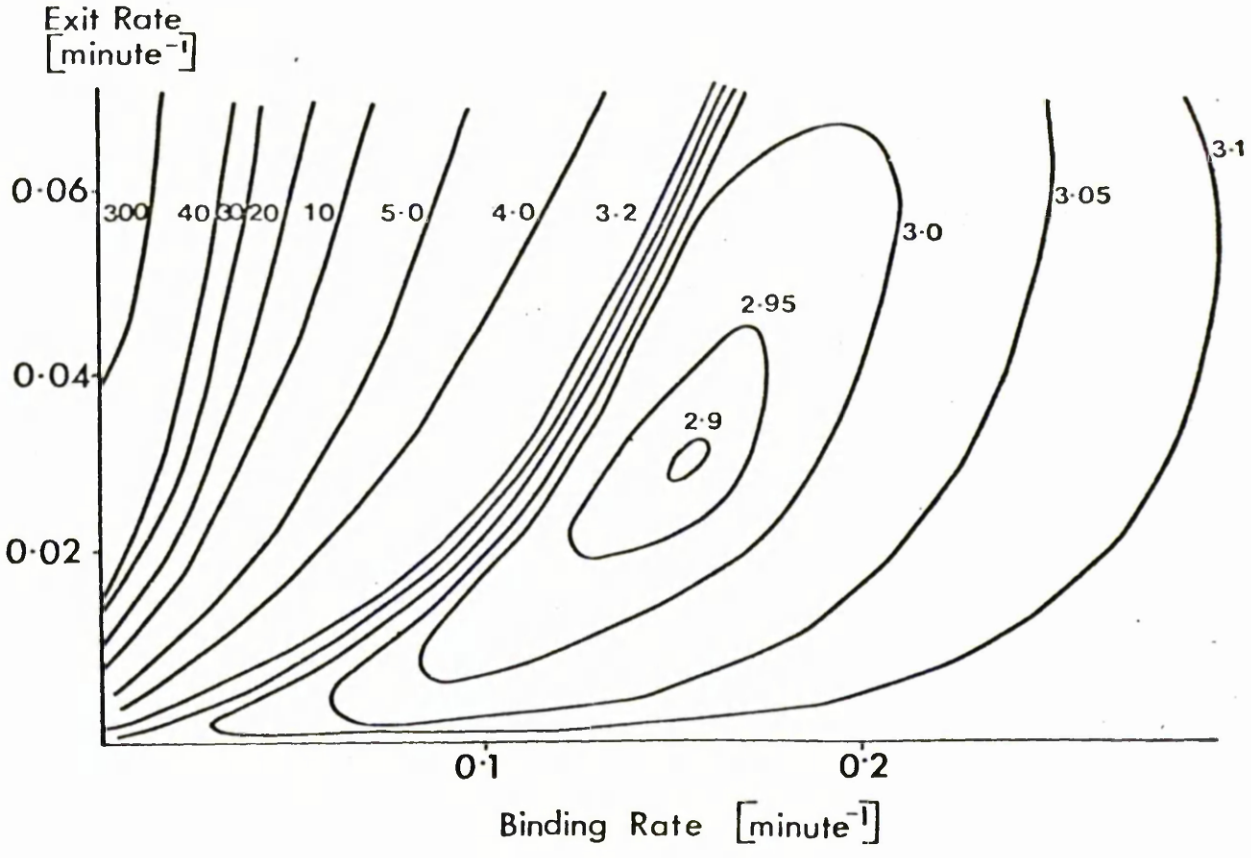


Figure 5.2 Contour map of sum of squares function in exit/binding rate space (radioiodide data from an untreated thyrotoxic subject).

TABLE 11

ANALYSIS OF RADIOIODIDE DATA FROM UNTREATED THYROTOXIC SUBJECTS: Parameter values at the minimum of the sum of squares function and optimum values of unidirectional clearance and initial free uptake with exit rate equal to zero

SUBJECT	PARAMETER VALUES (±SE) AT TRUE MINIMUM				OPTIMUM VALUES (±SE) WITH EXIT RATE=0	
	Unidirectional clearance, ml min^{-1}	Exit rate min^{-1}	Binding rate min^{-1}	Initial free uptake, % dose	Unidirectional clearance, ml min^{-1}	Initial free uptake, % dose
M.W.	6.8 ±36.2	-0.342 ±0.121	0.389 ±0.004	2.11 ±2.19	98.0 ±2.0	-1.08 ±0.46
D.McM	15.9 ±19.8	-0.251 ±0.122	0.350 ±0.136	1.63 ±0.49	65.7 ±2.1	0.70 ±0.79
M.L.	87.7 ±137.4	0.067 ±1.342	0.319 ±4.05	3.13 ±13.5	71.7 ±2.2	3.07 ±0.90
M.A.	106.4 ±37.4	0.031 ±0.090	0.154 ±0.180	0.78 ±0.87	87.4 ±1.1	1.30 ±0.30
D.K.	128.6 ±48.3	-0.037 ±0.020	0.146 ±0.026	0.20 ±19.21	177.0 ±2.7	-2.20 ±0.50
J.J.	187.8 ±92.6	0.294 ±0.356	0.266 ±0.120	-2.92 ±3.26	86.1 ±1.7	0.51 ±0.48
E.L.	198.7 ±78.4	0.010 ±0.034	0.057 ±0.082	-1.76 ±6.50	164.2 ±3.9	1.33 ±1.20
S.McP	270.0 ±287.0	0.572 ±1.538	0.246 ±0.311	-17.75 ±32.6	76.3 ±1.4	-1.88 ±0.70
M.C.	511.3 ±153.7	0.421 ±0.273	0.342 ±0.081	-3.81 ±3.30	218.1 ±2.5	2.49 ±0.50
D.W.	1091.0 ±431.8	0.496 ±0.290	0.340 ±0.070	-10.70 ±9.9	401.4 ±6.2	3.37 ±0.80

TABLE 12

ANALYSIS OF RADIOIODIDE UPTAKE DATA IN NORMAL AND THYROTOXIC SUBJECTS;

COMPARISON OF LEAST SUM OF SQUARED DIFFERENCES AND MINIMISED VALUE

WITH EXIT RATE EQUAL TO ZERO

Patient and thyroid status		Least sum of squared differences (S1) % dose^2	Sum of squared differences with exit rate = 0(S2) % dose^2	Ratio $\frac{S2}{S1}$
UNTREATED THYROTOXIC	W.C.	71.86	107.3	1.49
	R.D.	15.62	23.42	1.50
	M.R.	71.42	75.04	1.05
	D.N.	7.44	8.43	1.13
	M.H.	67.62	114.10	1.69
	M.L.	27.55	27.61	1.00
	D.McM.	20.89	21.87	1.05
	E.H.	15.62	17.37	1.11
	E.L.	39.34	40.89	1.04
	S.S.	10.27	22.80	2.22
	I.McA.	50.85	52.29	1.03
	M.McL.	23.72	36.58	1.54
	I.S.	25.71	28.45	1.11
	J.McA.	67.15	70.15	1.04
	S.McP.	10.13	13.05	1.29
	D.W.	15.65	23.05	1.47
	M.C.	5.93	10.89	1.84
	A.McG.	9.03	15.15	1.68
	D.K.	4.68	10.63	2.27
	M.A.	2.90	3.13	1.08
	A.Cg.	26.47	55.88	2.11
	J.J.	8.34	11.58	1.39
	C.I.	0.43	0.52	1.21
	M.W.	4.56	8.88	1.95
NORMAL	E.M.	8.83	9.36	1.06
	E.McF.	8.25	8.53	1.03
	E.McI.	12.44	13.79	1.11
	W.K.	8.20	8.32	1.01
	C.H.	3.55	4.83	1.36
	E.McP.	4.73	14.50	3.07
	I.M.	4.47	5.36	1.20
	A.W.	13.60	15.23	1.12

TABLE 13

COMPARISON OF IODIDE AND PERTECHNETATE KINETICS IN UNTREATED THYROTOXIC SUBJECTS

SUBJECT	SEX	20 MINUTE UPTAKE % dose		IODIDE		PERTECHNETATE			
		I	Tc	Unidirectional clearance (±se) $ml\ min^{-1}$	Initial free uptake (±se) % dose	Unidirectional clearance (±se) $ml\ min^{-1}$	Exit rate (±se) min^{-1}	Binding rate (±se) min^{-1}	Initial free uptake (±se) % dose
D.N.	F	9.7	7.0	19.7 ± 0.6	3.97 ± 0.32	22.9 ± 2.3	0.037 ± 0.005	0.0018 ± 0.0008	-0.13 ± 0.54
D.McM.	F	12.9	7.8	65.7 ± 2.1	0.70 ± 0.79	50.3 ± 7.2	0.066 ± 0.013	-0.0004 ± 0.0008	-0.66 ± 0.65
M.L.	F	17.6	3.3	71.7 ± 2.2	3.07 ± 0.87	23.6 ± 8.2	0.105 ± 0.030	0.0009 ± 0.0014	4.80 ± 0.55
E.H.	F	10.1	6.7	52.9 ± 1.7	0.39 ± 0.66	18.0 ± 2.5	0.022 ± 0.006	0.0130 ± 0.0025	0.38 ± 0.55
S.McP.	F	18.0	5.6	76.3 ± 1.4	-1.88 ± 0.71	16.6 ± 6.6	0.045 ± 0.016	0.0009 ± 0.0008	2.80 ± 1.12
S.S.	F	19.0	12.2	81.8 ± 1.9	1.80 ± 0.71	46.0 ± 5.4	0.027 ± 0.004	-0.0009 ± 0.0008	1.33 ± 0.92
M.McL.	F	22.3	7.1	103.7 ± 2.7	0.66 ± 0.90	18.7 ± 3.3	0.030 ± 0.005	0.0008 ± 0.0005	4.36 ± 0.44
I.McA.	F	24.3	4.4	116.8 ± 3.2	0.65 ± 1.21	26.2 ± 5.6	0.080 ± 0.017	0.0020 ± 0.0002	1.07 ± 0.73
M.R.	F	31.1	12.6	120.1 ± 3.2	3.30 ± 1.41	47.6 ± 17.3	0.063 ± 0.020	0.0017 ± 0.0003	7.91 ± 1.80
J.McA.	F	30.4	6.0	133.1 ± 3.4	4.81 ± 1.15	14.3 ± 10.8	0.038 ± 0.020	0.0011 ± 0.0004	6.29 ± 0.69
E.L.	F	31.1	12.5	164.2 ± 3.9	1.33 ± 1.20	118.2 ± 16.8	0.078 ± 0.012	0.0009 ± 0.0003	-1.68 ± 1.85
I.S.	F	31.8	7.3	183.5 ± 9.8	3.16 ± 1.81	25.9 ± 7.6	0.039 ± 0.009	-0.0003 ± 0.0028	5.64 ± 0.61
M.H.	F	54.9	-	322.6 ± 12.8	4.33 ± 2.58	-	-	-	-
R.D.	F	63.2	20.0	395.7 ± 7.0	11.25 ± 1.20	159.2 ± 43.2	0.062 ± 0.018	0.0019 ± 0.0006	7.75 ± 3.22
W.C.	M	45.4	23.8	503.1 ± 22.0	-0.32 ± 2.80	121.4 ± 21.3	0.026 ± 0.005	0.0007 ± 0.0004	13.85 ± 1.34
		Mean (±sd)		160.7 ± 138.5	2.48 ± 3.07	50.6 ± 47.0	0.051 ± 0.025	0.0017 ± 0.0034	3.87 ± 4.26

TABLE 14

COMPARISON OF IODIDE AND PERTECHNETATE KINETICS IN NORMAL SUBJECTS

SUBJECT	SEX	20 MINUTE UPTAKE % dose		IODIDE		PERTECHNETATE			
				Unidirectional clearance(±se) ml min^{-1}	Initial free uptake(±se) % dose	Unidirectional clearance(±se) ml min^{-1}	Exit rate(±se) min^{-1}	Binding rate(±se) min^{-1}	Initial free uptake (±se) % dose
E.M.	F	5.9	2.2	14.0 ±0.5	0.99 ±0.41	5.0 ±3.0	0.034 ±0.020	-0.0004 ±0.0015	1.28 ±0.51
E.McP.	F	5.7	2.5	14.5 ±0.8	1.11 ±0.54	10.4 ±1.9	0.056 ±0.011	-0.0018 ±0.0005	0.34 ±0.31
E.McI.	F	8.0	3.3	17.3 ±0.9	3.15 ±0.58	11.4 ±7.0	0.060 ±0.035	0.0018 ±0.0006	1.99 ±1.02
W.K.	M	5.9	2.0	21.0 ±1.0	1.60 ±0.45	11.3 ±2.5	0.057 ±0.016	0.0006 ±0.0009	-0.48 ±0.44
C.H.	M	3.6	1.1	21.6 ±0.9	-1.02 ±0.39	5.2 ±1.5	0.045 ±0.016	0.0017 ±0.0013	0.15 ±0.23
E.McF.	F	5.8	2.7	21.7 ±0.6	0.69 ±0.36	7.4 ±2.6	0.045 ±0.015	0.0001 ±0.0010	1.50 ±0.35
A.W.	M	5.5	1.7	34.3 ±1.6	-0.84 ±0.64	12.7 ±4.8	0.068 ±0.024	0.0010 ±0.0010	-0.50 ±0.76
I.M.	F	9.4	4.8	40.6 ±1.2	-0.56 ±0.45	15.3 ±2.8	0.035 ±0.007	-0.0026 ±0.0004	1.86 ±0.41
		Mean (± sd)		23.1 ± 9.5	0.64 ± 1.41	9.8 ± 3.7	0.050 ± 0.012	0.0001 ± 0.0016	0.77 ± 1.02

unidirectional clearance of iodide is 160.7±138.5 and 23.1±9.5 ml minute^{-1} in the thyrotoxic and normal group respectively. Corresponding values for pertechnetate are 50.6±47.0 and 9.8±3.7 ml minute^{-1}. The difference between the normal and thyrotoxic group for each anion is significant ($p<0.01$). When the data from all patients are combined, there is significant correlation ($p<0.05$) between the unidirectional clearances for iodide and pertechnetate (Figure 5.3). The unidirectional clearance for iodide is greater on average by a factor of 3.25±2.04 (sd), but there are considerable individual deviations from the mean.

There is no significant difference ($p>0.1$) in either the exit rate or binding rate of pertechnetate between the normal and thyrotoxic subjects. In all subjects the mean (±sd) exit rate is 0.051±0.019 minute^{-1} and the mean binding rate 0.0011±0.0030 minute^{-1}. The latter, in fact, is not significantly different from zero ($p>0.05$).

The mean (±sd) initial free uptake for iodide is 2.48±3.07 and 0.64±1.41 % dose, in the thyrotoxic and normal group respectively. For pertechnetate the corresponding values are 3.87±4.26 and 0.77±1.02 % dose. No significance can be attached at this stage, however, to these differences between normal and thyrotoxic subjects ($p>0.05$).

Regression analysis of the initial free uptakes for iodide and pertechnetate reveals a significant correlation ($p<0.05$) when the results from all cases (except subjects W.C. and R.D. where the errors are exceptionally large) are combined (Figure 5.4). The apparent difference between the initial free uptakes of the two anions is, however, not significant ($p>0.1$). (The relationship between the inital free uptakes of iodide and pertechnetate, and the possible relationship

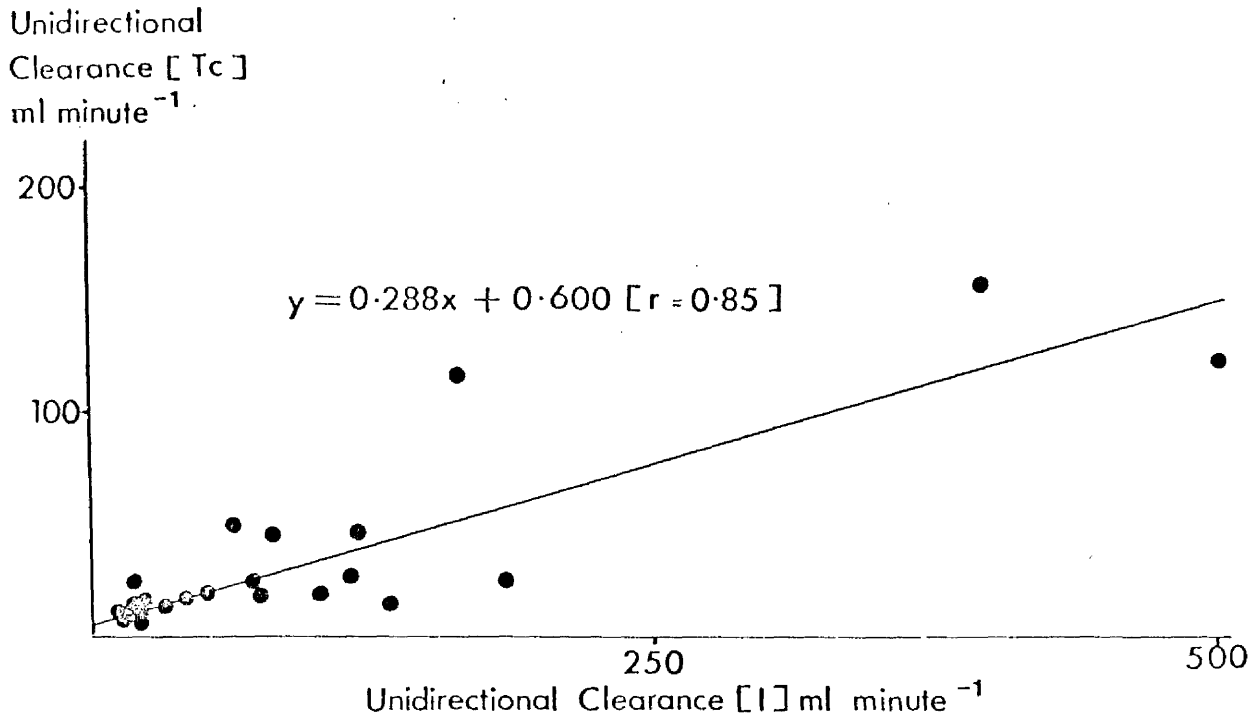


Figure 5.3 Correlation between the unidirectional clearances of iodide and pertechnetate in normal and untreated thyrotoxic subjects.

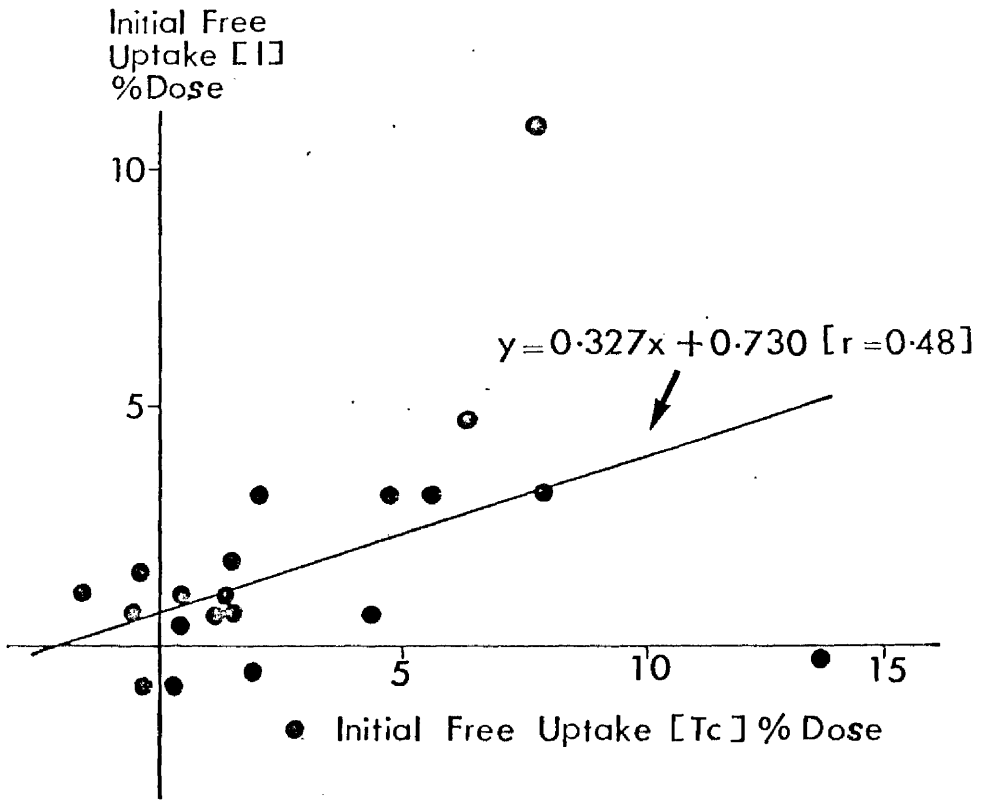


Figure 5.4 Correlation between the initial free uptakes of iodide and pertechnetate in normal and untreated thyrotoxic subjects.

between initial free uptake and gland activity, are explored further in Section 6.2).

Carbimazole had no effect, in the short-term at least, on the unidirectional clearance and initial free uptake of iodide (Table 15). Statistical analysis of the combined results from the thyrotoxic and normal subjects, before and after carbimazole, reveals no significant difference in these parameters ($p > 0.1$). The same is true of the unidirectional clearance, exit rate, binding rate and initial free uptake of pertechnetate ($p > 0.1$). After carbimazole, however, the binding rate for iodide is reduced to values that are considerably less than the estimated exit rate. In the 7 thyrotoxic subjects and 1 normal subject the mean ($\pm$sd) binding rate of iodide after carbimazole is -0.003 ± 0.008 minute^{-1}, which is not significantly different from zero ($p > 0.3$). The mean ($\pm$sd) exit rate is 0.033 ± 0.035 minute^{-1}.

5.5 Discussion

Numerical analysis of radioiodide uptake data, by the new method developed in this thesis, has produced results that are consistent with the binding rate of iodide being much greater than exit rate, in the uninhibited gland. The results confirm for the first time, by "least sum of squares" analysis, the finding of Gray (28) that the net clearance of radioiodide, in normal and thyrotoxic subjects, is virtually constant and equal to the unidirectional clearance. As in Gray's technique, the present method of analysis includes a correction for arterio/venous differences in plasma tracer levels.

Evidence for the constancy of radioiodide clearance lies in the occurrence of least sum of squares solutions with large errors due to lack of curvature in the sum of squares surface. Thus, when the

TABLE 15

EFFECT OF CARBIMAZOLE (120 mg over 24 hours) ON IODIDE AND PERTECHNETATE KINETICS

SUBJECT	BEFORE CARBIMAZOLE				AFTER CARBIMAZOLE			
	Unidirectional clearance(±se) ml min^{-1}	Exit rate(±se) min^{-1}	Binding rate(±se) min^{-1}	Initial free uptake(±se) % dose	Unidirectional clearance(±se) ml min^{-1}	Exit rate se) min^{-1}	Binding rate(±se) min^{-1}	Initial free uptake (±se) % dose
	IODIDE							
THYROTOXIC D.N.	19.7± 0.6	-	-	3.97± 0.32	57.1± 17.7	0.030 ±0.010	0.0025 ±0.0040	-1.28 ±1.20
THYROTOXIC A.McL.	40.2± 5.7	-	-	0.68± 1.60	48.1± 9.0	0.017 ±0.003	-0.0140 ±0.0100	-0.12 ±1.12
THYROTOXIC J.McA.	133.1± 3.4	-	-	4.81± 1.15	155.7± 19.8	0.116 ±0.023	0.0029 ±0.0040	-0.22 ±0.7C
THYROTOXIC R.D.	395.7± 7.0	-	-	11.25± 1.19	426.0± 89.8	0.041 ±0.011	0.0047 ±0.0020	10.98 ±3.96
THYROTOXIC W.L.	503.1± 22.0	-	-	-0.32± 2.80	357.6± 50.2	0.014 ±0.009	-0.0100 ±0.0050	5.42 ±2.20
THYROTOXIC P.Y.	-	-	-	-	265.4± 11.3	0.019 ±0.003	0.0022 ±0.0030	-1.61 ±1.51
THYROTOXIC M.McM.	-	-	-	-	592.5± 28.2	0.010 ±0.006	0.0007 ±0.0080	-3.80 ±2.10
* E.K.	24.6± 3.5	-	-	0.37± 0.51	18.4± 8.7	0.014 ±0.006	-0.0120 ±0.0050	-0.18 ±1.51
	PERTECHNETATE							
THYROTOXIC D.N.	22.9± 2.3	0.037± 0.005	0.0018 ±0.0008	-0.13± 0.54	32.8± 5.5	0.063 ±0.013	0.0041 ±0.0015	-0.02 ±1.17
THYROTOXIC I.McA.	26.2± 5.6	0.080± 0.017	0.0020±0.0002	1.07± 0.73	48.3± 11.8	0.146 ±0.037	0.0028 ±0.0020	0.67 ±1.14
THYROTOXIC A.McL.	35.5± 7.7	0.040± 0.028	0.0045±0.0210	-0.31± 0.87	41.6± 5.9	0.045 ±0.019	-0.0014 ±0.0140	-0.11 ±0.48
THYROTOXIC W.C.	121.4± 21.3	0.026± 0.005	0.0007±0.0004	13.85± 1.34	125.1± 42.9	0.016 ±0.005	-0.0160 ±0.0080	13.08 ±1.40
THYROTOXIC R.D.	159.2± 43.2	0.062± 0.018	0.0019±0.0006	7.75± 3.22	146.8± 18.9	0.037 ±0.005	0.0014 ±0.0008	16.38 ±1.13
THYROTOXIC S.S.	46.0± 5.4	0.027± 0.004	-0.0009±0.0008	1.33± 0.92	64.9± 8.6	0.030 ±0.007	0.0020 ±0.0020	1.58 ±0.87
THYROTOXIC J.J.	371.2± 71.2	0.078± 0.021	0.0080±0.0060	7.29± 2.80	270.2± 40.0	0.032 ±0.015	-0.0080 ±0.0130	-1.15 ±4.20
* S.A.	6.1± 1.3	0.004± 0.011	-0.0800±0.0900	-0.16± 0.29	13.0± 3.2	0.041 ±0.021	0.0041 ±0.0120	0.40 ±0.20
M.R.	6.1± 5.3	0.025± 0.020	-0.0220±0.0060	-0.07± 1.92	2.6± 1.5	0.018 ±0.008	-0.0190 ±0.0060	0.07 ±1.60
J.G.	19.3± 4.7	0.025± 0.005	-0.0100±0.0200	-0.63± 3.90	19.3± 5.1	0.039 ±0.023	0.0015 ±0.0060	0.07 ±0.14

* NORMAL

minimised sum of squares value, with the exit rate equal to zero (constant clearance condition), was compared with the least sum of squares, the former was never more than 3.07 times greater in 24 untreated thyrotoxic and 8 normal subjects. This is in keeping with earlier considerations of the analytical problems that might arise if the binding rate is much greater than the exit rate (Section 3.7). Under such circumstances the analysis can provide only estimates of unidirectional clearance and initial free uptake. Neither binding rate nor exit rate can be estimated reliably, since all values of these parameters, within certain limits, produce almost identical fits to the observed data.

A means of quantifying the binding rate of iodide in the uninhibited gland was discovered, unexpectedly, in the perchlorate discharge studies reported later (Section 6.4). In one exceptional case, an untreated thyrotoxic subject, there was a discharge of 7.5% of the radioiodide uptake at 30 minutes. The binding rate for that subject was estimated to be 0.150 minute^{-1}, using a method based on the observed discharge by perchlorate (Section 6.3 and Appendix 2). This serves, therefore, as an estimate of the lower limit of binding rate in normal and untreated thyrotoxic subjects.

A lower limit for the effective clearance of stable iodide (Section 2.7), in terms of unidirectional clearance, may also be determined for the uninhibited gland. This requires an estimate of exit rate which, for present purposes, may be taken to be the mean value (0.046 minute^{-1}) in all subjects studied with inhibited binding (Table 22, Chapter 6). The limit is 75% which is readily determined from the expression 100 $K_B/(K_{TP}+K_B)$ (Section 2.7) or from Figure 5.5

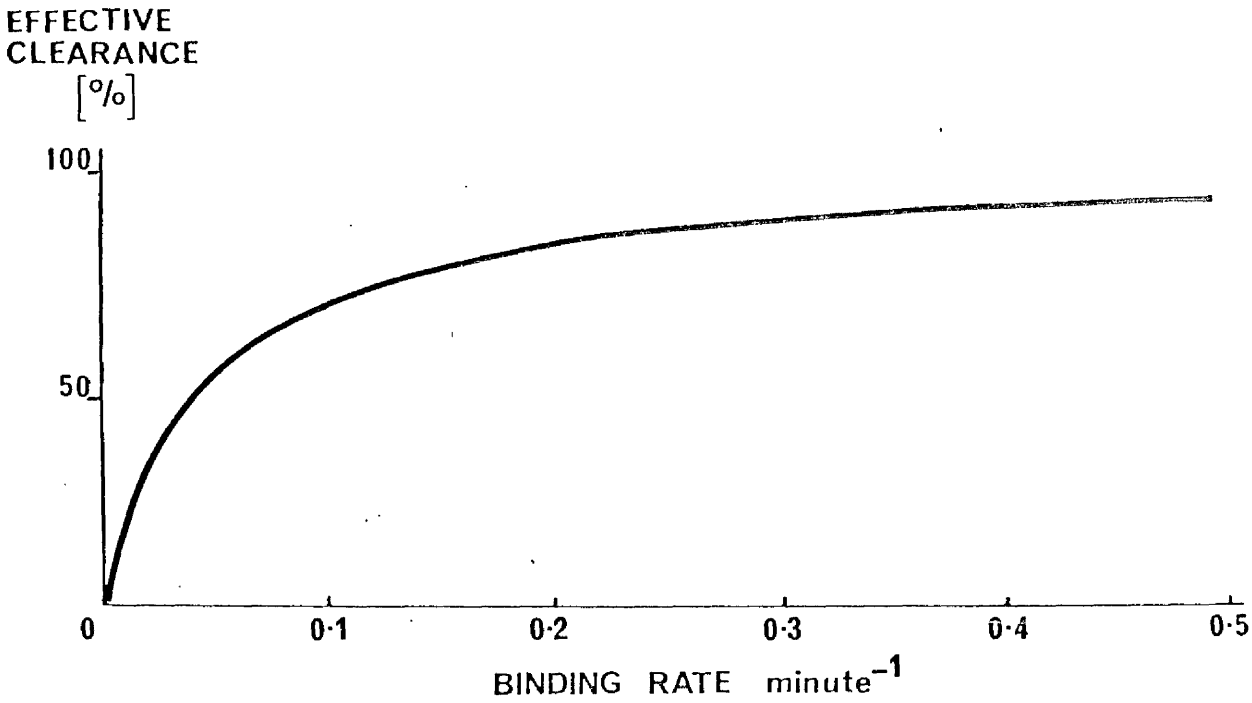


Figure 5.5 Variation of effective iodide clearance (as a percentage of unidirectional clearance) with binding rate, a value of 0.046 minute^{-1} being taken for the exit rate.

where effective clearance versus binding rate is plotted. Thus the present work suggests that,when there is no inhibition of binding, 75-100% of the iodide entering the gland is eventually organified.

In contrast to the results for iodide, the mean binding rate of pertechnetate (0.0011 minute^{-1}) in both normal and thyrotoxic subjects was not significantly different from zero. It would appear, therefore, that because of experimental errors the small extent to which pertechnetate is organified cannot be quantified reliably by the present technique. The estimated values indicate only the order of magnitude of the binding rate.

The mean unidirectional clearance of iodide and pertechnetate was found to be lower in normal subjects (23.1 and 9.8 ml minute^{-1} respectively) than in thyrotoxic subjects (160.7 and 50.6 ml minute^{-1}). On the other hand, the exit rate of ion from the gland would seem to be independent of thyroid status, as can be deduced from the results for pertechnetate. These findings, which merely confirm previously reported work (Table 1), suggest that the greater uptake of both ions in the thyrotoxic state (27, 116) is a consequence of greater blood flow and trapping, and not of reduction in leakage of trapped material. (Evidence to be discussed later in Section 6.2 suggests that the exit rate in individual subjects may have some dependence upon unidirectional clearance).

Whilst the unidirectional clearance of iodide in normal and thyrotoxic subjects was greater than that for pertechnetate (by a factor of 3.25 on average), the same was not true of the initial free uptake. The observed data suggested that the initial free uptake for pertechnetate was at least as great as that for iodide. However, if,

as indicated earlier (Section 3.3), the initial free uptake is the fraction of the injected bolus of tracer that is trapped by the thyroid, that parameter should have been greater for iodide on the basis of greater unidirectional clearance. In view of this inconsistency between observed and expected results, the phenomenon of initial free uptake, and its relationship to unidirectional clearance, was studied further and the outcome is reported in Section 6.2.

The relationship between the unidirectional clearance for iodide and that for pertechnetate merits some further comment. Other workers (Table 1) have reported iodide/pertechnetate clearance ratios of 1.4-2.4, which are lower than the present value of 3.25 ± 2.04 (sd). These discrepancies can be explained by differences in analytical technique and by the fact that the relative affinity of the gland for the two ions may vary markedly from patient to patient. (In a further 6 thyrotoxic patients studied (Section 6.2), the mean iodide/pertechnetate clearance ratio was 1.71 ± 0.50).

Carbimazole (120 mg in divided doses over 24 hours) was found to affect only the binding of iodide, which was reduced to negligible levels. This meant that exit rate could be estimated. (The exit rate estimates are combined with additional results in Section 6.2 to obtain a grand mean in all subjects studied in the present work). Any possible effect on the binding of pertechnetate would certainly have been beyond the limits of detection of the analysis technique. There is no evidence from the results to support the finding of Robertson (27) that carbimazole may increase both unidirectional clearance and exit rate.

CHAPTER SIX

CONTROL OF THYROTOXICOSIS WITH ANTITHYROID DRUGS

6.1 Introduction

The thiocarbamide drugs, namely, methimazole, carbimazole and propylthiouracil, have an important role in the treatment of thyrotoxicosis, particularly in the younger patient (131-133). These drugs prevent the biosynthesis of thyroid hormone by inhibiting, firstly, the formation of monoiodotyrosine and diiodotyrosine and, secondly, the coupling of these iodotyrosines to form the iodothyronines, T_3 and T_4 (9, 86, 134, 135). Thus treatment with thiocarbamide drugs results in reduced levels of circulating thyroid hormone; a euthyroid clinical state being achieved, usually, within 4-8 weeks from commencement of treatment (9,136). However, to minimise the risk of hypothyroidism, the dose of drug is usually not sufficient to completely inhibit organification of iodide (137, 138). There is no clear evidence to suggest that antithyroid drugs have a direct effect on the underlying disease process (132, 135, 139) and treatment is continued for 12-24 months in the hope that remission of the disease will occur spontaneously during therapy (131, 133).

Most studies have shown that, in approximately 50% of patients so treated, thyrotoxicosis either continues or recurs, usually within 2 years of completing treatment (131, 132, 136, 138, 140). In one study, however, the failure rate was approximately 80%, but this was thought to be due to an increased iodine intake in the population under

investigation (141). Long-term remission of the disease occurs in the remainder but, in a proportion of these, some abnormality in thyroid function may still exist at a subclinical level (139, 142). Recurrence of the disease has been observed in an occasional patient as long as 22 years after a first course of antithyroid drug therapy (140).

As yet, no way has been found of determining, before antithyroid drug therapy commences, whether the subject will remain euthyroid or relapse after therapy. However, limited success has been achieved in predicting the outcome of treatment, once it has started, by observing various aspects of thyroid function. The likelihood of long-term remission has been found to be greater where there is a reduction in goitre size (143-145). Remission is also more likely if there is a return to normal suppressibility of uptake by the action of exogenous hormone (112, 116, 133, 140, 146-151), or if there is a spontaneous reduction in thyroid uptake (138, 152). On the other hand, if the blood level of thyroid stimulating immunoglobulin remains high, there may be a greater risk of relapse (153, 154).

It is particularly important to recognise,as early as possible, those patients who are destined to have persistent disease so that destructive therapy can be arranged with as little delay as possible. In an interim plan for treatment of thyrotoxicosis, McLarty and Alexander (140) change to destructive therapy if, after 6 months treatment with antithyroid drugs, the 20 minute radioiodide uptake has either risen, is above 30%, or cannot be suppressed by T3 to 50% of the control value. Goolden et al (138) found the 20 minute uptake of ^{99m}Tc-pertechnetate during the first 6 months of treatment to be of limited value, but found that failure to achieve a normal uptake at the

end of treatment indicated impending relapse. It may well be that if all relevant parameters, including immunological and biochemical factors, were considered together, better prediction of the results of antithyroid drug therapy might be possible (146).

In view of the continuing use of early iodide and pertechnetate uptake in the assessment of thyroid function during antithyroid drug therapy, the present work included some detailed studies of the uptake kinetics of these two anions in thyrotoxic subjects receiving drug treatment. These studies were intended to serve as an extension to previously reported work on the subject (115,116).

There were a number of objectives. In particular, kinetic analysis would provide hitherto unknown information about those parameters which describe the trapping and binding functions of the gland, during the course of drug-treated thyrotoxicosis. It was hoped that these parameters might be useful in predicting the outcome of therapy. Furthermore, there was a need to explain some interesting differences in the behaviour of the two anions, such as variability in relative uptake with the occurrence of uptake values for pertechnetate that sometimes exceed those for iodide (155). Another problem considered was that of occasional resistance to antithyroid drugs due to a lower than normal inhibition of iodide organification (156). Some observations were also made of the short-term response to varying doses of drug. The object was to find the smallest dose necessary for control of the disease - a factor that is important in the treatment of the pregnant thyrotoxic patient when placental transfer of the drug (157) should be minimised. Possible long-term effects of antithyroid drugs on the binding function of the gland were also investigated.

6.2 Iodide and Pertechnetate Kinetics in Long-Term Antithyroid Drug Therapy

Patients and Methods. Six previously untreated thyrotoxic subjects were selected for the simultaneous study of iodide and pertechnetate during routine drug therapy (133). Five were treated with carbimazole (30-40 mg d^{-1} initially, reducing to 15-20 mg d^{-1}) and T3 (80 ug d^{-1}). Drug therapy was discontinued after 4-12 months but patients continued to receive T3. The remaining subject received only propylthiourucil, in an initial dose of 400 mg d^{-1} reducing to 100 mg d^{-1}.

Thyroidal uptake of intravenously administered ^{132}I-iodide and ^{99m}Tc-pertechnetate was estimated using an uptake counter, adhering to the procedure described earlier (Section 4.2). Samples of venous blood, for estimation of plasma radioactivity, were taken at appropriate times after the administration of the tracers (Section 3.9). Each subject was investigated before and at various times during the course of therapy. There were at least 3 investigations within the first 6 months of treatment, but the frequency was less thereafter. A perchlorate discharge test (Section 3.9) was introduced later as an additional procedure in the investigation of each subject.

Repeated studies of the kinetics of ^{99m}Tc-pertechnetate alone were performed in one further thyrotoxic subject who was treated with carbimazole and T3. Thyroid uptake was measured using a gamma camera (Section 4.3) but the remaining aspects of the study were as for the other subjects.

Data were analysed, as described previously (Section 3.9), to provide estimates of the various parameters of the open three-compartment binding model of the thyroid gland. Analysis of the

perchlorate discharge phase (Section 2.6) permitted results from the uptake phase to be verified. Statistical analysis of the results was performed using non-parametric methods, whenever possible, as in Section 5.3.

Results. Because the longest period of observation in the present work was only 30 months, it was not possible to finally classify the response to therapy after the fashion of Alexander et al(133). They studied patients for at least 4 years from the start of treatment. Thyrotoxicosis was found in their experience to follow one of three courses:- (a) remission of the disease after one course of antithyroid drugs, (b) continuance or recurrence of the disease, and (c) remission of the disease after two courses of antithyroid drugs. The following clinical summary of the subjects studied in the present work can be considered only as an interim report.

Two subjects (D.K. and M.C. in the results tables to follow) were still in remission 12 and 24.5 months, respectively, after a first course of antithyroid drugs. Only time will tell whether the disease in these subjects will follow course (a), (b), or (c). In one of these cases (D.K.) the 20 minute uptake of ^{132}I-iodide had suppressed into the normal range ($< 8\%$) on the last day of treatment, but in the other (M.C.) the uptake was still elevated. Four subjects (A.McG., A.C., D.W. and M.A.) relapsed 0-11 months after therapy and a second course of antithyroid drug therapy was commenced. All of these subjects had an elevated uptake of ^{132}I-iodide on the last day of the first course of treatment. In these four subjects the disease may eventually follow course (b) or (c). The last subject (S.D.) had not completed a first course of antithyroid drug therapy when the present results were computed.

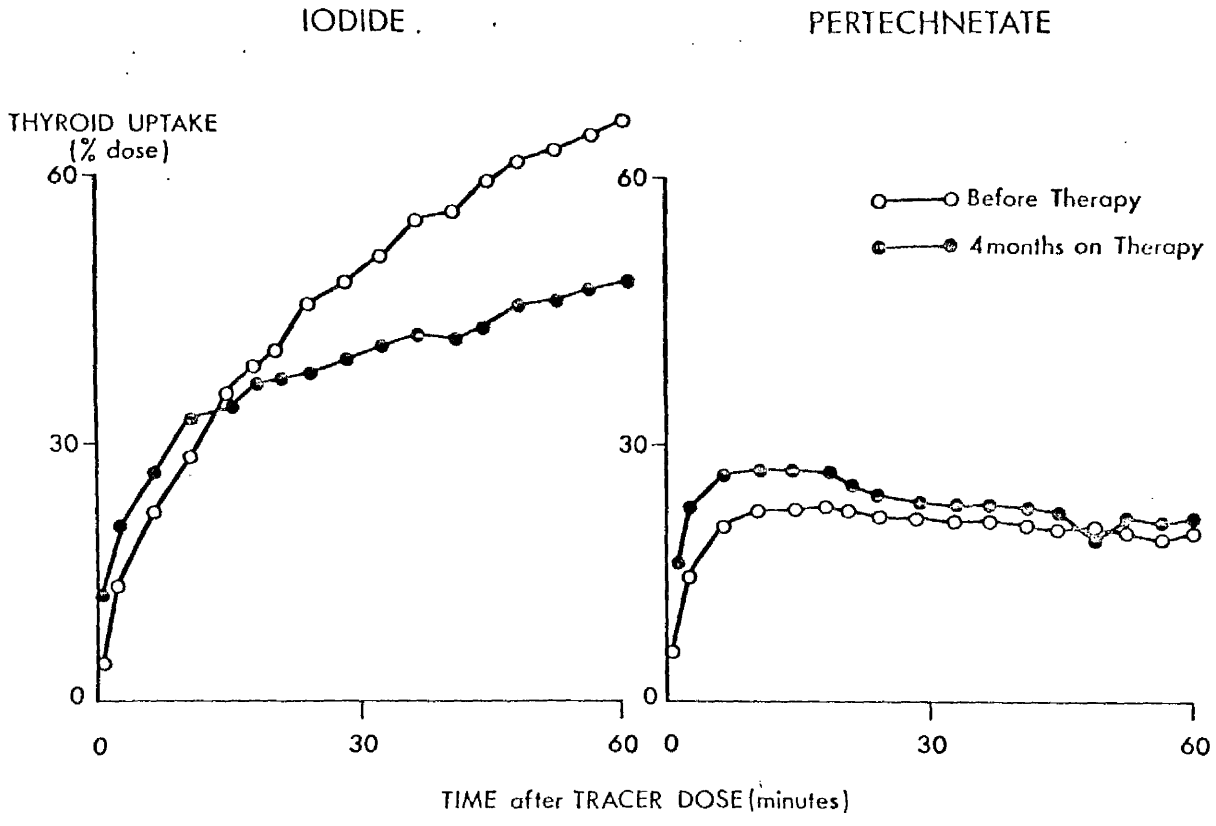
Thyroid uptake curves for both ^{132}I and ^{99m}Tc at various times during the period of observation are shown in Figure 6.1 for each of the subjects who received both tracers. Similar data are given in Figure 6.2 for the one subject who received only ^{99m}Tc-pertechnetate. In most cases the iodide curves, once treatment had started, can be seen to be similar to the pertechnetate curves, having reached a maximum value before 60 minutes. However, in the case of subjects A.McG. and M.C. the pattern is one of continuously increasing radioiodide uptake off or on treatment.

Results of the kinetic analysis along with the clinical and biochemical response to therapy are presented for each subject in Table 16. These results comprise estimates of unidirectional clearance, exit rate and initial free uptake for iodide and pertechnetate, and estimates of binding rate for iodide. Included also are the estimated 20 minute uptakes of the two tracers and estimates of the free thyroxine index (158).

In terms of immediate response to therapy the most relevant result is the observed reduction in binding rate of iodide from a pre-treatment value of > 0.150 minute^{-1} to a mean value ($\pm$sd) for all subjects of 0.009 ± 0.007 minute^{-1} during treatment. The effective clearance of iodide (Section 2.7) is thereby reduced from >75% (Section 5.5) to a mean value ($\pm$sd) of $17.6 \pm 14.5\%$ of the unidirectional clearance (Table 17). Both the mean binding rate and effective clearance of iodide during drug therapy are significantly greater than zero ($p<0.05$).

Subject A.McG. has the lowest exit rate and subject M.C. the greatest binding rate of iodide during therapy. This may explain the failure of radioiodide uptake in those two cases to reach a peak value

Subject M.C.



SUBJECT M.A.

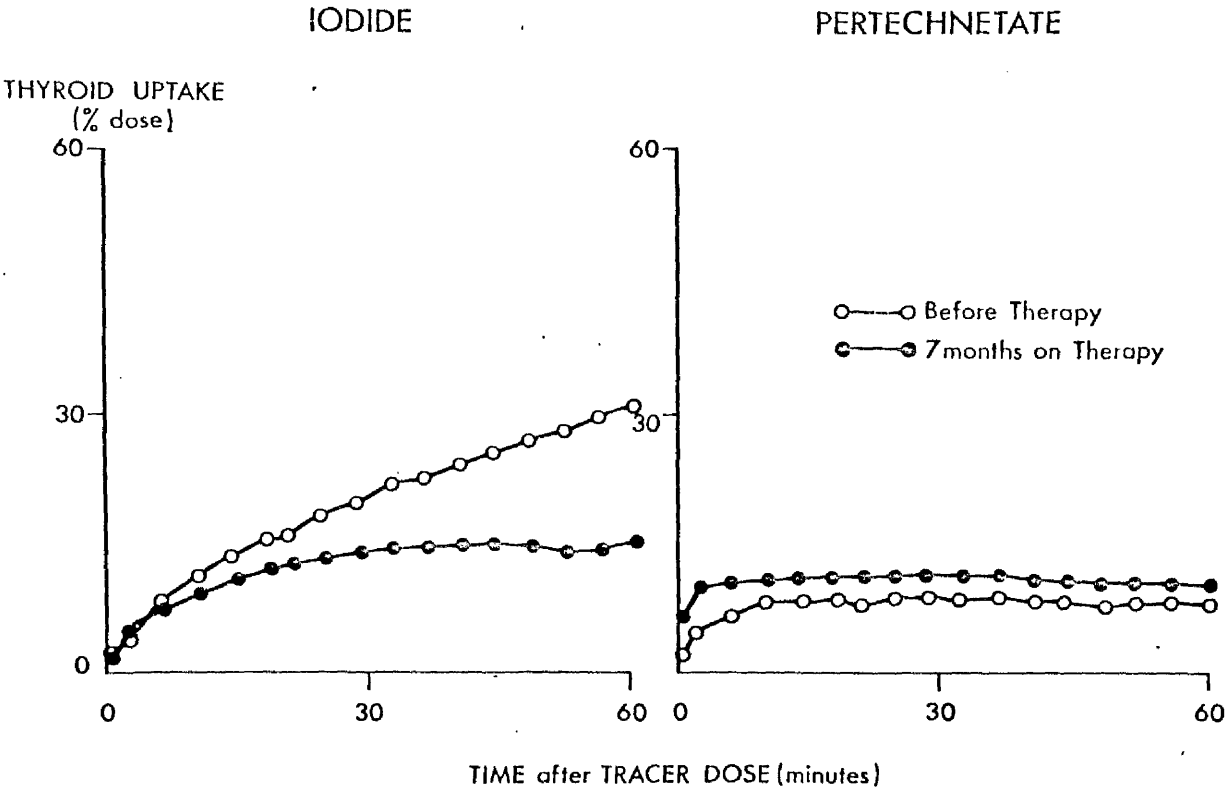
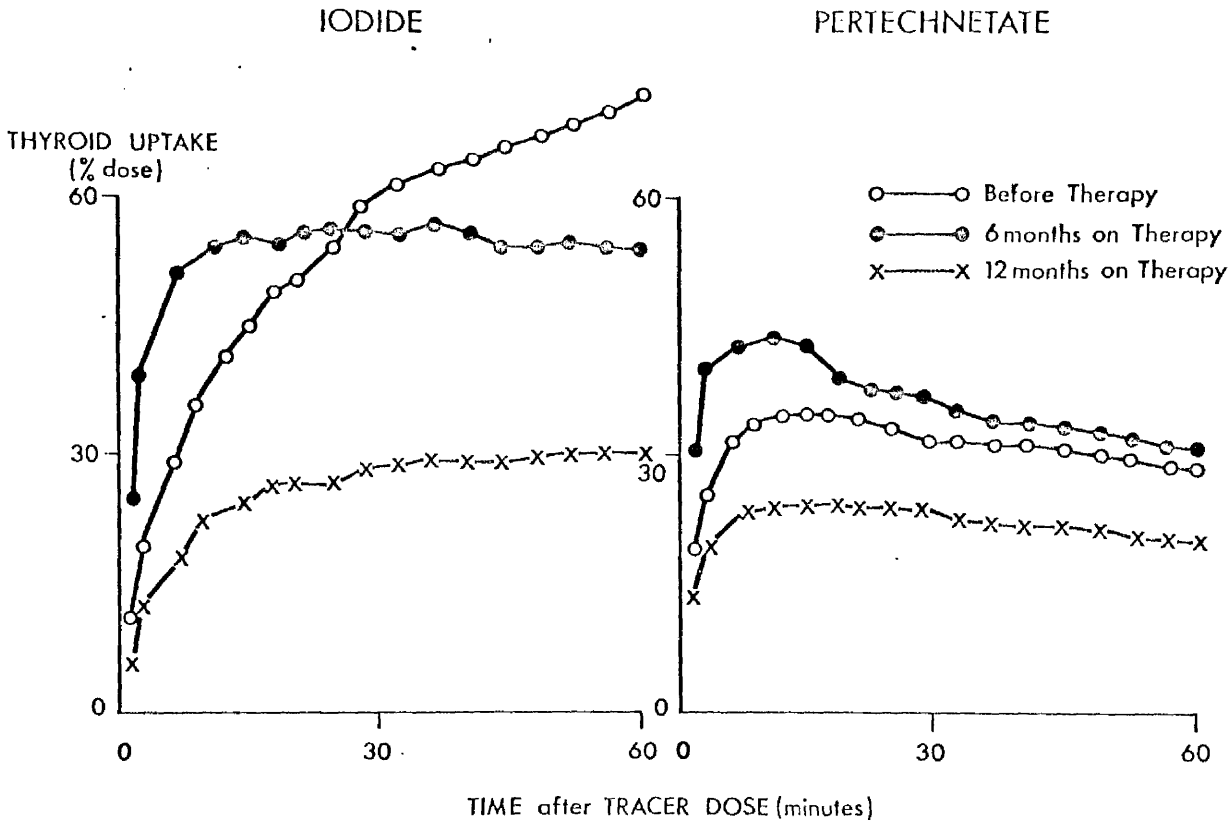


Figure 6.1 Thyroidal uptake variations of iodide and pertechnetate in antithyroid drug therapy.

Subject D. W.



Subject A.McG.

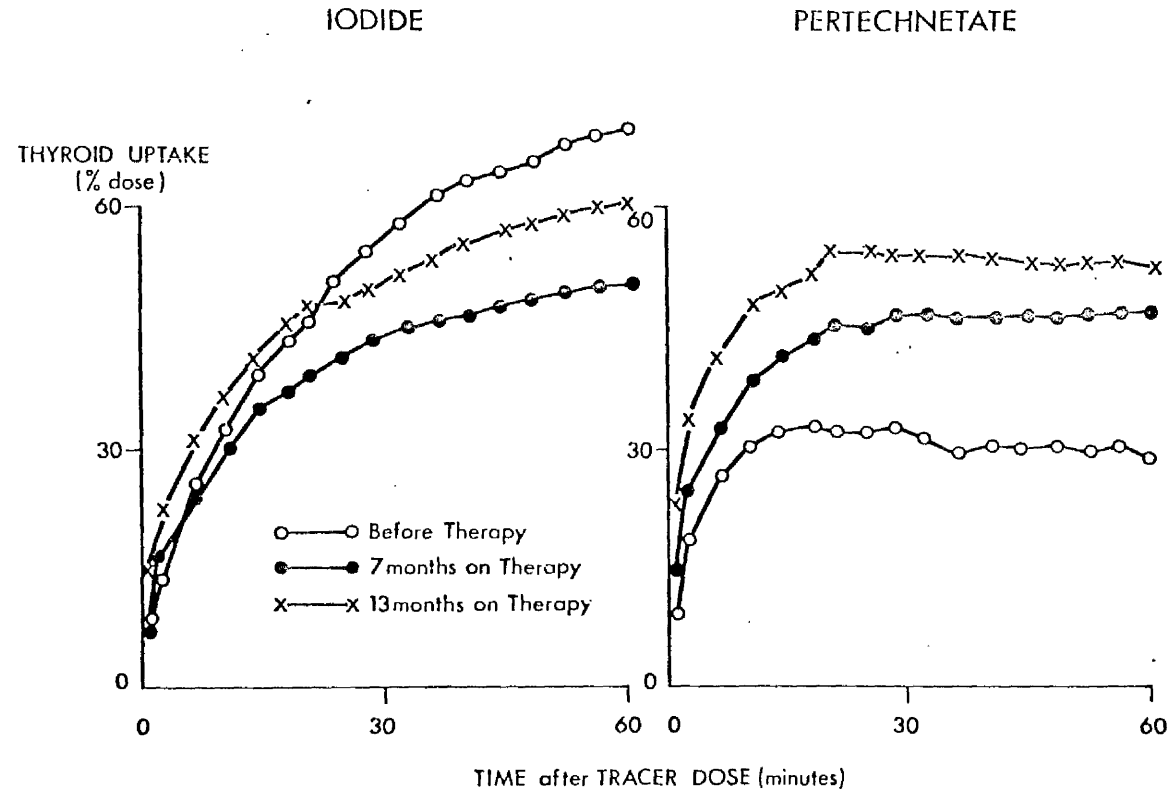
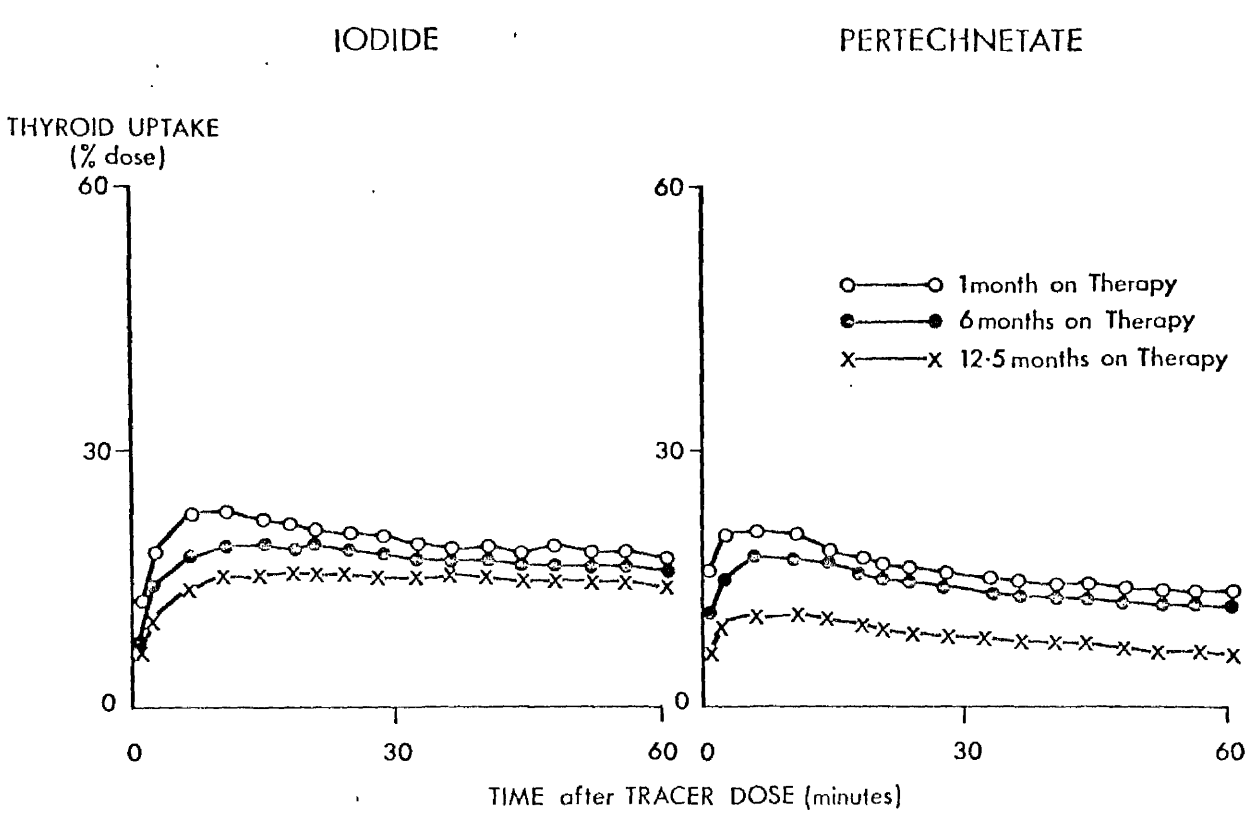


Figure 6.1 cont'd

Subject A.C.



Subject D.K.

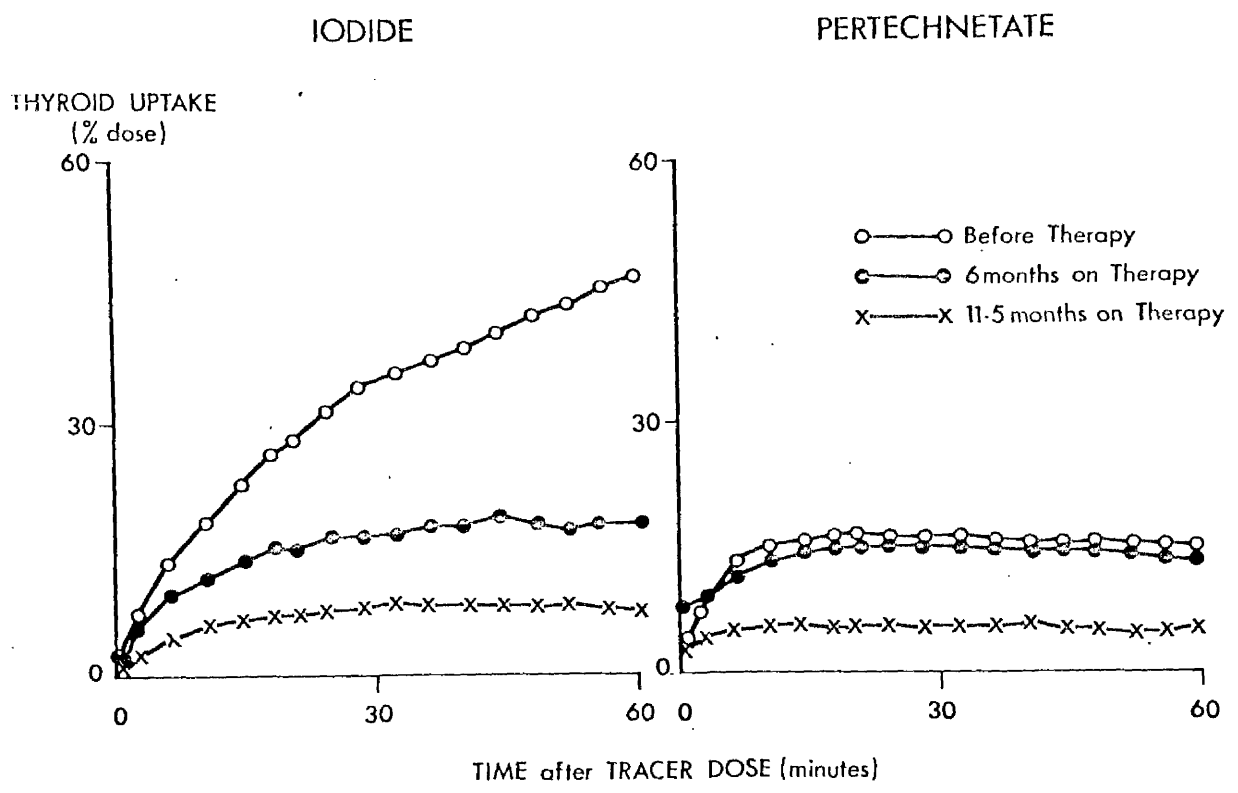


Figure 6.1 cont'd

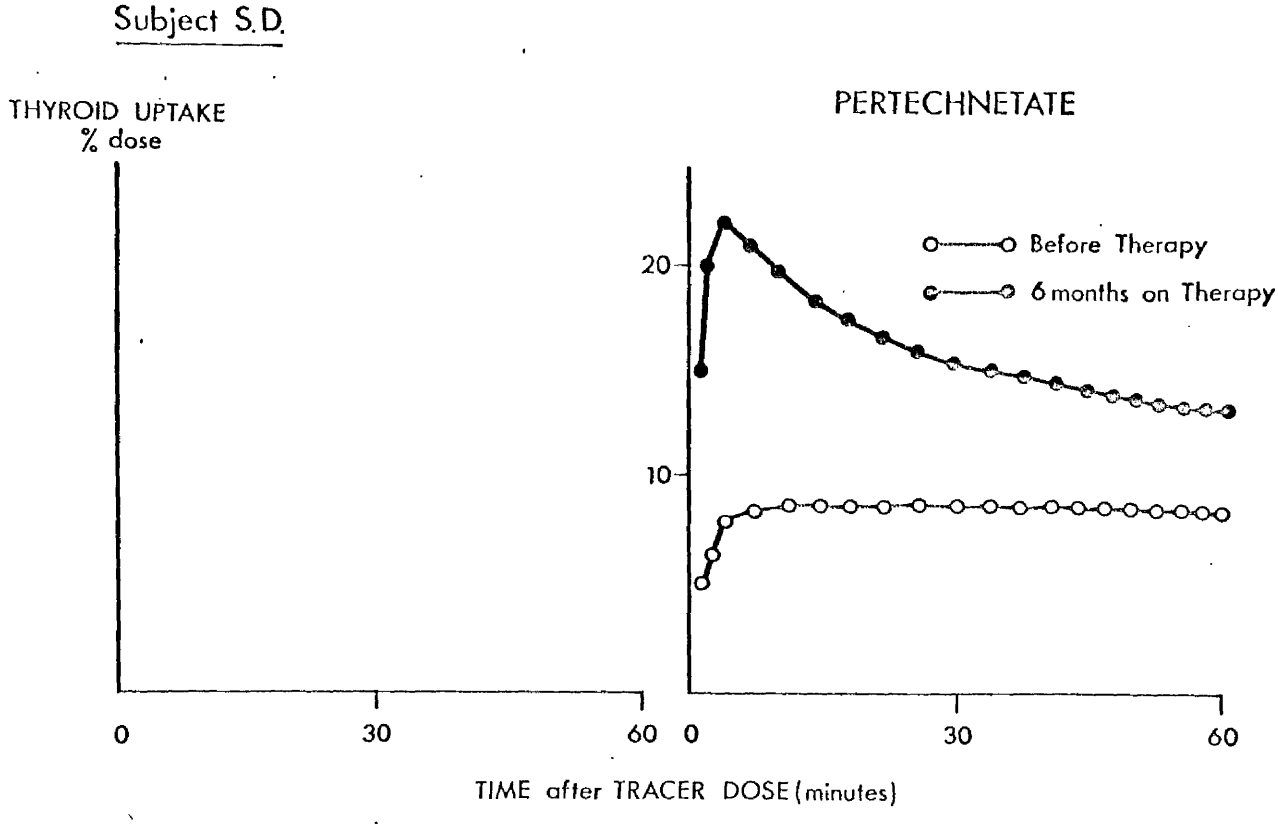


Figure 6.2 Thyroidal uptake variations of pertechnetate in antithyroid drug therapy. (Subject S.D. did not receive radioiodide tracer).

TABLE 16

IODIDE AND PERTECHNETATE KINETICS DURING ANTITHYROID DRUG THERAPY

MONTH OF STUDY	FREE T_4 INDEX, * CLINICAL STATUS	20 MIN. UPTAKE % dose		UNIDIRECTIONAL CLEARANCE (±SE) ml min^{-1}		EXIT RATE (±SE) min^{-1}		BINDING RATE (±SE) min^{-1}	INITIAL FREE UPTAKE (±SE) % dose	
		I	Tc	I	Tc	I	Tc	I	I	Tc
0	1646, T	40.8	22.4	218.1± 2.5	230.6±14.8	-	0.129±0.010	>0.150	2.5±0.5	0.6±0.9
1	193, E	34.4	24.9	267.8±19.4	232.8± 9.4	0.048±0.006	0.128±0.006	0.011±0.002	2.1±1.2	5.7±0.6
2	<79, E	39.8	26.9	326.3±29.2	256.7±23.7	0.047±0.008	0.110±0.011	0.016±0.003	7.4±1.2	10.4±1.1
4	<79, E	37.2	25.0	384.5±50.7	241.2±21.7	0.091±0.020	0.129±0.012	0.041±0.004	11.8±1.0	11.8±1.0
6	<79, E	41.8	19.0	137.1± 4.4	138.8±28.2	-	0.096±0.020	>0.150	6.5±7.0	11.5±1.3
8	104, E	24.0	12.9	94.5± 2.8	110.3±10.3	-	0.098±0.010	>0.150	-0.1±0.9	5.5±0.5
14	370, E	26.0	18.8	133.2± 1.6	73.4±13.2	-	0.038±0.007	>0.150	-1.1±0.4	7.7±0.7
17	426, E	22.5	9.3	109.1± 3.3	50.4± 7.9	-	0.078±0.014	>0.150	-0.5±0.9	4.1±0.5
22.5	764, E	12.7	6.4	53.0± 1.4	26.8± 2.8	-	0.049±0.008	>0.150	-0.3±0.5	0.8±0.3
28.5	363, E	6.3	3.7	22.0± 0.7	16.2± 3.3	-	0.061±0.010	>0.150	0.1±0.3	1.2±0.4

SUBJECT:- M.C.(FEMALE), TREATMENT:- CARBIMAZOLE + T_3

* Normal range 276-591

TABLE 16 CONT'D

MONTH OF STUDY	FREE T4 INDEX, * CLINICAL STATUS	20 MIN. UPTAKE % dose		UNIDIRECTIONAL CLEARANCE ($ml\ min^{-1}$) (±SE)		EXIT RATE (min^{-1}) (±SE)		BINDING RATE (min^{-1}) (±SE)	INITIAL FREE UPTAKE (±SE) % dose	
		I	Tc	I	Tc	I	Tc	I	I	Tc
0	713, T	15.2	7.6	87.4± 1.1	77.4± 9.0	-	0.105±0.017	>0.150	1.3±0.3	-0.2±0.6
1	355, E	14.6	10.5	89.4±11.1	62.1± 9.8	0.018±0.008	0.062±0.014	0.003±0.010	0.4±0.6	2.0±0.6
2	220, E	12.8	8.9	66.6± 9.9	38.8± 3.7	0.015±0.036	0.044±0.006	-0.004±0.010	1.4±3.1	3.4±0.3
4.5	127, E	14.1	12.3	81.1± 8.2	38.0± 9.2	0.022±0.006	0.029±0.008	0.003±0.006	0.4±0.6	4.5±1.6
7	121, E	12.9	10.7	87.9± 6.0	47.0±13.2	0.036±0.005	0.057±0.017	0.007±0.002	0.7±0.4	6.4±0.7
11	265, E	8.4	2.4	38.8± 1.0	15.0± 2.4	-	0.077±0.017	>0.150	-0.1±0.3	-0.4±0.3
18	770, T	18.5	10.0	91.9± 1.7	38.2± 2.9	-	0.034±0.004	>0.150	0.4±0.4	2.0±0.3
23	142, E	12.8	7.3	97.1± 6.5	40.1± 2.9	0.046±0.006	0.070±0.006	0.009±0.002	1.1±0.4	3.1±0.2

SUBJECT:- M.A.(FEMALE) TREATMENT:- CARBIMAZOLE + T_3

* Normal range 276-591

TABLE 16 CONT'D

MONTH OF STUDY	FREE T_4 INDEX, * CLINICAL STATUS	20 MIN. UPTAKE % dose		UNIDIRECTIONAL CLEARANCE (±SE) ml min^{-1}		EXIT RATE (±SE) min^{-1}		BINDING RATE (±SE) min^{-1}	INITIAL FREE UPTAKE (±SE) % dose	
		I	Tc	I	Tc	I	Tc	I	I	Tc
0	1200, E	50.7	34.1	401.4± 6.2	339.5±36.6	-	0.085±0.011	>0.150	3.4±0.8	9.8±1.8
1	1300, T	34.7	28.5	330.8±27.4	208.1±13.2	0.021±0.004	0.055±0.005	0.002±0.004	6.1±1.8	9.7±0.6
2	189, E	46.2	33.8	731.5±55.3	353.3±33.6	0.058±0.007	0.086±0.009	0.010±0.002	10.0±1.3	16.8±1.1
4	126, E	52.2	38.4	909.8±64.3	711.9±54.0	0.061±0.006	0.152±0.012	0.009±0.001	16.1±1.4	20.5±1.2
6	83, E	55.5	37.9	640.5±46.0	387.6±34.5	0.046±0.005	0.093±0.008	0.008±0.002	17.8±1.5	23.9±1.3
9	263, E	41.6	26.9	360.0±18.9	186.6±25.7	0.041±0.003	0.083±0.011	0.010±0.001	10.7±0.9	17.2±1.4
12	319, E	26.5	23.6	188.9±12.8	124.2±17.3	0.032±0.005	0.059±0.009	0.012±0.003	3.8±0.7	11.1±1.1
14	402, E	12.1	12.5	48.0± 1.2	18.4±13.3	-	0.020±0.040	>0.150	2.7±0.3	10.9±2.5
17	430, E	9.1	8.0	29.5± 0.8	9.4± 4.3	-	0.020±0.006	>0.150	2.5±0.2	8.2±1.9
22	1480, T	60.1	26.2	529.0± 7.7	326.0±14.2	-	0.108±0.005	>0.150	4.9±0.9	7.8±0.6
30	218, E	13.2	8.6	87.9± 4.7	43.8± 3.0	0.037±0.004	0.061±0.004	0.020±0.002	1.9±0.2	4.4±0.2

SUBJECT:- D.W.(MALE) TREATMENT:- PROPYLTHIOURACIL

* Normal range 276-591

TABLE 16 CONT'D

MONTH OF STUDY	FREE T_4 INDEX, * CLINICAL STATUS	20 MIN. UPTAKE % dose		UNIDIRECTIONAL CLEARANCE (±SE) ml min^{-1}		EXIT RATE (±SE) min^{-1}		BINDING RATE (±SE) min^{-1}	INITIAL FREE UPTAKE (±SE) % dose	
		I	Tc	I	Tc	I	Tc	I	I	Tc
0	1916, T	46.0	32.0	324.4± 4.1	303.1±13.7	-	0.073±0.005	>0.150	1.6±0.7	2.9±0.7
1.5	89, E	43.6	34.9	360.0±13.7	246.6±15.3	0.035±0.003	0.068±0.005	0.008±0.001	6.2±0.7	13.1±0.9
2.5	341, E	37.8	35.1	233.6±11.6	196.3±17.4	0.017±0.001	0.057±0.006	-0.001±0.003	7.6±2.5	11.8±1.2
4.5	115, E	42.3	44.2	270.3±15.6	235.8±11.5	0.018±0.002	0.049±0.003	0.002±0.004	7.6±2.0	14.2±0.8
7	134, E	38.5	44.2	237.5±10.7	202.9± 6.5	0.016±0.003	0.034±0.002	0.016±0.004	3.3±0.6	9.0±0.5
10	163, E	40.9	44.9	196.5±17.2	155.3±11.0	0.007±0.004	0.019±0.002	0.006±0.016	7.5±5.0	12.2±3.0
13	292, E	45.8	53.9	250.0±13.8	222.0±12.3	0.011±0.002	0.026±0.002	0.010±0.004	11.2±2.0	16.9±1.4
16	1534, T	56.0	44.2	373.6± 5.0	264.0±13.1	-	0.032±0.003	>0.150	2.4±0.8	6.9±1.0
19.5	158, E	33.5	33.6	115.9± 4.0	119.7± 3.8	0.004±0.001	0.030±0.002	0.002±0.006	4.0±2.1	4.4±0.4
21.5	315, E	22.9	23.6	116.7± 6.6	84.7± 1.9	0.007±0.004	0.016±0.001	0.014±0.013	0.5±0.5	1.5±0.3
26	920, T	23.1	21.1	105.8± 8.8	85.5± 2.7	0.005±0.004	0.026±0.002	-0.004±0.003	1.7±0.5	2.8±0.3

SUBJECT:- A.McG(FEMALE), TREATMENT:- CARBIMAZOLE + T_3

* Normal range 276-591

TABLE 16 CONT'D

MONTH OF STUDY	FREE T_4 INDEX, * CLINICAL STATUS	20 MIN. UPTAKE % dose		UNIDIRECTIONAL CLEARANCE (±SE) ml min^{-1}		EXIT RATE$_1$ (±SE) min^{-1}		BINDING RATE (±SE) min^{-1}	INITIAL FREE UPTAKE (±SE) % dose	
		I	Tc	I	Tc	I	Tc	I	I	Tc
0	1330, T	-	-	-	-	-	-	-	-	-
1	192, E	21.1	17.2	301.6±34.5	126.4±28.0	0.079±0.010	0.074±0.015	0.007±0.001	9.6±1.1	15.1±1.3
2	<79, E	19.7	15.0	405.1±39.1	156.2±39.8	0.113±0.009	0.095±0.021	0.006±0.001	7.4±0.9	14.3±1.4
4	110, E	25.9	18.1	254.9±19.5	149.5± 8.2	0.049±0.006	0.078±0.004	0.013±0.002	5.8±0.9	10.0±0.5
6	<79, E	19.0	15.2	263.8±25.9	156.6±15.3	0.086±0.010	0.113±0.011	0.008±0.001	4.0±0.9	8.6±0.7
10	187, E	19.4	17.8	215.0±20.4	83.8±10.0	0.070±0.008	0.051±0.006	0.007±0.001	5.3±0.8	12.2±0.6
12.5	<79, E	15.7	9.2	199.5±16.3	92.5±10.0	0.077±0.008	0.087±0.010	0.014±0.001	3.3±0.6	5.3±0.5
15	465, E	26.6	8.4	137.4± 2.9	49.2± 6.2	-	0.064±0.009	>0.150	0.9±0.7	3.6±0.4
17	932, T	36.2	9.9	259.3± 7.3	114.6±15.7	-	0.111±0.017	>0.150	0.4±1.2	3.4±0.8
19	790, T	41.2	15.9	327.3± 4.5	162.8±13.2	-	0.085±0.008	>0.150	-0.3±0.7	2.9±0.7
25	89, E	23.8	13.4	244.9±17.2	113.2±10.0	0.064±0.006	0.097±0.008	0.006±0.001	5.1±0.8	7.7±0.6

SUBJECT:- A.C.(MALE) TREATMENT:- CARBIMAZOLE + T_3

* Normal range 276-591

TABLE 16 CONT'D

MONTH OF STUDY	FREE T_4 INDEX, * CLINICAL STATUS	20 MIN. UPTAKE % Dose		UNIDIRECTIONAL CLEARANCE (±SE) ml min^{-1}		EXIT RATE $_1$ (±SE) min^{-1}		BINDING RATE (±SE) min^{-1}	INITIAL FREE UPTAKE (±SE) % dose	
		I	Tc	I	Tc	I	Tc	I	I	Tc
0	1322, T	27.5	16.6	177.0± 2.7	103.4± 3.8	-	0.062±0.004	>0.150	-2.2±0.5	1.2±0.3
1	309, E	25.4	19.1	187.9± 6.4	108.3± 5.8	0.016±0.002	0.041±0.004	0.001±0.003	-0.8±0.6	1.3±0.5
2	159, E	24.2	19.1	181.9±11.3	108.1± 3.9	0.030±0.005	0.052±0.003	0.006±0.003	-0.3±0.8	3.2±0.3
4	<79, E	17.0	19.8	108.1±31.1	86.2± 8.4	0.012±0.007	0.035±0.005	-0.008±0.009	2.5±1.4	7.1±0.7
6	<79, E	15.5	14.8	114.5±12.0	79.9± 7.1	0.027±0.007	0.052±0.006	0.004±0.005	1.6±0.6	5.4±0.4
8.5	<79, E	9.7	16.3	56.5± 7.1	51.8±10.5	0.023±0.006	0.034±0.007	0.007±0.005	2.3±0.5	11.3±1.1
11.5	<79, E	7.8	6.1	82.7± 6.0	43.8± 5.2	0.054±0.007	0.084±0.012	0.004±0.002	0.2±0.3	2.1±0.2
15	206, E	7.8	3.4	40.3± 1.0	21.7± 3.3	-	0.068±0.015	>0.150	-0.1±0.3	0.1±0.3
17	348, E	8.1	3.7	38.9± 0.7	18.5± 2.1	-	0.039±0.007	>0.150	0.9±0.2	0.2±0.2
24	538, E	13.8	6.8	79.8± 2.7	49.1± 5.3	-	0.060±0.007	>0.150	0.1±0.6	3.6±0.2

SUBJECT:- D.K.(MALE) TREATMENT:- CARBIMAZOLE + T_3

* Normal range 276-591

TABLE 16 CONT'D

MONTH OF STUDY	FREE T_4 INDEX, * CLINICAL STATUS	20 MIN. UPTAKE % dose		UNIDIRECTIONAL CLEARANCE (±SE) ml min^{-1}		EXIT RATE (±SE) min^{-1}		BINDING RATE (±SE) min^{-1}	INITIAL FREE UPTAKE (±SE) % dose	
		I	Tc	I	Tc	I	Tc	I	I	Tc
0	1195, T	-	8.6	-	62.0± 6.8	-	0.096±0.013	-	-	2.4±0.4
1	693, T	-	8.0	-	59.8± 2.0	-	0.093±0.004	-	-	0.7±0.2
2	552, E	-	8.3	-	90.3± 3.5	-	0.130±0.006	-	-	0.7±0.2
6	134, E	-	16.7	-	486.6±15.1	-	0.339±0.011	-	-	8.4±0.4
SUBJECT:- S.D. (FEMALE), TREATMENT:- CARBIMAZOLE + T_3						* Normal range 276-591				

TABLE 17

EXIT AND BINDING RATES, AND EFFECTIVE CLEARANCE OF IODIDE DURING ANTITHYROID DRUG THERAPY

Subject	Mean exit rate (±se) min^{-1}	Mean binding rate (±se) min^{-1}	Mean effective clearance (±se) % *
M.C.	0.062±0.015	0.023±0.009	27.0±9.1
M.A.	0.027±0.006	0.004±0.002	12.0±6.7
D.W.	0.043±0.006	0.010±0.002	19.0±3.0
A.McG.	0.013±0.003	0.006±0.002	30.2±9.4
A.C.	0.077±0.008	0.009±0.001	10.1±1.7
D.K.	0.027±0.006	0.002±0.002	8.2±7.1
Mean (±sd)	0.042±0.025	0.009±0.007	17.6±14.5

* as a percentage of unidirectional clearance

before 60 minutes, during therapy (Figure 6.1). In spite of manifesting a relatively high uptake of ^{132}I--iodide, after antithyroid drugs were discontinued, subject M.C. remained clinically euthyroid. Triiodothyronine was also discontinued of the subject's own accord. Concurrent estimates of plasma inorganic iodide (159) revealed abnormally low values, which suggested a state of iodide deficiency and hence greater uptake of radioiodide.

Some validation of the binding rate estimates for iodide during therapy is provided by the results given in Table 18. Shown there are the results of the perchlorate discharge tests in the present group of thyrotoxic subjects, combined with similar results from those other subjects with inhibited organification of iodide, studied in the present work. Analysis of the uptake phase of the studies provided estimates of the fraction of uptake that would be discharged by perchlorate (Section 2.6) and these are compared in the table with the observed values. In fact, the binding rate largely determines these dischargeable fractions (Section 6.3). The mean computed value ($\pm$ sd), $60.0\pm29.7\%$, in 19 subjects is not significantly different ($p > 0.1$) from the mean observed value, $58.9\pm25.8\%$. Thus although there may occur substantial discrepancies in individual cases, the kinetic analysis seems to provide a reliable estimate of the mean binding rate of iodide in a group of subjects.

A similar comparison is presented for pertechnetate in Table 19, but statistical analysis reveals a significant difference ($p<0.05$) between the mean computed dischargeable fraction ($\pm$sd), $77.8\pm9.0\%$, and the observed value, $92.5\pm6.5\%$, in the seven subjects studied.

TABLE 18

PROPORTION (%) OF RADIOIODIDE UPTAKE DISCHARGED BY PERCHLORATE AT 60 MINUTES; COMPARISON OF OBSERVED AND COMPUTED VALUES IN SUBJECTS WITH DEFECTIVE BINDING DUE TO DRUGS OR DISEASE

Subject	Observed discharge (±se) %	Computed discharge (±se) %
M.A.	78.6±2.3	80.8±16.0
D.W.	54.2±1.5	40.3± 5.4
A.McG.	75.9±4.9	53.7±11.9
A.C.	69.1±3.9	46.9±4.2
D.K.	83.7±3.0	89.0±16.5
P.Y.	85.3±0.9	86.8±17.5
M.D.	47.0±18.1	57.7±22.5
S.W.	11.0±0.5	13.8±5.0
W.McC.	52.3±0.6	49.5±3.0
F.McT.	39.0±4.4	86.6±26.8
L.McL.	98.1±0.5	69.9±6.6
M.S.	64.8±2.5	64.3±7.5
JA.S.	11.0±0.4	15.7±3.3
B.S.	25.2±0.7	17.7±4.6
MY.S.	29.5±0.9	30.8±3.1
J.J.	71.2±2.4	50.1±7.9
C.I.	81.3±1.6	62.6±7.0
C.McI.	81.1±2.6	105.5±24.4
M.W.	62.4±4.3	118.4±37.0
Mean (±sd)	58.9±25.8	60.0±29.7

TABLE 19

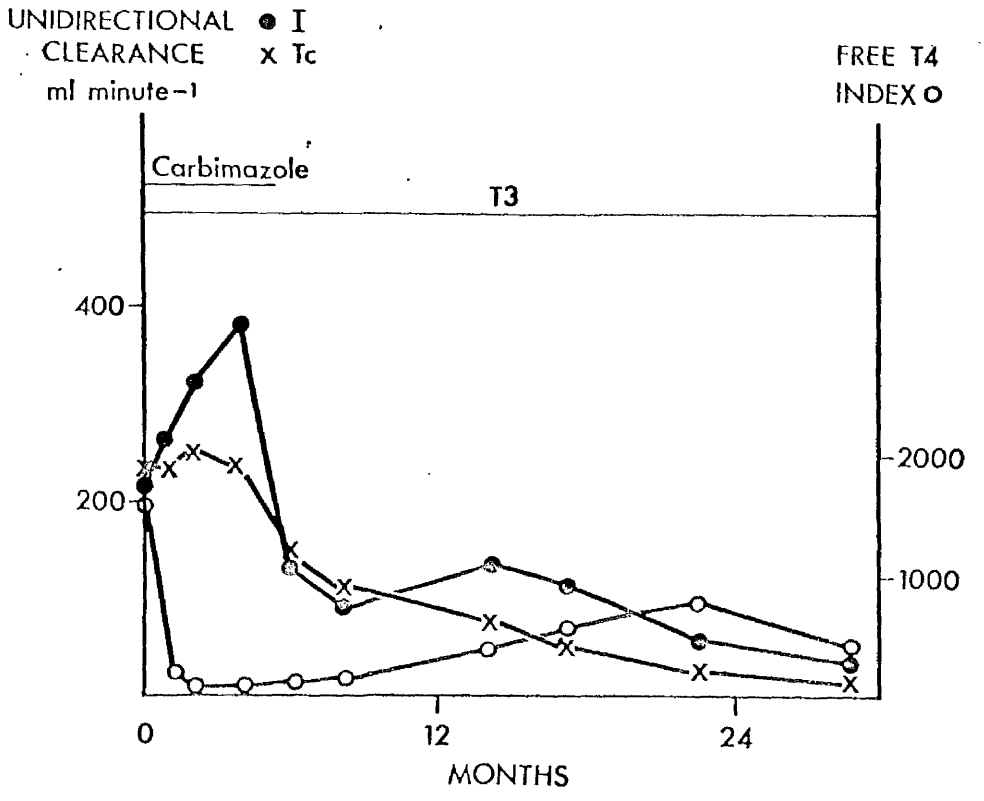
BINDING RATE AND PROPORTION (%) OF ^{99m}Tc-PERTECHNETATE UPTAKE DISCHARGED BY PERCHLORATE AT 60 MINUTES IN THYROTOXIC SUBJECTS; COMPARISON OF OBSERVED AND COMPUTED VALUES OF THE LATTER

Subject	Binding rate (±se) min^{-1}	Observed discharge (±se) %	Computed discharge (±se) %
M.C.	0.005±0.001	95.2±12.7	73.6±5.2
M.A.	0.003±0.002	82.2± 3.5	80.9±8.5
D.W.	0.003±0.001	89.8± 6.5	88.3±12.1
A.McG.	0.004±0.001	96.4±2.8	77.3±8.2
A.C.	0.004±0.001	87.1±3.9	70.2±3.0
D.K.	0.002±0.001	101.4±7.4	89.0±7.7
S.D.	0.007±0.001	95.1±0.8	65.0±4.4
Mean(±sd)	0.004±0.002	92.5±6.5	77.8±9.0

This suggests that the estimated binding rate for the group (±sd), 0.004 ± 0.002 minute^{-1}, is too large by a factor of approximately 3. (The individual values for binding rate given in Table 19 are the means of all the estimated values for each subject. There was no significant difference ($p > 0.1$) between the estimated binding rates for pertechnetate on and off antithyroid drugs).

Figure 6.3 shows the variation of unidirectional clearance and 20 minute uptake of iodide and pertechnetate, along with the free thyroxine index, during the course of management of each subject. The pattern of change is basically the same for each anion; both unidirectional clearance and 20 minute uptake being greater in the case of iodide. However, in subjects D.K. and A.McG. there are occasions when the uptake of pertechnetate exceeds that for iodide. As a rule both unidirectional clearance and 20 minute uptake decrease or remain constant with time, unless there is clinical relapse off antithyroid drugs, or an increase in plasma concentration of thyroid stimulating hormone (TSH), as in subjects S.D. and D.W. (Subject S.D. had just suffered a severe domestic crisis before the final investigation and there was doubt as to whether T3 was being taken as prescribed. Subject D.W. did not receive T3.) In subject A.McG. there is discrepancy between the changes in unidirectional clearance and those in 20 minute uptake.

Histograms of the 60 minute/20 minute uptake ratios for ^{132}I-iodide on and off antithyroid drugs are compared in Figure 6.4; the results for all subjects being combined. More than 95% of the values during therapy are ≤ 1.38, whilst in the untreated state the range is 1.42-2.22. Estimation of the 60 minute/20 minute uptake ratio or radioiodide would seem to be a possible method of checking whether



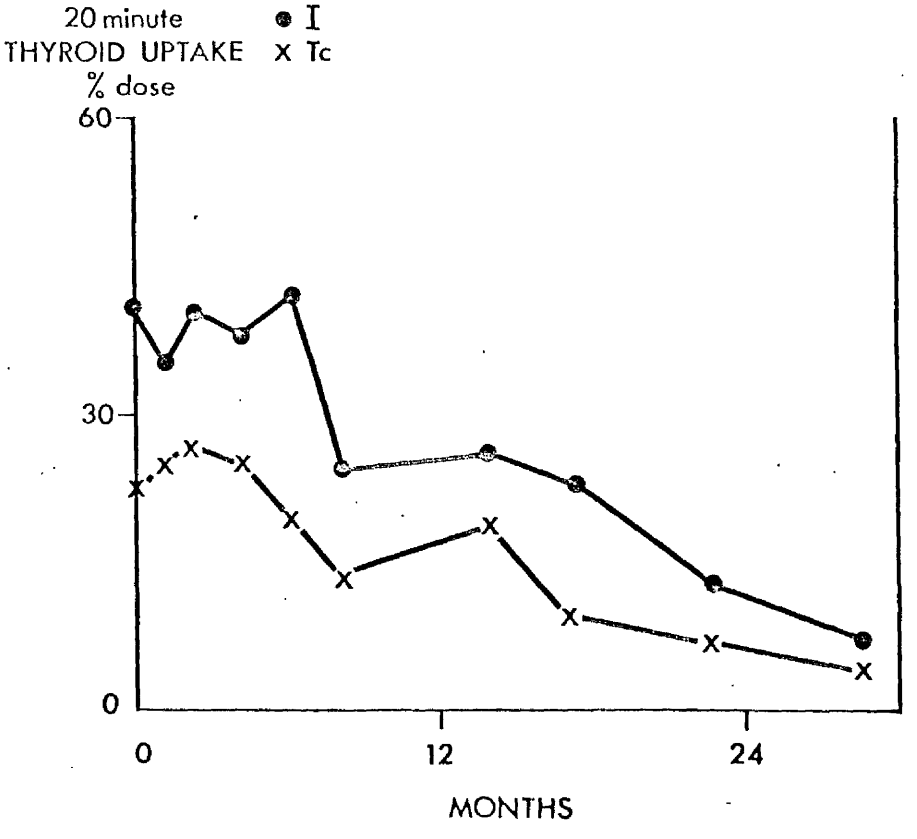
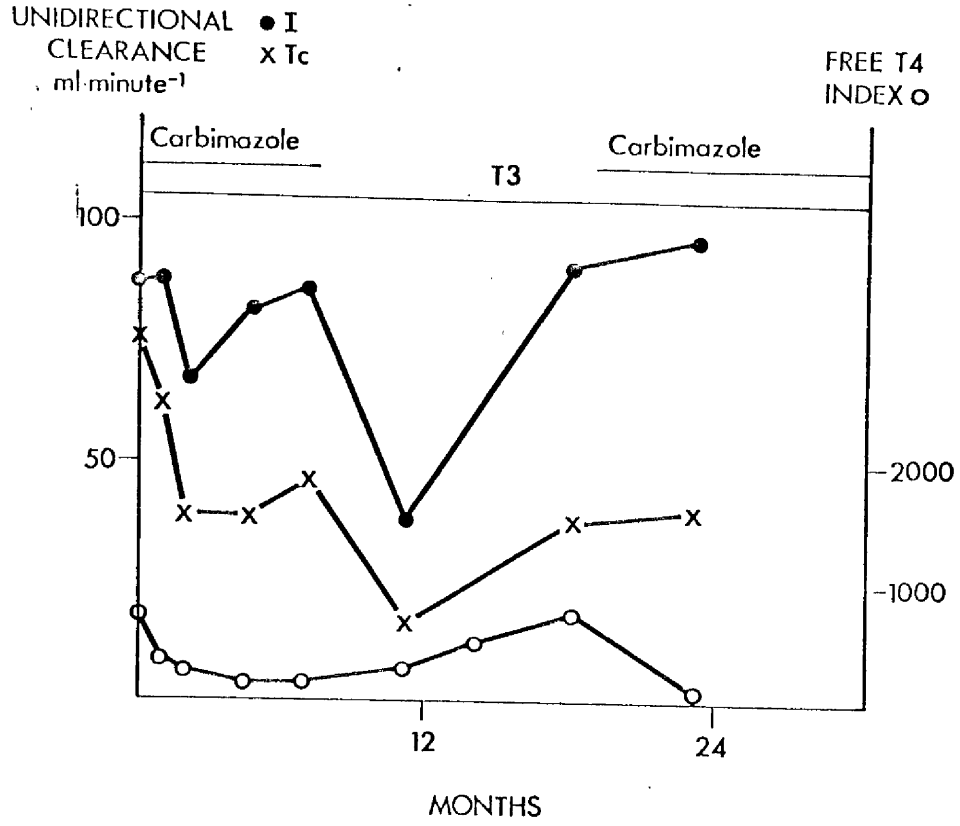


Figure 6.3 Changes in unidirectional clearance and 20 minute uptake of iodide and pertechnetate, and in the T4 index, in antithyroid drug therapy.



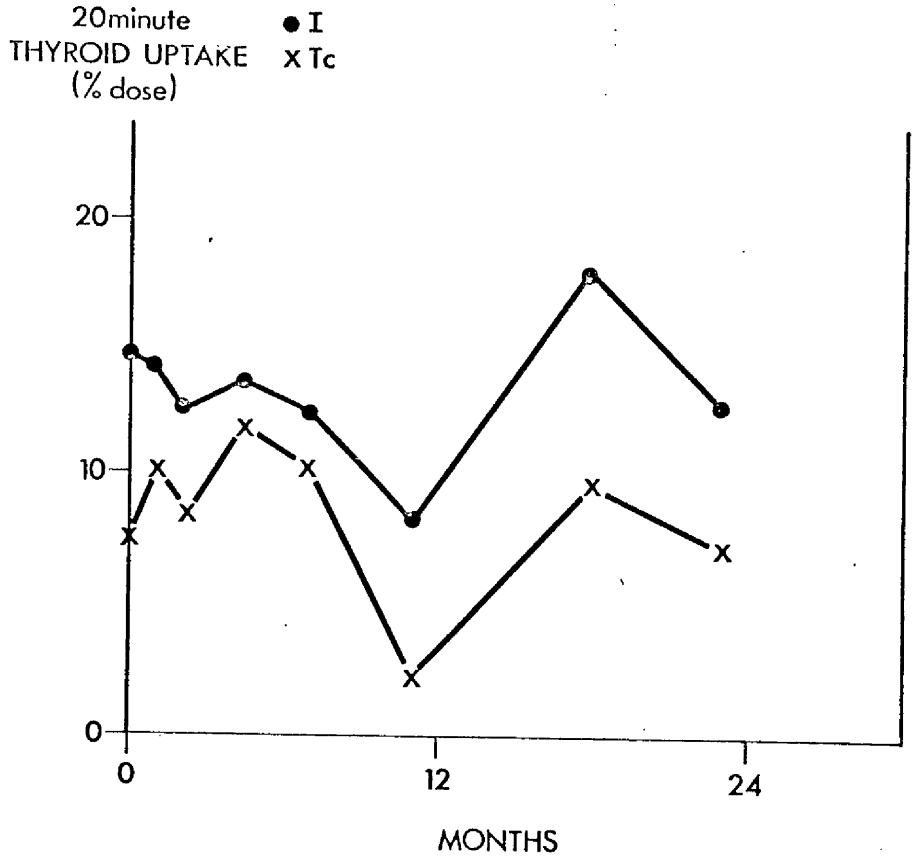
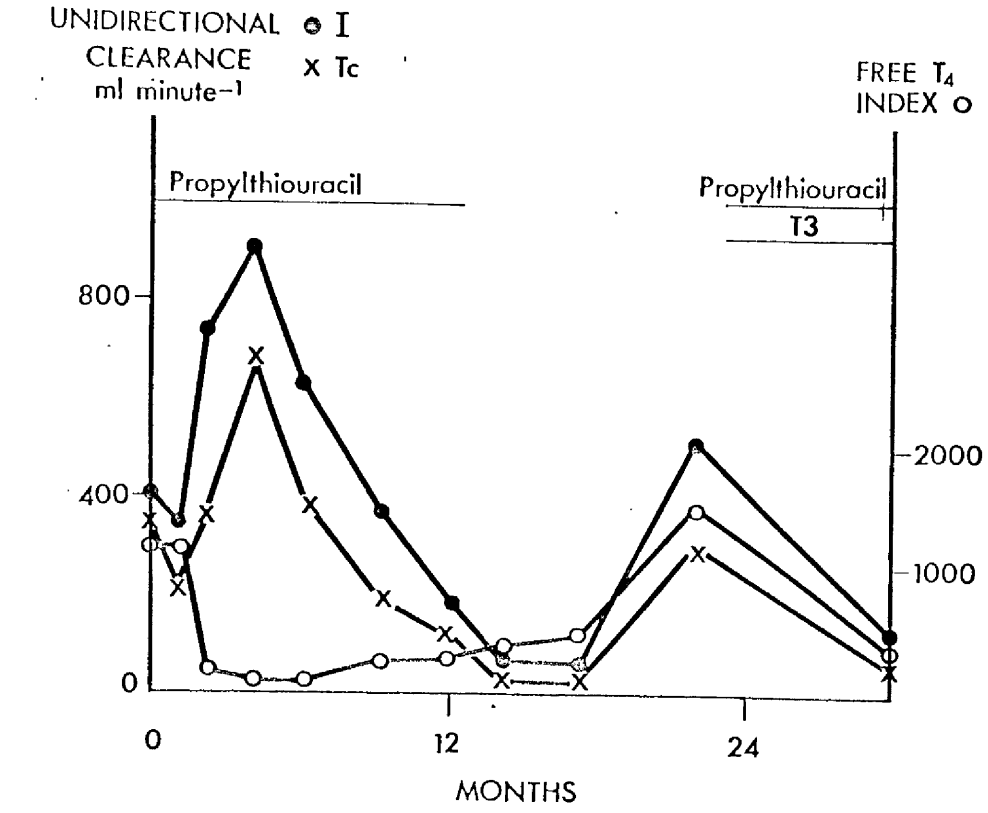
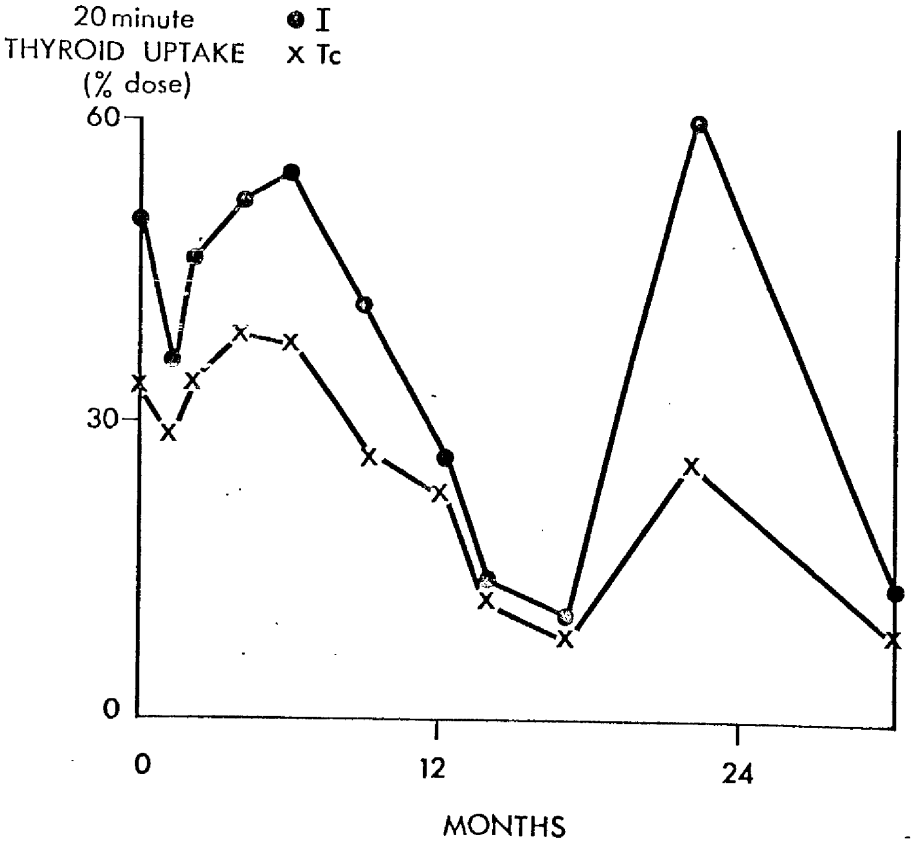


Figure 6.3 cont'd





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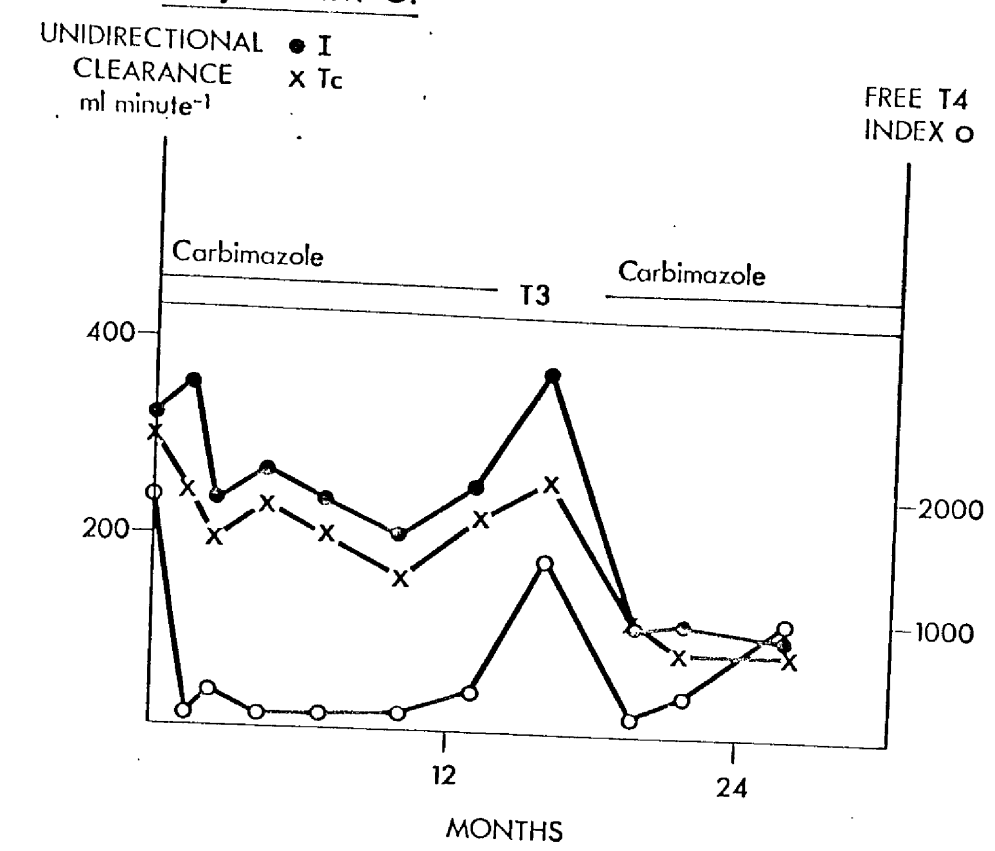

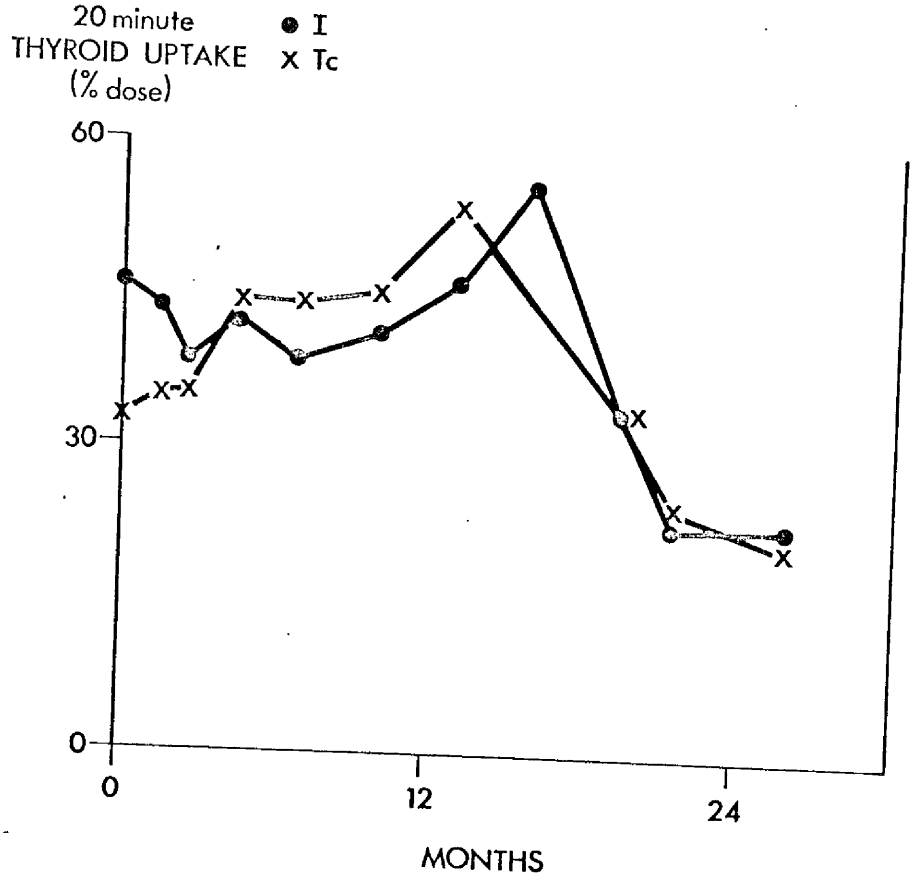

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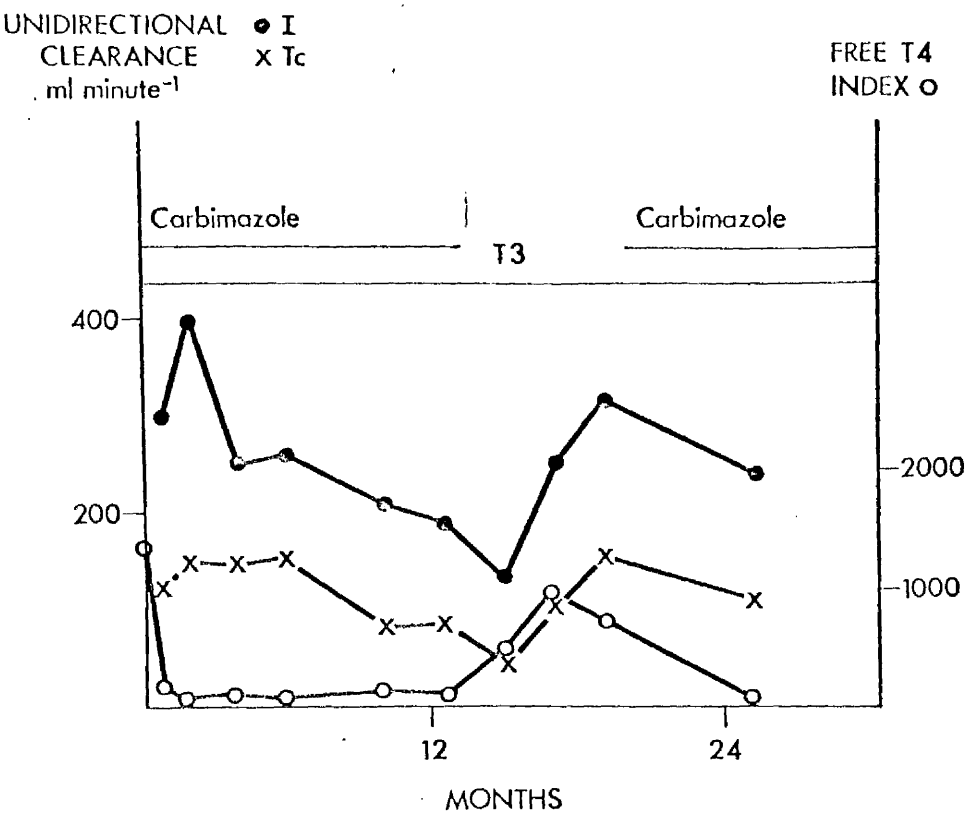

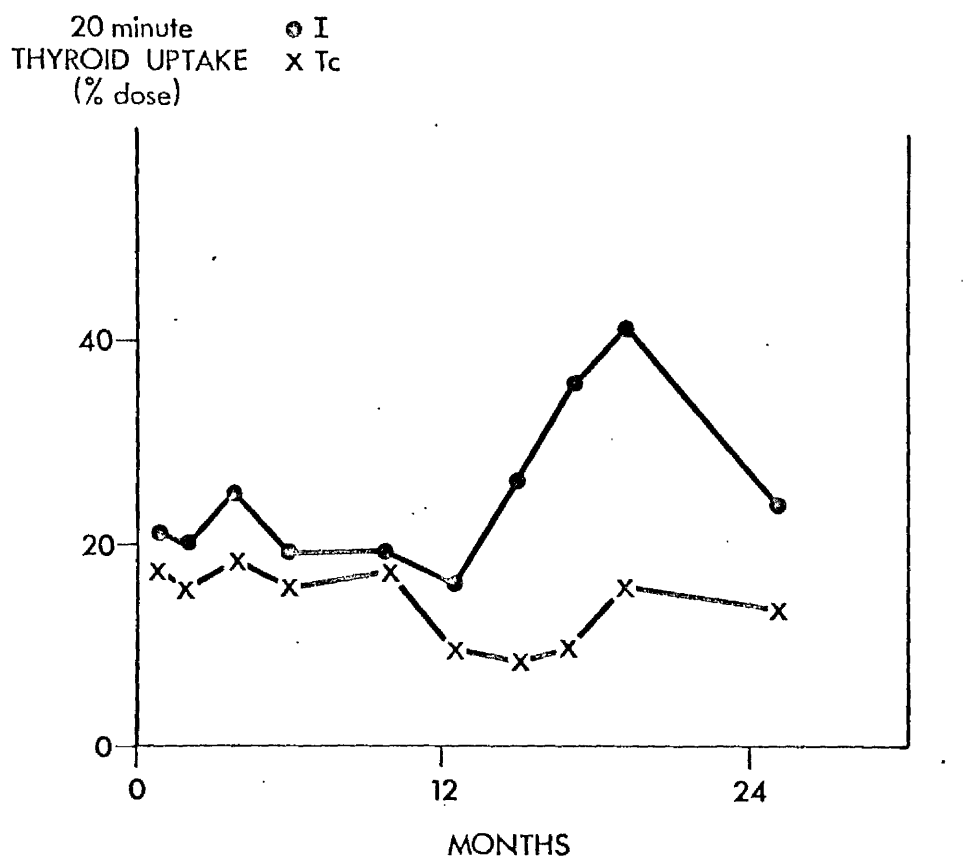

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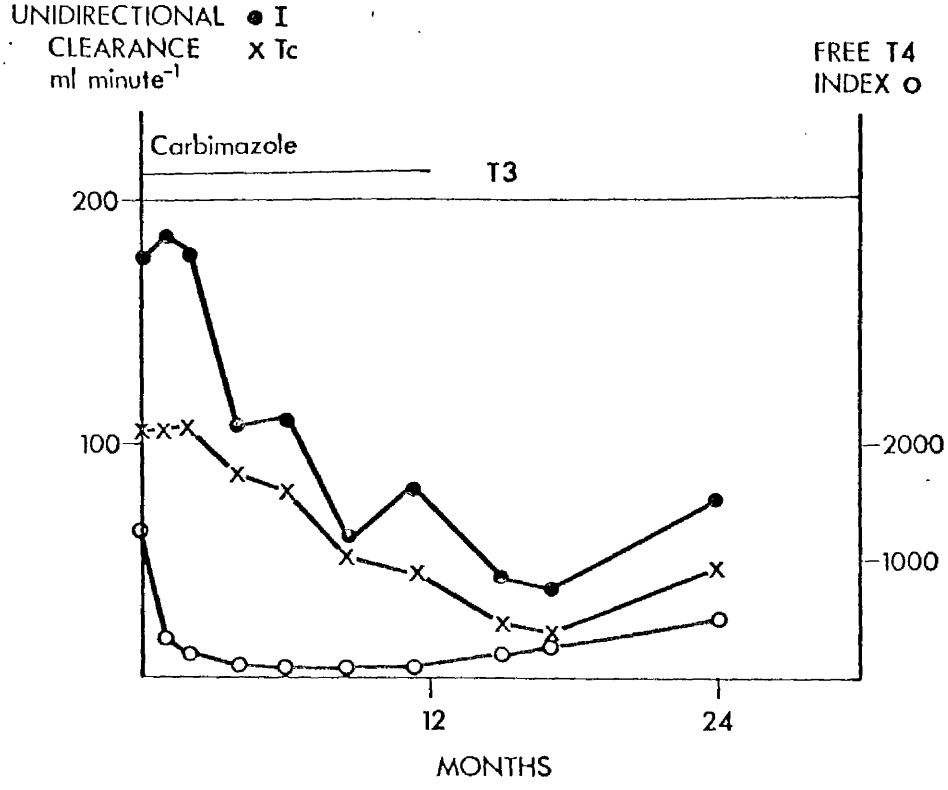

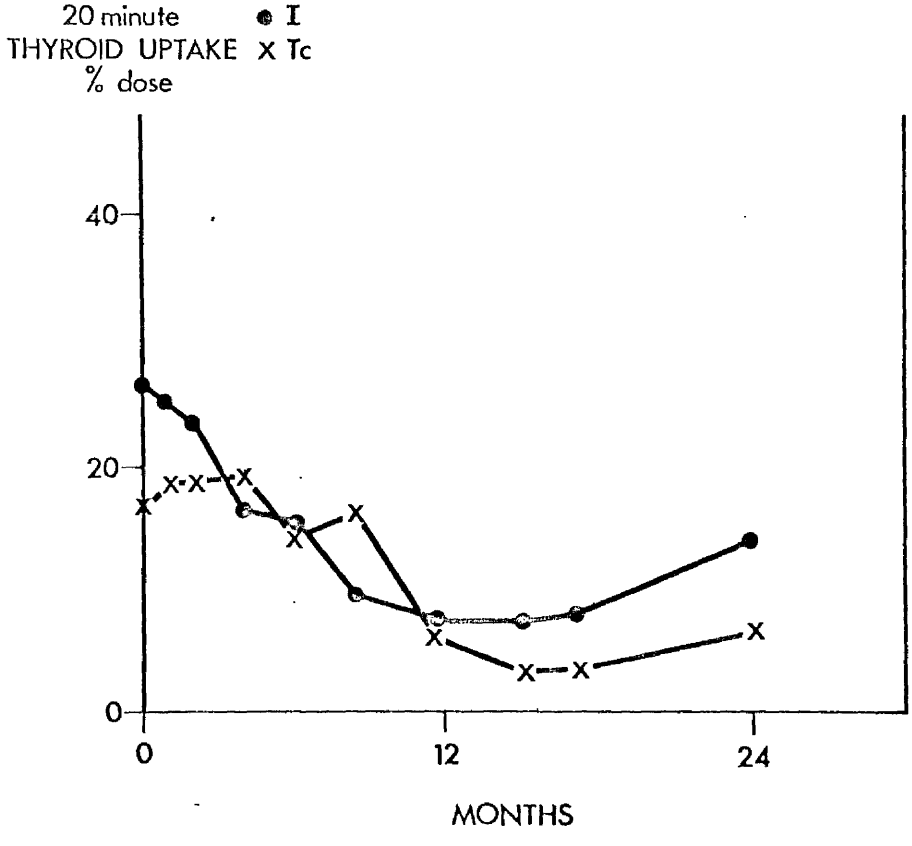

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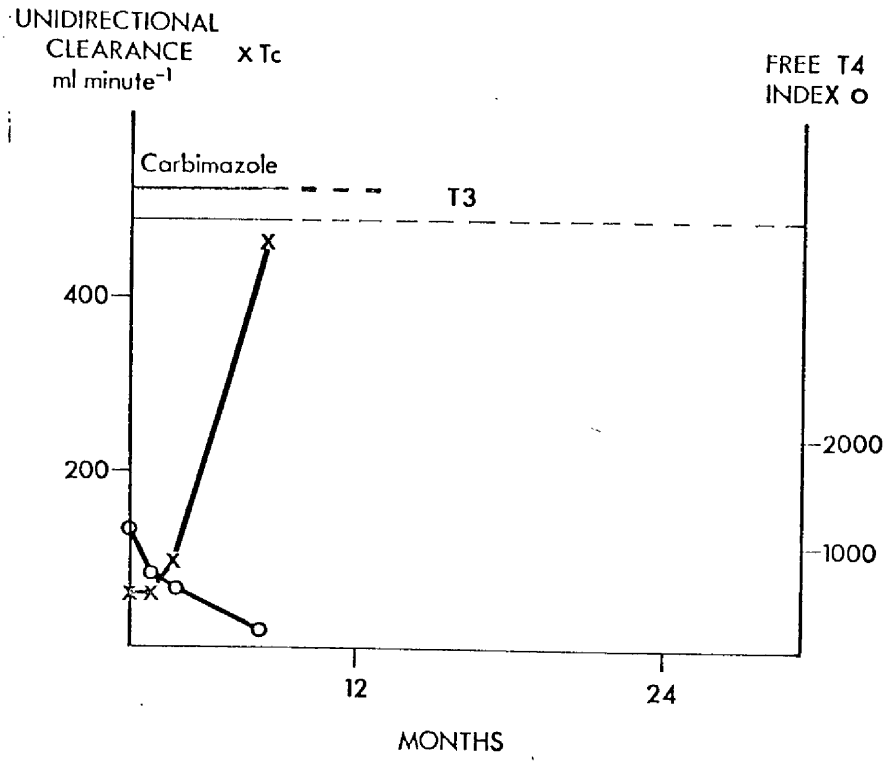

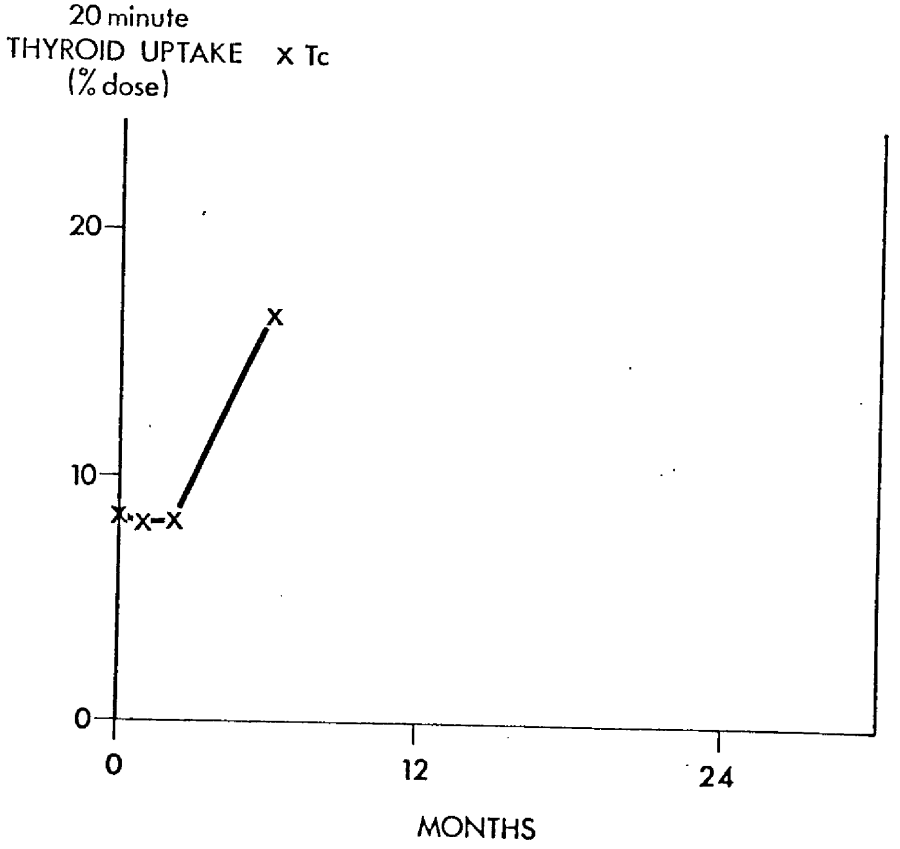

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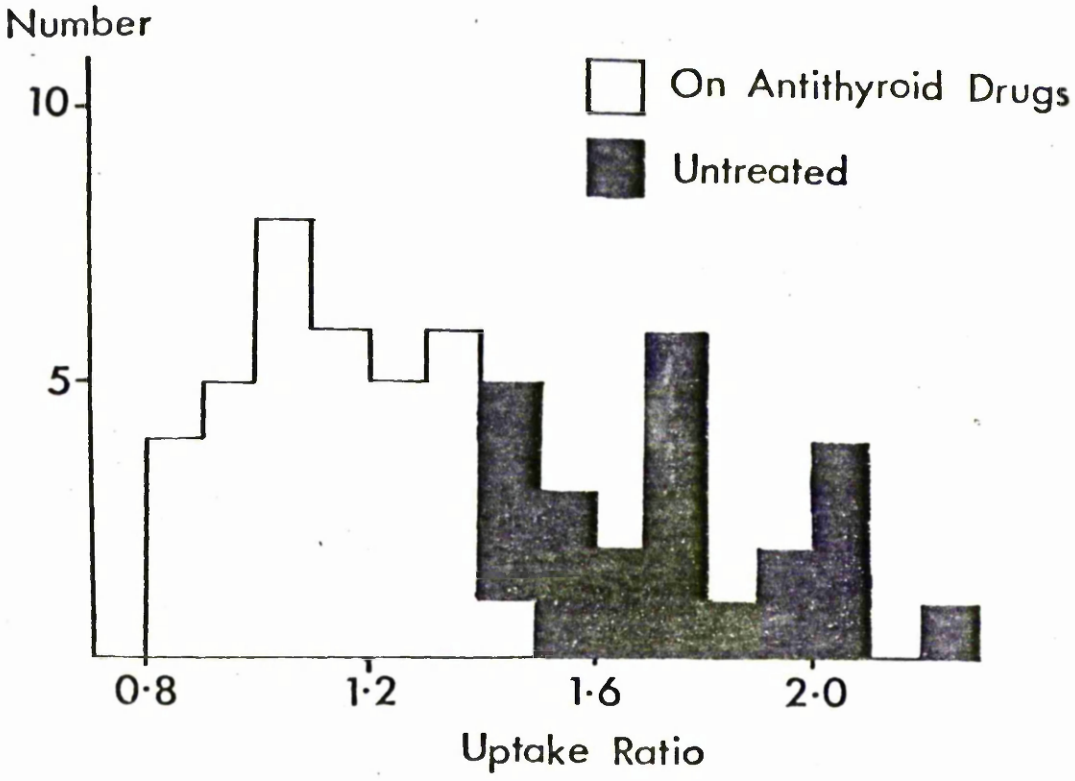


Figure 6.4 Histograms of the 60 minute/20 minute uptake ratios of radioiodide in thyrotoxic subjects on and off antithyroid drug therapy.

the prescribed antithyroid drug is being taken.

Correlation in the group of subjects as a whole between the unidirectional clearances, 20 minute uptakes and exit rates for iodide and pertechnetate is demonstrated in Figures 6.5-6.7, respectively. There is a good linear relationship ($p<0.001$) between the unidirectional clearances for the two anions (Figure 6.5), with the clearance for iodide being greater by a factor of 1.71 ± 0.50 (sd) on average. The 20 minute uptakes for iodide and pertechnetate, whilst the subjects were receiving antithyroid drugs, are similarly well correlated ($p<0.001$) (Figure 6.6), with the uptake for iodide being on average 1.25 ± 0.27 (sd) times greater. There is also linear correlation ($p<0.001$) between the exit rates for iodide and pertechnetate (Figure 6.7), but there are considerable individual deviations from the best-fit line. The exit rate for pertechnetate is on average 2.22 ± 1.26 (sd) times greater than that for iodide.

Some attempt was made to obtain relationships between the various parameters describing the behaviour of each anion. Figures 6.8 and 6.9 are plots of 20 minute uptake versus unidirectional clearance for iodide (on antithyroid drugs) and pertechnetate, respectively; the results for all subjects being combined. As can be seen there is significant linear correlation ($p<0.001$) between these two parameters for each anion, but there is considerable scatter about the best-fit lines. Relationship between initital free uptake and unidirectional clearance, when the subjects were clinically euthyroid (under the action of the antithyroid drug), is shown in Figures 6.10 and 6.11 for iodide and pertechnetate, respectively. In the case of iodide there is good linear correlation between the two parameters ($p<0.001$) with

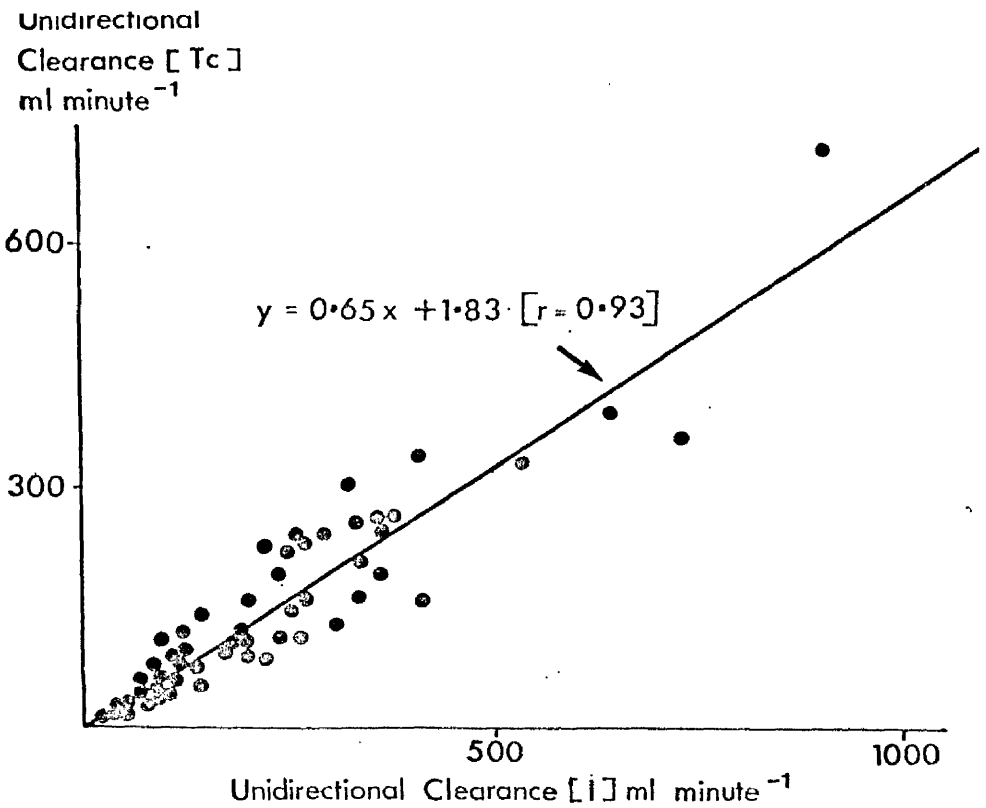


Figure 6.5 Correlation between the unidirectional clearances of iodide and pertechnetate in thyrotoxic subjects on and off antithyroid drug therapy.

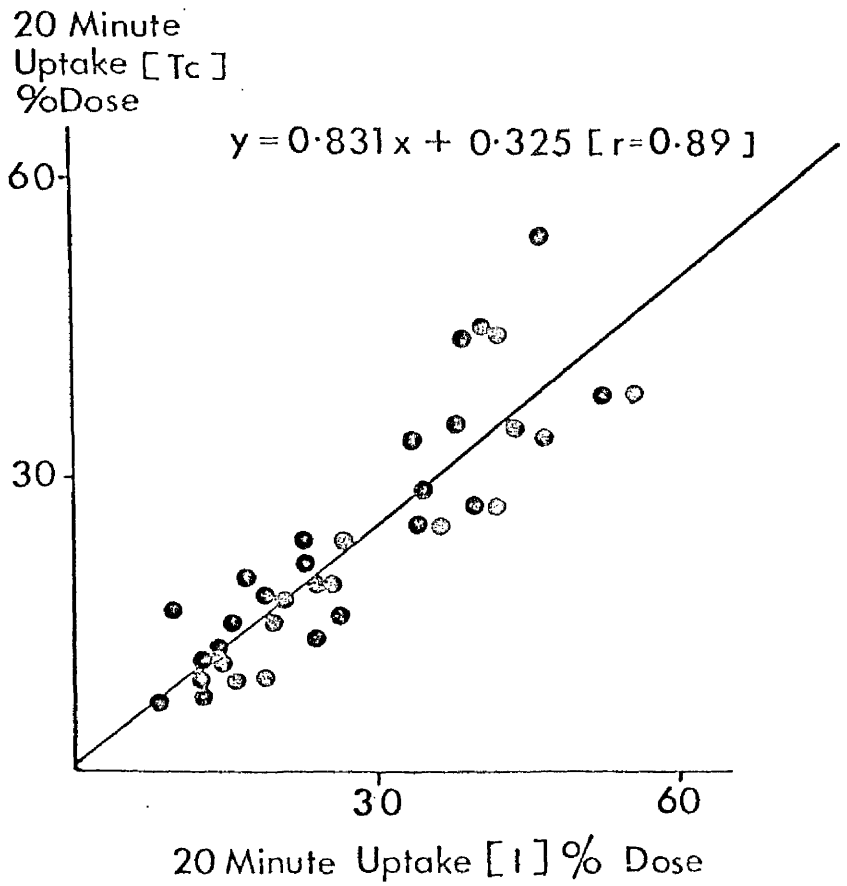


Figure 6.6 Correlation between the 20 minute thyroid uptakes of iodide and pertechnetate in thyrotoxic subject receiving antithyroid drugs.

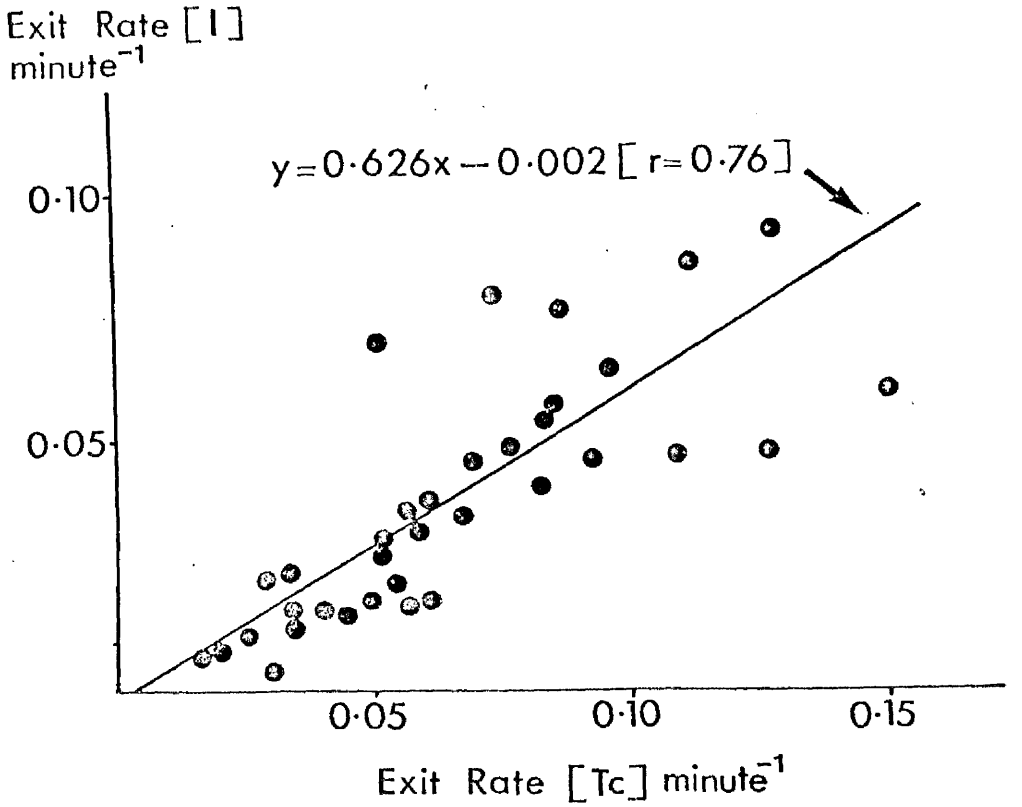


Figure 6.7 Correlation between the exit rates of iodide and pertechnetate in thyrotoxic subjects receiving antithyroid drugs.

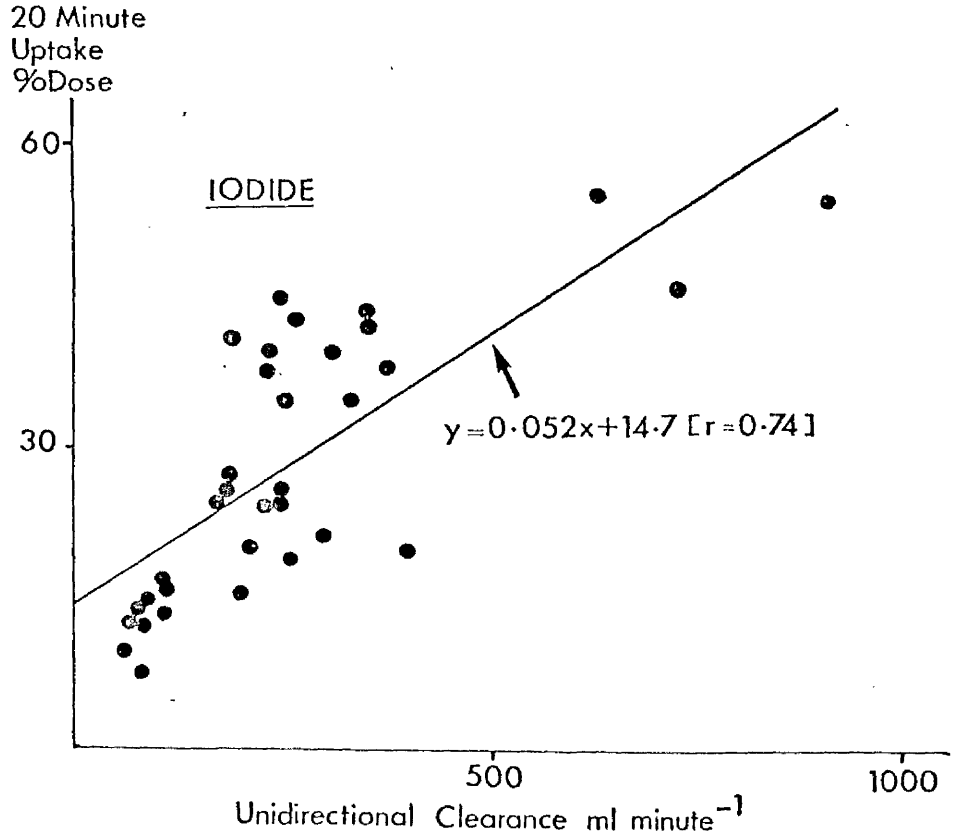


Figure 6.8 Correlation between the 20 minute thyroid uptake and unidirectional clearance of iodide in thyrotoxic subjects receiving antithyroid drugs.

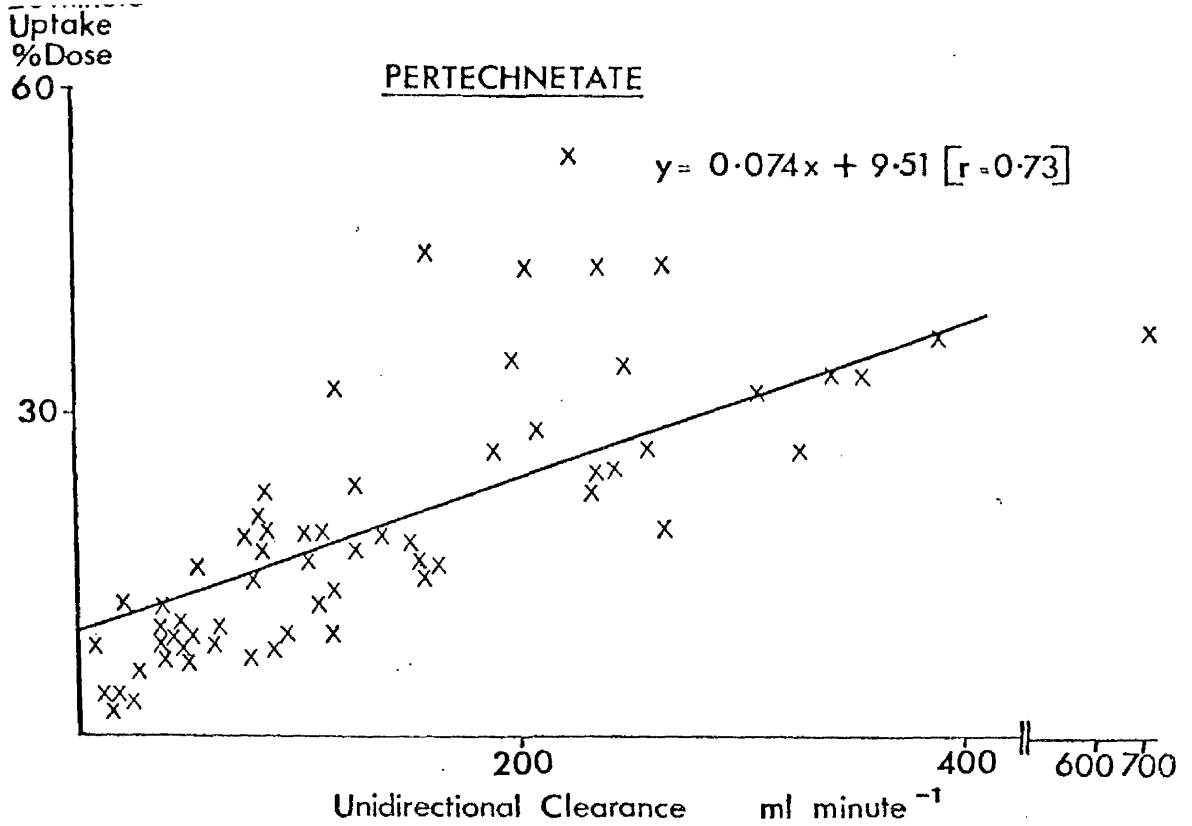


Figure 6.9 Correlation between the 20 minute thyroid uptake and unidirectional clearance of pertechnetate in thyrotoxic subjects on and off antithyroid drugs.

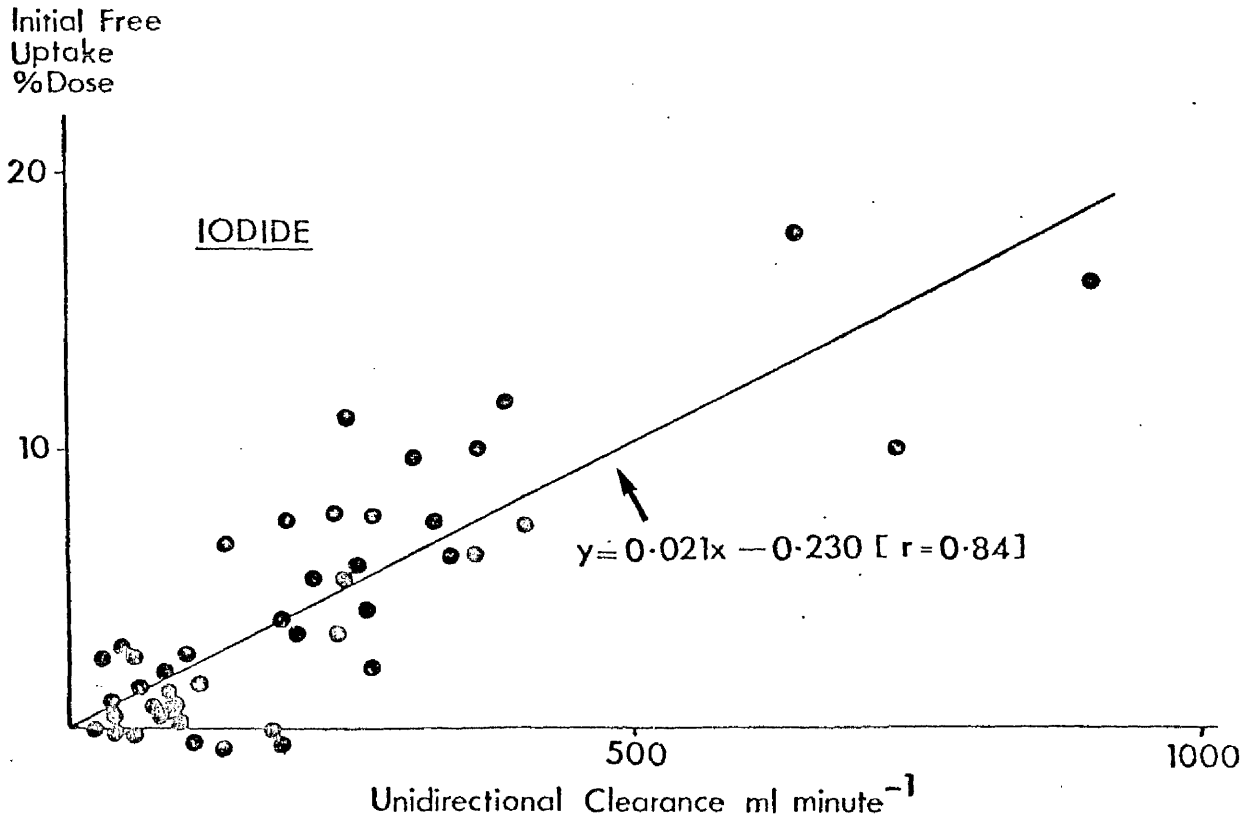


Figure 6.10 Correlation between the initial free uptake and unidirectional clearance of iodide in thyrotoxic subjects rendered euthyroid by the action of antithyroid drugs.

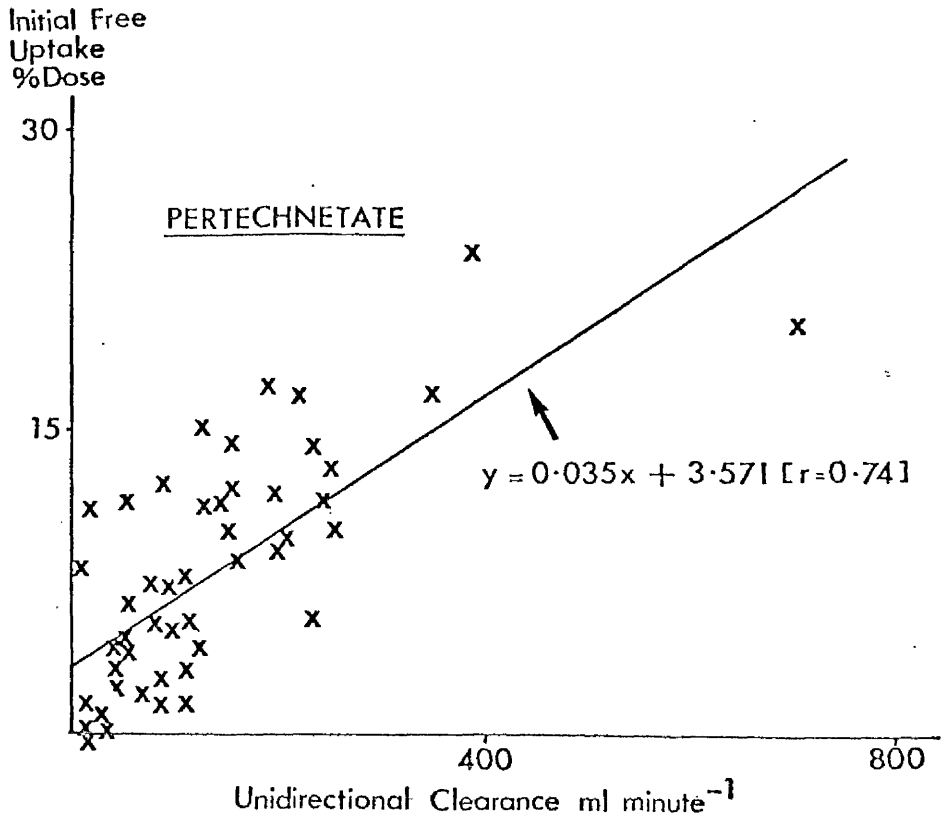


Figure 6.11 Correlation between the initial free uptake and unidirectional clearance of pertechnetate in thyrotoxic subjects rendered euthyroid by the action of antithyroid drugs.

the best-fit line virtually passing through the origin. There is also linear correlation ($p<0.001$) between the initial free uptake and unidirectional clearance for pertechnetate, but the best-fit line does not pass through the origin. The reason for this is evidently the occurrence of substantial initial free uptake when the estimated clearance is relatively low.

Figure 6.12 is a combined plot of the initial free uptakes for iodide versus those for pertechnetate when the subjects were clinically euthyroid. There is good linear correlation between the two parameters ($p<0.001$) but the best-fit line fails to pass through the origin due to there being substantial initial free uptake of pertechnetate, when the iodide value is low. The mean initial free uptake for pertechnetate is approximately twice as great as that for iodide.

The reason for dealing separately with the initial free uptake whilst the subjects were clinically euthyroid, is apparent from the results plotted in Figure 6.13. Shown there is the variation with time of the mean initial free uptake of iodide and pertechnetate during drug therapy. Statistical analysis shows that after 2-4 months therapy the values for both iodide and pertechnetate are significantly greater ($p<0.05$) than before treatment commenced. (Even after 1 month the values are greater, but the difference is not significant.) By these times all subjects had become clinically euthyroid under action of the antithyroid drugs.

In certain cases there is positive linear correlation between exit rate and unidirectional clearance (Table 20). Subjects M.C., D.W., A.McG. and S.D. manifest this relationship for pertechnetate ($p<0.02$) and subjects D.W. and A.McG. for iodide ($p<0.02$).

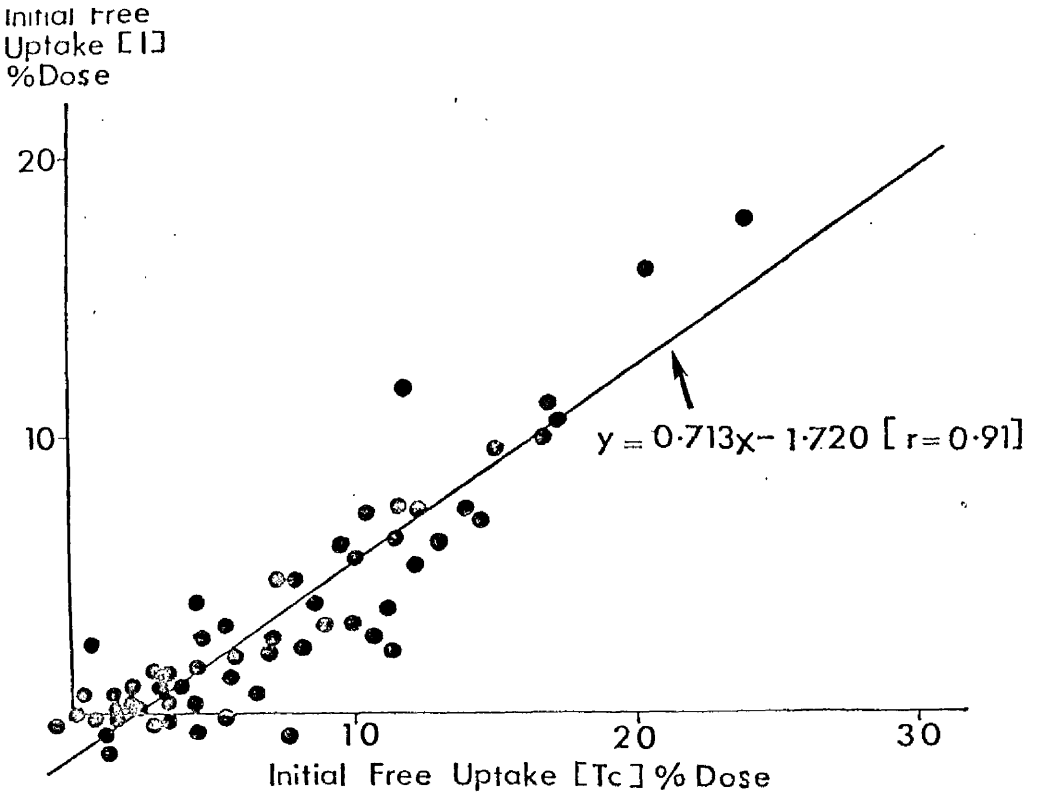


Figure 6.12 Correlation between the initial free uptakes of iodide and pertechnetate in thyrotoxic subjects rendered euthyroid by the action of antithyroid drugs.

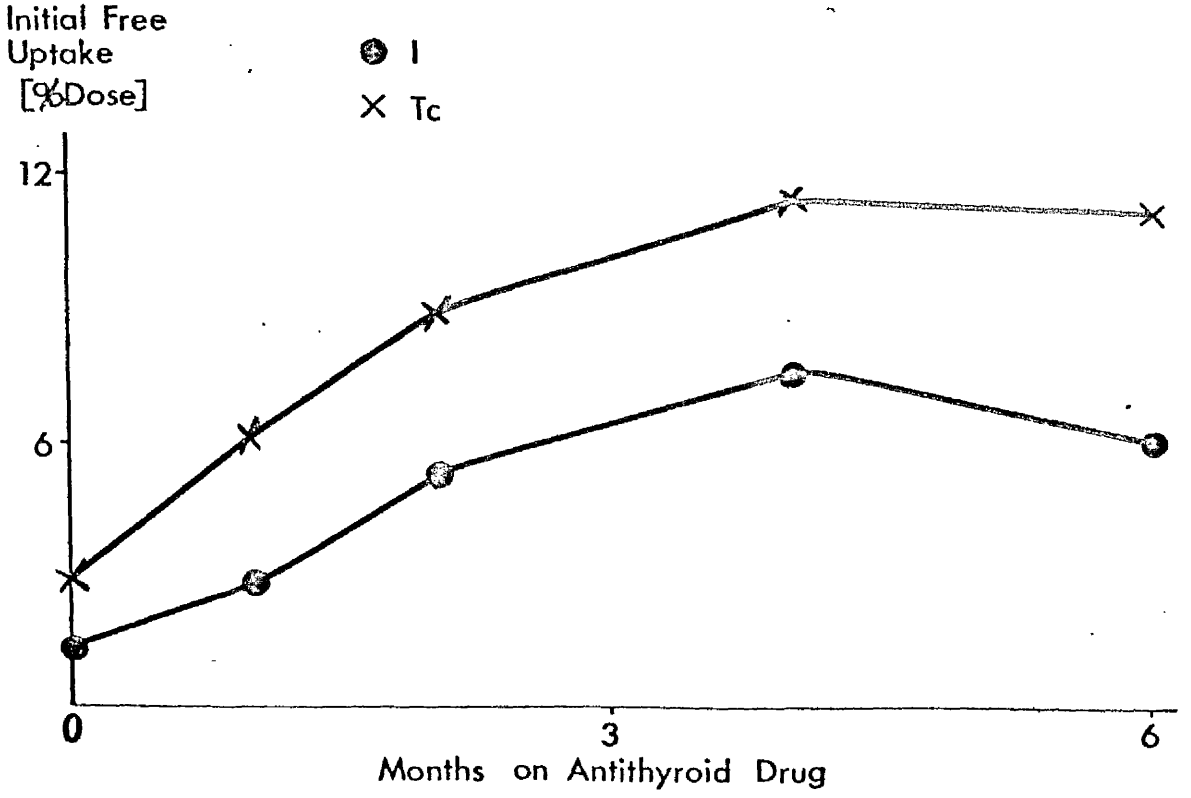


Figure 6.13 Variation of the mean initial free uptake of iodide and pertechnetate, with duration on antithyroid drugs, in thyrotoxic subjects.

TABLE 20

STATISTICAL ANALYSIS FOR POSITIVE LINEAR CORRELATION BETWEEN EXIT RATE AND UNIDIRECTIONAL CLEARANCE IN THYROTOXIC SUBJECTS

Subject	Iodide	Pertechnetate
	Significance level	Significance level
M.C.	$p>0.3$	$p<0.001$
M.A.	$p>0.2$	$p>0.2$
D.W.	$p<0.02$	$p<0.001$
A.McG.	$p<0.001$	$p<0.02$
A.C.	$p>0.1$	$p>0.1$
D.K.	$p>0.5$	$p>0.1$
S.D.	-	$p<0.005$

Mean exit rates from the uptake phase in each subject are compared in Table 21 with the mean values obtained during perchlorate discharge. Perchlorate increases exit rate significantly ($p<0.01$), by mean factors ($\pm$sd) of 3.42 ± 2.25 and 4.23 ± 2.98 for iodide and pertechnetate respectively. (The results for iodide are combined in Table 22 with those from all other subjects studied in the present work.)

Discussion. These serial kinetic studies have provided, for the first time, detailed information on the thyroidal uptake kinetics of iodide and pertechnetate during routine drug treatment of thyrotoxicosis. The results show that the course of drug-treated thyrotoxicosis is quite variable, and that there is no clear association between the parameters of the three-compartment model and the final outcome of therapy. There are insufficient data to confirm whether the likelihood of remission is greater when T3 suppression of uptake or unidirectional clearance is achieved (112, 116, 133, 140, 146-151). Furthermore it cannot be argued from the results that failure to achieve a normal uptake of iodide or pertechnetate is suggestive of impending relapse (138, 152). Thus the studies have not fulfilled earlier expectations that detailed kinetic analysis would provide better prediction of long-term response to antithyroid drug treatment. However the work is of considerable value because it provides further information on the short-term response to antithyroid drugs and on the relative behaviour of iodide and pertechnetate.

Carbimazole and propylthiouracil in the usual therapeutic dosage were found to reduce the binding rate of iodide to less than 6% of the value in the uninhibited gland. As a result the effective clearance (Section 2.7) was reduced from 75-100% of the unidirectional clearance

TABLE 21

EXIT RATE BEFORE AND AFTER PERCHLORATE IN THYROTOXIC SUBJECTS

Subject	IODIDE		PERTECHNETATE	
	Exit rate (±se) before perchlorate min^{-1}	Exit rate (±se) after perchlorate min^{-1}	Exit rate (±se) before perchlorate min^{-1}	Exit rate (±se) after perchlorate min^{-1}
M.C.	0.062 ± 0.015	-	0.092 ± 0.011	0.379 ± 0.123
M.A.	0.027 ± 0.006	0.079 ± 0.013	0.063 ± 0.010	0.156 ± 0.013
D.W.	0.042 ± 0.005	0.075 ± 0.011	0.075 ± 0.011	0.227 ± 0.032
A.McG.	0.013 ± 0.003	0.091 ± 0.010	0.039 ± 0.006	0.420 ± 0.149
A.C.	0.077 ± 0.008	0.108 ± 0.009	0.086 ± 0.006	0.312 ± 0.017
D.K.	0.027 ± 0.006	0.108 ± 0.011	0.053 ± 0.005	0.180 ± 0.014
S.D.	-	-	0.165 ± 0.059	0.351 ± 0.198
Mean (±sd)	0.041 ± 0.024	0.092 ± 0.016	0.082 ± 0.041	0.289 ± 0.103

TABLE 22

EXIT RATE OF IODIDE BEFORE AND AFTER PERCHLORATE IN SUBJECTS WITH DEFECTIVE BINDING DUE TO DRUGS OR DISEASE

Subject	Exit rate (±se) before perchlorate min^{-1}	Exit rate (±se) after perchlorate min^{-1}
M.C.	0.062±0.015	-
M.A.	0.027±0.006	0.079±0.013
D.W.	0.042±0.005	0.075±0.011
A.McG.	0.013±0.003	0.091±0.010
A.C.	0.077±0.008	0.108±0.009
D.K.	0.027±0.006	0.108±0.011
D.N.	0.030±0.010	-
A.McL.	0.017±0.003	-
J.McA.	0.116±0.023	-
R.D.	0.041±0.011	-
W.C.	0.014±0.009	-
P.Y.	0.019±0.003	0.088±0.004
M.McM.	0.010±0.006	0.049±0.008
E.K.	0.014±0.006	-
J.J.	0.118±0.010	0.142±0.010
C.I.	0.068±0.006	0.130±0.019
M.W.	0.008±0.001	0.062±0.006
J.McI.	0.024±0.004	0.094±0.016
M.D.	0.018±0.006	0.127±0.009
M.S.	0.060±0.007	0.116±0.016
B.S.	0.053±0.005	0.061±0.018
MY.S.	0.069±0.006	0.139±0.018
JA.S.	0.027±0.003	0.095±0.016
W.McC.	0.077±0.006	0.107±0.007
F.McT.	0.054±0.018	0.142±0.050
L.McL.	0.121±0.016	0.245±0.007
S.W.	0.040±0.005	0.116±0.030
Mean (±sd)	0.046±0.031	0.109±0.040

to a mean value of 17.6% during drug therapy. There was good agreement, on average, between the predictions of the kinetic analysis for iodide and the observations after perchlorate. The fact that both binding rate and effective clearance of iodide were significantly greater than zero support the accepted view that antithyroid drugs, in usual dosages, do not completely inhibit binding (137, 138).

Since there is some degree of inverse relationship between the two parameters (Section 2.7), any reduction in exit rate would increase effective clearance. This probably explains the resistance to antithyroid drugs ultimately manifested in subject A.McG. (Table 16) who was found to have an exceptionally low exit rate of iodide.

Measurement of the 60 minute/20 minute uptake ratio of radioiodide would seem to offer possibilities as a means of checking whether the patient is taking the prescribed antithyroid drug. In 97% of the measurements during drug therapy this ratio never exceeded 1.38. The observed values merely reflect the fact that when binding is inhibited the slope of the uptake curve is markedly reduced after 20 minutes. Off antithyroid drugs, however, the uptake ratio was never less than 1.42.

As in published work in rats (3), the estimated binding rate of pertechnetate was found not to be affected by antithyroid drugs. The observed degree of pertechnetate binding, as determined by perchlorate discharge, is almost identical to that found by Burke et al (7) in thyrotoxic subjects. Compartmental analysis, however, was found to overestimate the binding of pertechnetate in the present study. This may be a consequence of the arterio/venous approximation used in the

analysis (Section 3.2), exaggerated because of the small number of subjects. Earlier studies in a larger group of subjects (Section 5.4) produced a considerably lower mean value for the estimated binding rate of pertechnetate.

Unidirectional clearances of iodide and pertechnetate were found either to remain virtually constant or to decrease during drug therapy, except when plasma TSH was elevated. There was good correlation between the unidirectional clearances of the two anions, with that for iodide being greater on average by a factor of 1.71. Comparison with earlier studies (Section 5.4), where the mean ratio was 3.25, suggests that the relative magnitude of the unidirectional clearances for these two anions may be quite variable.

The observed correlation between the 20 minute uptake and unidirectional clearance, for both iodide and pertechnetate, justifies the current practice of accepting early thyroidal uptake as an index of trapping during drug therapy (2, 116). Furthermore there was good correlation between the 20 minute uptakes of the two anions. Thus suppression of the 20 minute uptake of either iodide or pertechnetate, during drug therapy, reflects suppression of the fundamental trapping mechanism of the gland.

One of the most interesting and unique features of the present study is the observation of significant initial free uptake of both anions, especially when the subjects became clinically euthyroid under the action of the antithyroid drug. The initial free uptake in the euthyroid state was linearly related to unidirectional clearance. This is what would be expected if the initial free uptake were a measure of the fraction of the injection bolus that reached the thyroid gland (Section 3.3); a direct relationship being assumed between unidirectional

clearance and thyroidal blood flow. In the transition from the thyrotoxic to the euthyroid state, cardiac output is known to decrease (160-162), thus a larger fraction of the injected bolus would reach the gland initially, provided thyroidal blood flow remained constant. The fact that initial free uptake was found to increase as the symptoms of thyrotoxicosis were suppressed, lends support to this theory.

Initial free uptake of pertechnetate was found to be greater than that of iodide, in spite of the fact that the estimated unidirectional clearance of the latter was greater. An answer may lie in the fact that earlier work, on the affinity of the thyroid gland for various anions, suggested that the affinity for pertechnetate should be greater than that for iodide (1, 2). However, pertechnetate, more so than iodide, is bound to plasma protein (Section 3.1) and hence its availability for thyroidal uptake is reduced. Initial free uptake may be a better index of the inherent affinity of the gland for a specific anion, because it probably reflects circumstances before any protein binding has time to be completed. Occurrence of a much greater initial free uptake of pertechnetate would explain the occasional finding of a 20 minute pertechnetate uptake that exceeds that for iodide.

The present findings that the exit rate of pertechnetate is greater than that of iodide, and that perchlorate increases the exit rate of both anions, are in agreement with the results of other studies in man (2, 27, 28, 163). An original observation in the present study, however, is that in some cases exit rate may be directly related to unidirectional clearance.

6.3 Short-Term Changes in Iodide Kinetics with Varying Doses of Carbimazole.

Patients and methods. Four previously untreated thyrotoxic subjects were maintained for consecutive periods of one week on a range of carbimazole dosages. In three cases the dosage in the first week was 5 mg d^{-1}, followed by 10, 20, 30 and finally 40 mg d^{-1} during the fifth week of study. The final subject received 40 mg d^{-1} initially which was reduced to 30, 20 and, finally, 10 mg d^{-1}. None of the patients received T3. The plan was to study iodide kinetics before carbimazole was given and at the end of each week's treatment with the specified dosages. In one case, however, a study of iodide kinetics was made one week after carbimazole was discontinued, rather than before drug treatment commenced. Each daily dose of carbimazole was divided into two equal portions, given orally at 08.00 and 20.00 hours. The kinetic investigations were performed at 14.00 hours on the day of study.

Thyroidal uptake of intravenous ^{132}I-iodide was studied and analysed using the same methods as before (Section 6.2). Each study was terminated by an intravenous perchlorate discharge test (Section 3.9). An alternative method of estimating binding rate was used which utilises the perchlorate discharge results. This was considered necessary because kinetic analysis of radioiodide uptake data may produce individual estimates of binding rate that are considerably different from the true value (Section 6.2).

The method exploits an empirical finding that the fraction of radioiodide uptake susceptible to discharge by perchlorate, at any time, is much more dependent on binding rate than on any of the other parameters.

This almost exclusive dependence on binding rate was elicited by generating a large series of thyroidal uptake curves using the digital computer. These curves were constructed for a range of exit and binding rates, unidirectional clearances and simulated arterial tracer levels. The fraction of uptake that would be discharged by perchlorate at any given time was also calculated (Section 2.6).

Figure 6.14 displays the relationship between the dischargeable fraction at 60 minutes and binding rate. The curve is independent of all parameters except for some dependence on the exit rate of iodide before and after perchlorate. Thus the solid line in the figure is the relationship when these exit rates are set equal to the mean values, 0.046 and 0.109 $minute^{-1}$, respectively, in all subjects studied in the present work (Table 22). The dotted area includes 95% of all possible variations from the mean curve, which were determined from knowledge of the 95% range of the observed exit rates before and after perchlorate. Clearly, an estimate of binding rate may be obtained by reference to that relationship if the dischargeable fraction at 60 minutes is determined empirically. Similar relationships for the dischargeable fractions at 10 and 30 minutes are displayed in Appendix 2. (In all these derivations, perchlorate is assumed not to affect binding (Sections 2.6 and 3.8). If binding is taken to be completely inhibited after perchlorate is administered, the estimated fractional discharge can be shown to be greater. The resulting relationship between dischargeable fraction and binding rate would lead to a greater estimate of the latter for a given discharge.)

Statistical analysis of the results was performed using the same methods as before (Section 5.3).

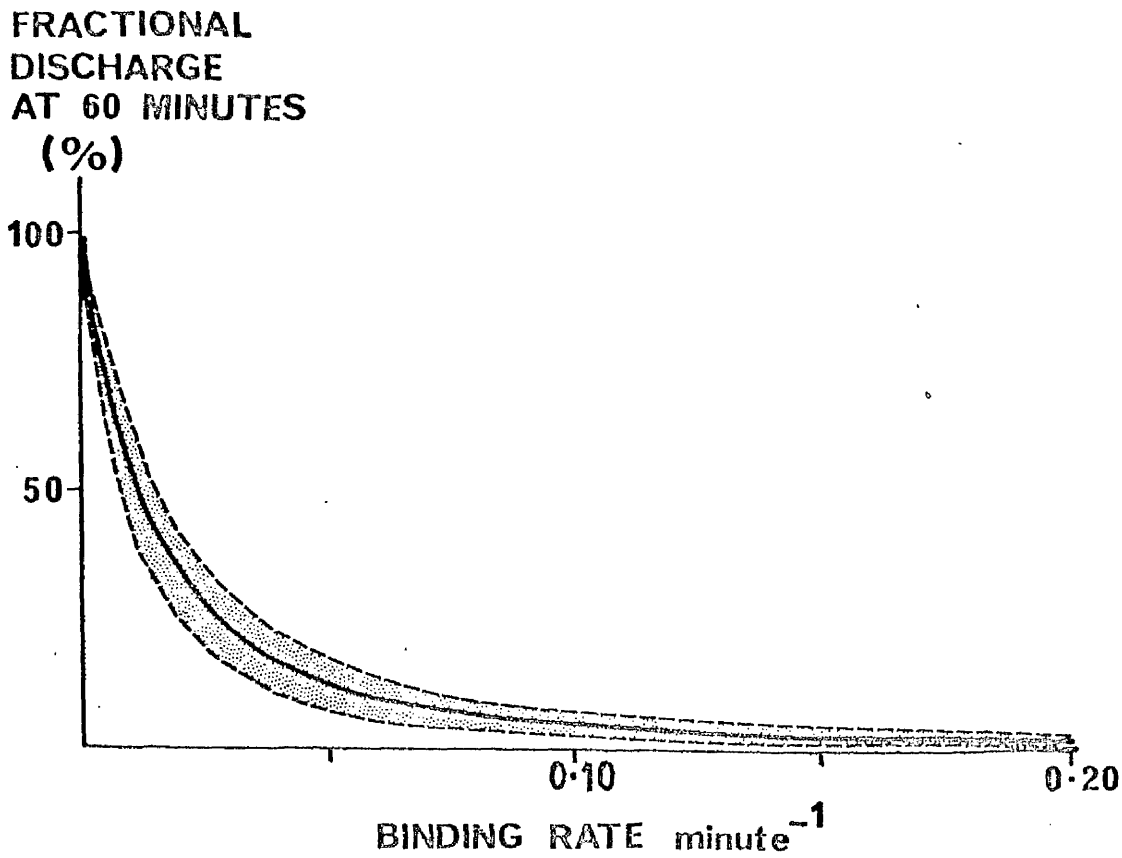


Figure 6.14 Relationship between the fraction of radioiodide uptake at 60 minutes dischargeable by perchlorate, and binding rate. The solid line is the relationship with the exit rates, before and after perchlorate, equal to the mean values 0.046 and 0.109 minute^{-1} respectively. The dotted area included 95% of all possible variations from the mean relationship.

Results. The time course of thyroidal uptake of radioiodide, during treatment with the greatest and least dosages of carbimazole, is compared with that in the untreated state in Figure 6.15. Even at the lowest dosage the uptake curve in all subjects can be seen to be markedly affected by the antithyroid drug.

Results of the kinetic analyses of uptake and perchlorate discharge in each subject are presented in Table 23. These consist of estimates of unidirectional clearance, exit rate and binding rate from the uptake phase, each with appropriate random errors. Included also are the observed perchlorate discharges at 60 minutes. The second estimates of iodide binding rate given in the table are those derived from Figure 6.14, making use of the observed dischargeable fractions. These latter estimates are considered to be more reliable than the binding rate estimates from compartmental analysis of the uptake phase. The variation of free thyroxine index (158) with dosage of carbimazole is also shown.

The results do not demonstrate any consistent effect of carbimazole upon unidirectional clearance and exit rate. Changes in these parameters, which cannot be explained by random error, did occur but there is no clearly recognisable trend. The drug, however, has a marked effect on binding rate. At the lowest dosage (5 mg d^{-1}) the binding rate is reduced from a value of $\geqslant 0.150$ $minute^{-1}$, before treatment, to an average value of 0.021 $minute^{-1}$. In two subjects, J.J. and C.I., there is a slight reduction in free thyroxine index. Greater dosages of carbimazole reduce the binding rate still further, but except in subject J.J., there is no convincing change in effectiveness over the range 10-40 mg d^{-1}. In subject J.J. however, the binding rate continues to decrease, so much

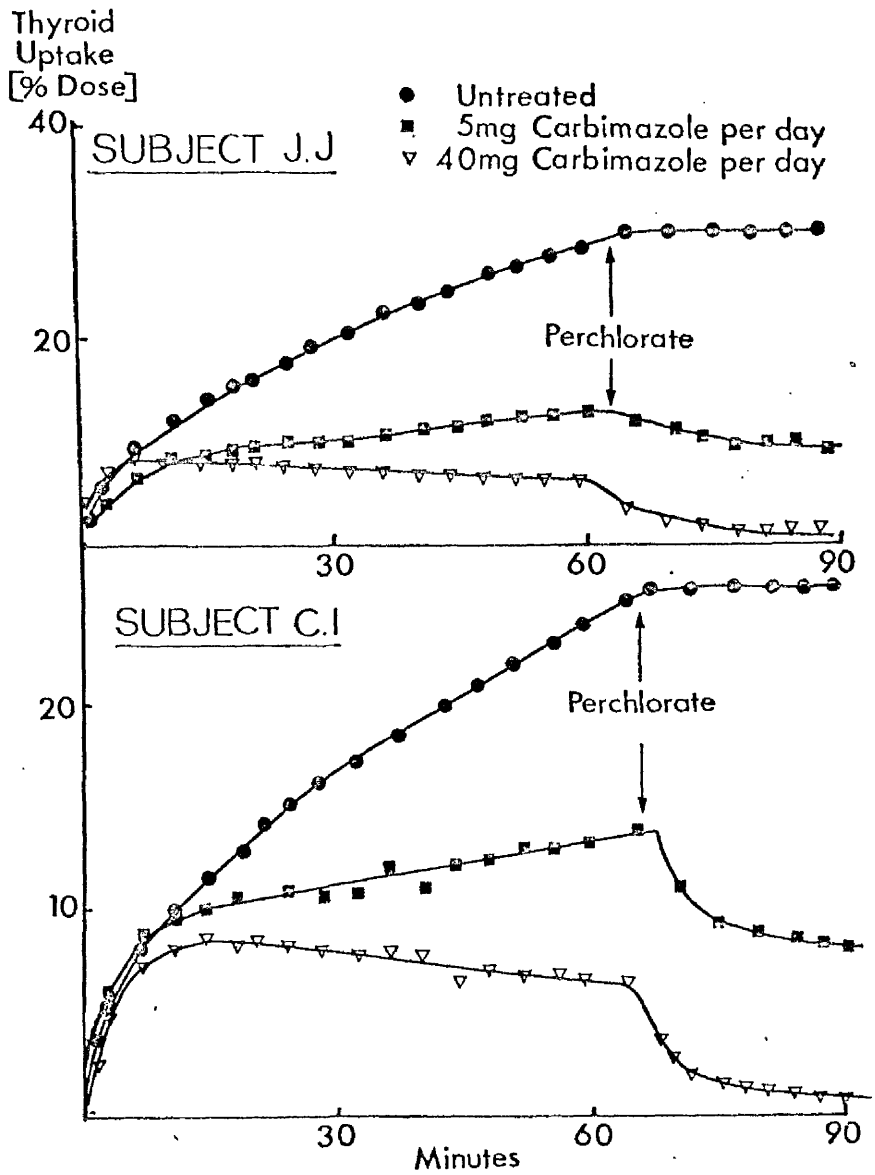


Figure 6.15 Variation of thyroidal uptake of radioiodide with time in thyrotoxic subjects on two different dosages of carbimazole. The solid lines are the best-fit curves during the uptake and perchlorate discharge phases.

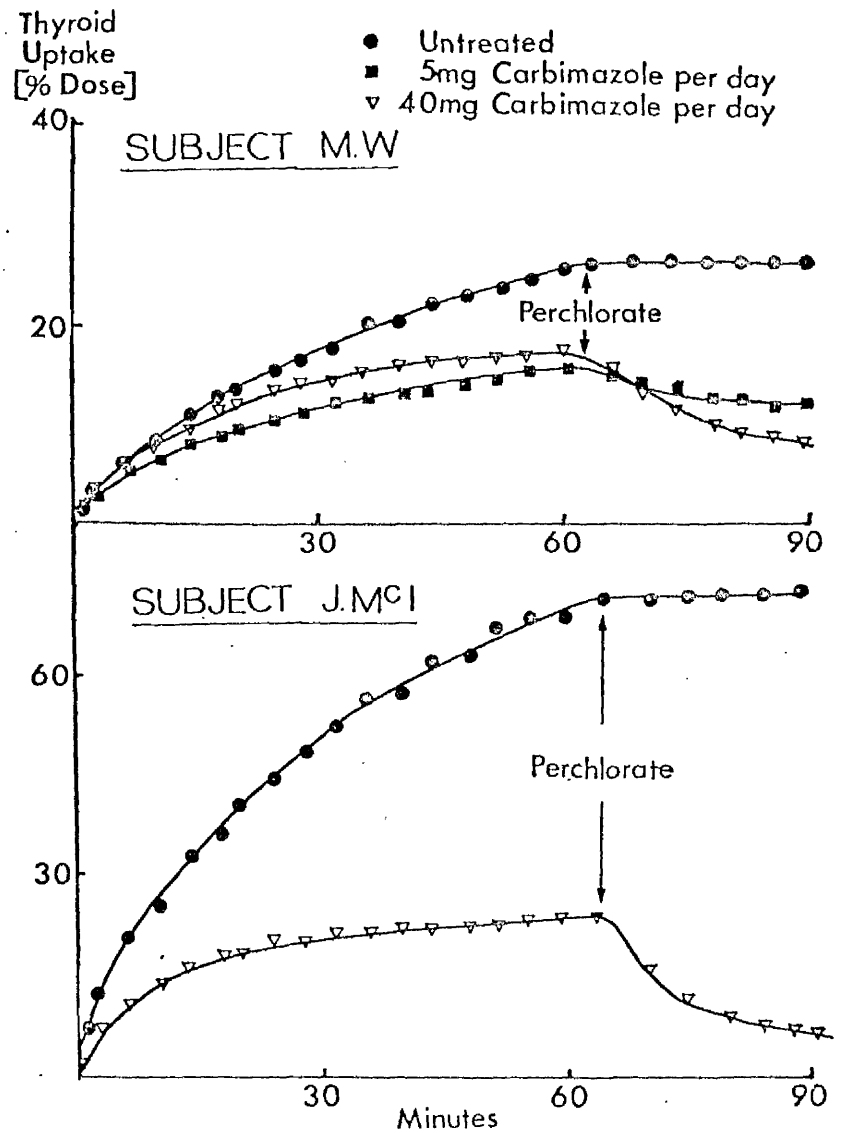


Figure 6.15 cont'd

TABLE 23

KINETIC ANALYSIS OF RADIOIODIDE UPTAKE, AND DISCHARGE BY PERCHLORATE, IN THYROTOXIC SUBJECTS ON VARYING DOSAGES OF CARBIMAZOLE

SUBJECT	Carbimazole dosage mg d^{-1}	Free T_4 index *	ANALYSIS OF UPTAKE PHASE			ANALYSIS OF DISCHARGE PHASE	
			Unidirectional clearance (±se) ml min^{-1}	Exit rate (±se) min^{-1}	Binding rate (±se) min^{-1}	Fraction of 60 minute uptake discharged (±se) %	Estimated binding rate with 95% confidence limits min^{-1}
J.J. (F)	0	710	86.0 ± 1.7	-	> 0.150	0	-
	5	560	115.1 ± 12.1	0.134 ± 0.024	0.042 ± 0.004	27.4 ± 4.6	0.025 ± 0.012
	10	332	94.7 ± 12.9	0.081 ± 0.017	0.011 ± 0.003	54.6 ± 1.2	0.009 ± 0.003
	20	387	158.1 ± 16.3	0.123 ± 0.017	0.011 ± 0.002	80.7 ± 1.2	0.002 ± 0.001
	30	221	127.0 ± 12.0	0.111 ± 0.014	0.008 ± 0.001	94.2 ± 2.9	0.001 ± 0.001
	40	71	142.7 ± 14.8	0.140 ± 0.017	0.006 ± 0.001	99.3 ± 2.2	0.000 ± 0.001
C.I. (F)	0	1400	72.2 ± 1.1	-	> 0.150	0	-
	5	925	107.7 ± 17.9	0.079 ± 0.020	0.027 ± 0.003	46.2 ± 1.3	0.012 ± 0.003
	10	663	89.1 ± 10.5	0.078 ± 0.012	0.013 ± 0.002	81.3 ± 3.0	0.002 ± 0.001
	20	769	81.5 ± 5.9	0.050 ± 0.005	0.004 ± 0.001	96.1 ± 2.0	0.001 ± 0.001
	30	686	107.7 ± 3.9	0.071 ± 0.004	0.006 ± 0.001	86.1 ± 0.5	0.002 ± 0.001
	40	631	104.6 ± 7.0	0.060 ± 0.006	0.001 ± 0.001	96.9 ± 1.4	0.001 ± 0.001

* Normal range 276-591

TABLE 23 CONT'D.

SUBJECT	Carbimazole dosage	Free T_4 index *	ANALYSIS OF UPTAKE PHASE			ANALYSIS OF DISCHARGE PHASE	
			Unidirectional clearance (±se)	Exit rate (±se)	Binding rate (±se)	Fraction of 60 minute uptake discharged (±se)	Estimated binding rate with 95% confidence limits
	mg d^{-1}		ml min^{-1}	min^{-1}	min^{-1}	%	min^{-1}
M.W. (F)	0	1152	98.0 ± 2.0	-	> 0.150	0	-
	5	1280	48.9 ± 0.8	-	> 0.150	26.6 ± 7.8	0.025 $^{+ 0.031}_{- 0.011}$
	10	890	42.4 ± 1.2	-	> 0.150	49.5 ± 1.4	0.011 ± 0.003
	20	811	53.5 ± 7.1	0.007 ± 0.001	0.001 ± 0.012	63.1 ± 6.2	0.006 $^{+ 0.005}_{- 0.003}$
	30	868	54.4 ± 6.4	0.006 ± 0.002	-0.005 ± 0.009	66.7 ± 4.5	0.005 $^{+ 0.004}_{- 0.002}$
	40	945	84.0 ± 7.4	0.011 ± 0.001	-0.007 ± 0.006	57.3 ± 2.3	0.008 ± 0.003
J.McI. (F)	0	2210	-	-	-	-	-
	40	1610	69.2 ± 6.1	0.018 ± 0.004	-0.000 ± 0.005	78.9 ± 1.9	0.003 $^{+ 0.002}_{- 0.001}$
	30	1395	92.7 ± 8.7	0.016 ± 0.002	-0.007 ± 0.004	86.2 ± 2.7	0.002 ± 0.001
	20	1070	92.6 ± 7.3	0.032 ± 0.006	0.006 ± 0.004	83.4 ± 3.7	0.002 ± 0.001
	10	854	102.7 ± 8.6	0.031 ± 0.004	0.001 ± 0.002	75.8 ± 2.0	0.003 $^{+ 0.002}_{- 0.001}$
	0	902	156.3 ± 2.4	-	> 0.150	0	-

* Normal range 276-591

so thatthere is biochemical evidence of hypothyroidism towards the end of the study.

Exit rate could not be estimated in subject M.W. when the lower dosages of carbimazole were being taken. However, as can be seen, that parameter was found eventually to have an exceptionally low value - considerably lower than the estimated binding rates for carbimazole dosages of 5-10 mg d^{-1}. In these circumstances, problems with the compartmental analysis are to be expected (Section 3.7).

A greater problem, however, as far as response to therapy is concerned, is the effect of a low exit rate upon the effective clearance of iodide. This is shown by the results given in Table 24, which include estimates of the effective clearance of iodide (Section 2.7), as a percentage of unidirectional clearance,for each subject. As can be seen, the results for patient M.W. are uncharacteristic with effective clearance remaining relatively high, even at 40 mg d^{-1} carbimazole. That patient responded least, biochemically and clinically, in the short-term to antithyroid drug therapy. The variation of effective clearance and binding rate, with dosage of carbimazole,is shown in Figure 6.16. Evidently there is little to be gained by increasing the dosage beyond 10 mg d^{-1}.

Discussion. Different methods have been used in the past to test the effectiveness of various treatment regimes of antithyroid drugs. These have included comparative measurements of thyroidal uptake of radioiodide, before and after treatment (12, 164), and determinations of the degree of binding inhibition by perchlorate or thiocyanate discharge studies (165-166). The duration of action and degree of binding inhibition of antithyroid drugs have been tested in humans by sampling thyroid tissue, and assaying in vitro the fraction of radioiodide

TABLE 24

EFFECTIVE CLEARANCE OF IODIDE IN THYROTOXIC SUBJECTS ON VARYING DOSAGES OF CARBIMAZOLE

Subject	Carbimazole dosage mg d^{-1}	Effective clearance with 95% confidence limits * % unidirectional clearance
J.J.	0	75 - 100
	5	17.5±7.0
	10	7.1±2.5
	20	1.7±0.8
	30	0.8±0.8
	40	0.0±0.8
C.I.	0	75 - 100
	5	15.0±3.9
	10	2.9±1.5
	20	1.4±1.5
	30	2.9±1.5
	40	1.4±1.5
M.W.	0	75 - 100
	5	75.8±12.0
	10	57.9±13.9
	20	42.9±15.0
	30	38.5±14.4
	40	50.0±15.6
J.McI.	40	11.1±6.1
	30	7.7±4.3
	20	7.7±4.3
	10	11.1±6.1
	0	75 - 100

* Based on the mean exit rate and binding rate estimate from the perchlorate discharge test.

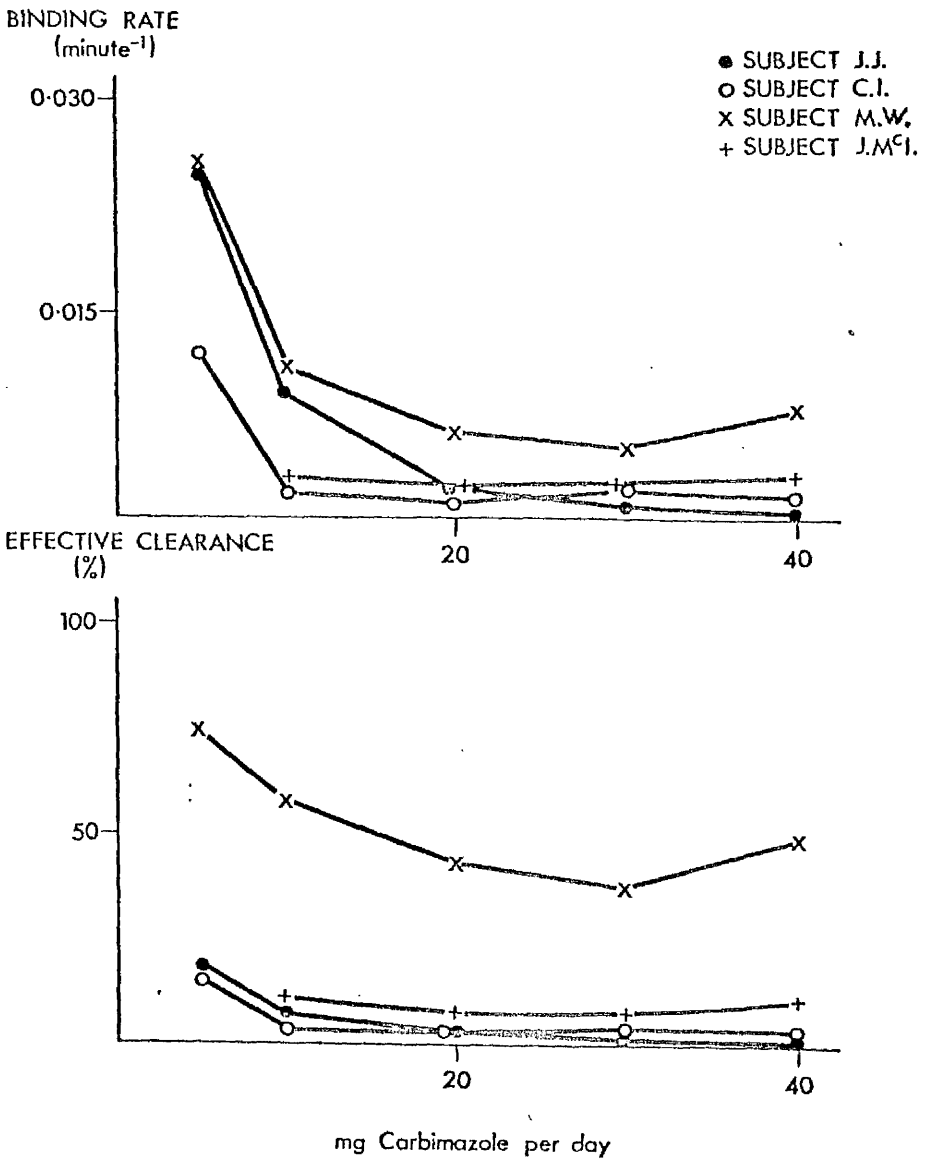


Figure 6.16 Variation of binding rate, and of effective clearance of iodide, with dosage of carbimazole in thyrotoxic subjects.

that is organically bound (156). Other workers have used compartmental analysis of radioiodide uptake to assess the degree of binding inhibition after single doses of drug (50).

The present work was an attmept to quantify the rate of iodide organification during the short-term treatment of thyrotoxicosis with varying doses of antithyroid drug, and to compare this with biochemical and clinical response to therapy. In so doing it was hoped that some useful predictions could be made as to the minimum dosage regimes necessary, in the long-term, for effective control of the disease. Unlike previous work on the problem, all essential aspects of iodide accumulation by the thyroid gland were investigated, which was of benefit in explaining some observed variability in response to the drug.

The technique that was employed provides an estimate of organification rate only over the period of each kinetic investigation. This may differ in value from the rate at other times if, as in the studies, the drug is given in discrete portions. However, any difference in observed response to the various dosages of drug, due to this effect, were minimised by performing each kinetic study at exactly the same time between the two daily portions of the drug. The results, therefore, reflect the relative effectiveness of the various drug regimes but the observed estimates of binding rate and effective clearance of iodide may not, in fact, be mean values for a given drug dosage. (There is some indirect evidence (167) to suggest that the drug level in blood, at the time chosen for the kinetic studies, may be a reasonable estimate of the mean daily value.)

Binding of iodide was found to be markedly affected by carbimazole even at the lowest dosage of 5 mg d^{-1}; the binding rate being at least a factor of 7 less than the value in the untreated state. In two out of

three cases there was a biochemical response to 5 mg d^{-1} carbimazole. The lack of biochemical response in the third subject was probably due to the effective clearance of iodide not being sufficiently suppressed. This in turn was the consequence of a relatively low exit rate.

There was no detectable difference in effectiveness of carbimazole for dosages in the range 10-40 mgd^{-1} in three out of four cases, as borne out by the results of the kinetic analysis and to some extent by biochemical response. In the remaining case, binding rate and effective clearance both continued to decrease with increasing drug dosage. By the end of the period of investigation the subject had become biochemically hypothyroid.

These short-term studies have indicated that dosages of carbimazole not exceeding 10 mg d^{-1} might prove to be successful in controlling thyrotoxicosis. The results conflict with earlier less detailed work (12) which suggested that dosages of this magnitude would not effectively reduce iodide binding. In order to resolve this difficulty, response to long-term administration of these lower dosages of carbimazole is currently being investigated. The present work, has, however, demonstrated the value of detailed kinetic analysis of radioiodide uptake in explaining variability of response to antithyroid drug therapy.

6.4 Investigation of Binding Defects Following Antithyroid Drug Therapy.

Patients and Methods. Perchlorate discharge studies were performed in 21 untreated thyrotoxic subjects and in 29 thyrotoxic subjects who had completed antithyroid drug therapy. Investigations in the latter

group were performed at 1 week to 30 months after drug treatment and, at the time of study, the subjects were receiving triiodothyronine supplements of 80 ug d^{-1}.

Twenty-nine of the subjects were in fact originally studied by Robertson (27, 130) who used a radioisotope scanning technique to measure thyroidal uptake of radioiodide (Section 5.2). Perchlorate was given orally in a dose of 500 mg, 20 minutes after intravenous administration of the tracer, and the study continued for a further 30 minutes. Administration of ^{99m}Tc-pertechnetate, along with the radioiodide, permitted the efficiency of the perchlorate to be verified. More rigorous statistical analysis is applied here to the results of these studies.

The remaining subjects were investigated using either an uptake counter (Section 4.2) or gamma camera (Section 4.3) to measure thyroidal uptake of intravenous radioiodide. Perchlorate was given intravenously as sodium perchlorate in a dose of 300 mg (Section 3.8), 30-60 minutes after the tracer. Each study was continued for a further 30 minutes after the perchlorate was administered.

Particular attention was paid to the statistical testing of uptake variations after perchlorate. The method that was finally adopted consisted of performing a linear regression on the uptake points after perchlorate and evaluating the "t" statistic (80). This allowed the significance of negative gradients, i.e. of apparent discharge, to be quantified. Discharge was considered to have occurred when the significance level was 95% or greater. More complex curve fitting did not improve sensitivity because the amounts discharged were low and/or because there were considerable random variations in some of the uptake data.

Repeated studies of iodide kinetics were performed in two drug-treated thyrotoxic subjects who were found to have a significant discharge. The studies were a follow-up to earlier work on these two subjects (85). Thyroid uptake of radioiodide was estimated using a gamma camera (Section 4.3) and data were subjected to compartmental analysis (Section 3.9). Each study was terminated by an intravenous perchlorate discharge test which provided an alternative, more reliable estimate of binding rate (Section 6.3).

Results. For more precise analysis of the results, the discharge studies are classified as either "early" or "late" (Table 25). Included in the first group are those studies where perchlorate was given orally at 20 minutes or intravenously at 30 minutes. (The simultaneous studies with ^{99m}Tc-pertechnetate revealed that oral perchlorate acts within 3-8 minutes. It seemed reasonable, therefore, to combine the results of the 20 minute oral and 30 minute intravenous perchlorate discharge studies.) The second group comprises the remaining studies where perchlorate was given intravenously 50-60 minutes after the tracer.

Out of 15 untreated thyrotoxic subjects who had "early" discharge studies, there is only one with significant discharge of radioiodide. This amounted to 7.5±1.4 (±se)% of the uptake just before perchlorate. Although there are 3 out of 18 drug-treated subjects with significant "early" discharge, the difference between the untreated and drug-treated group is not significant by the chi-square test ($p>0.3$). The discharges (±se) in the latter group were 22.8±8.1, 42.2±8.4 and 61.6±5.5%; 12, 3 and 9 months, respectively, after antithyroid drug therapy. In the last two cases, however, no discharge was found in

TABLE 25

PERCHLORATE DISCHARGE STUDIES IN UNTREATED THYROTOXIC SUBJECTS AND DRUG-TREATED THYROTOXIC SUBJECTS 1 WEEK - 30 MONTHS OFF THERAPY

	EARLY DISCHARGE STUDIES		LATE DISCHARGE STUDIES	
	Number with discharge	Number without discharge	Number with discharge	Number without discharge
Untreated thyrotoxic subjects	1	14	0	6
Drug-treated thyrotoxic subjects after therapy	3	15	2	9

atudies 7-8 months later.

The "late" discharge studies reveal no significant difference ($p>0.8$) between the frequency of discharge in untreated thyrotoxic and drug-treated thyrotoxic subjects after therapy. None of the 6 untreated cases manifested significant discharge of radioiodide. Two out of 11 subjects in the drug treated group manifested significant discharges; 74.4±1.7 and 12.1±1.9 (±se)%, 19 and 30 months respectively after drug therapy.

These last two cases were, in fact, the subject of detailed follow-up studies. Results of the kinetic analyses along with the free thyroxine index (158) and 20 minute radioiodide uptake, at the time of each study, are presented in Table 26. The second estimate of binding rate given in the table is that derived from Figure 6.14, using the observed dischargeable fraction. This latter estimate is considered to be more reliable than the binding rate estimate from compartmental analysis of the uptake phase (Section 6.3). Thyroid uptake curves for each subject, typical of those observed during the follow-up period, are plotted in Figure 6.17.

The discharges manifested by subject J.P. are relatively small, with some fluctuation being apparent during the period of study. Kinetic analysis of uptake in all studies of that subject suggested the existence of an iodide binding rate which is considerably greater than the exit rate (Section 3.7). In such circumstances neither parameter can be determined by compartmental analysis. Estimates of binding rate were, however, obtained from the observed discharges. There is probably some increase in binding rate with time but, because of relatively large errors, accurate assessment of the increase is difficult. Taking the mean value of 0.065 minute^{-1} and a value of 0.046 minute^{-1}

TABLE 26

REPEATED KINETIC ANALYSES OF RADIOIODIDE UPTAKE AND DISCHARGE IN TWO DRUG-TREATED THYROTOXIC SUBJECTS AFTER THERAPY

SUBJECT	Time after initial drug therapy	Free T_4 index *	20 minute radioiodide uptake % dose	ANALYSIS OF UPTAKE PHASE			ANALYSIS OF DISCHARGE PHASE	
				Unidirectional clearance (±se)	Exit rate (±se)	Binding rate (±se)	Fraction of 60 minute uptake discharged (±se)	Estimated binding rate with 95% confidence limits
	months			ml min^{-1}	min^{-1}	min^{-1}	%	min^{-1}
J.P.	30, on T_3	505	19.0	56.2 ± 2.0	-	> 0.150	12.1 ± 1.9	0.052 ± 0.023
	78,÷ off T_3	259	11.1	35.0 ± 1.9	-	> 0.150	11.5 ± 3.9	$0.055 \begin{smallmatrix}+0.065\\-0.025\end{smallmatrix}$
	79, on T_3	276	12.8	43.8 ± 1.1	-	> 0.150	26.8 ± 3.8	$0.025 \begin{smallmatrix}+0.011\\-0.008\end{smallmatrix}$
	88, off T_3	457	26.2	88.5 ± 1.0	-	> 0.150	5.1 ± 0.4	$0.096 \begin{smallmatrix}+0.018\\-0.030\end{smallmatrix}$
	91, off T_3	520	23.5	67.6 ± 1.2	-	> 0.150	4.8 ± 1.4	$0.098 \begin{smallmatrix}+0.018\\-0.030\end{smallmatrix}$
M.D.	19, on T_3	95	32.5	426.1 ± 16.4	0.022 ± 0.004	-0.003 ± 0.005	74.4 ± 1.7	0.004 ± 0.002
	30, on T_3	640	33.7	307.5 ± 18.8	0.011 ± 0.006	0.010 ± 0.020	27.6 ± 3.5	0.024 ± 0.008
	68,+ on T_3	182	63.7	368.1 ± 13.1	0.005 ± 0.003	0.015 ± 0.024	5.8 ± 0.7	$0.088 \begin{smallmatrix}+0.018\\-0.030\end{smallmatrix}$
	69,÷ off T_3	300	63.8	448.2 ± 7.2	-	> 0.150	0.0	-
	80, on T_3	150	49.3	424.5 ± 7.1	0.032 ± 0.002	0.008 ± 0.001	80.1 ± 0.8	0.002 ± 0.001

* Normal range 276-591; +Further antithyroid drug therapy had just been completed three months before the study; ÷Off T_3 for one month before the study.

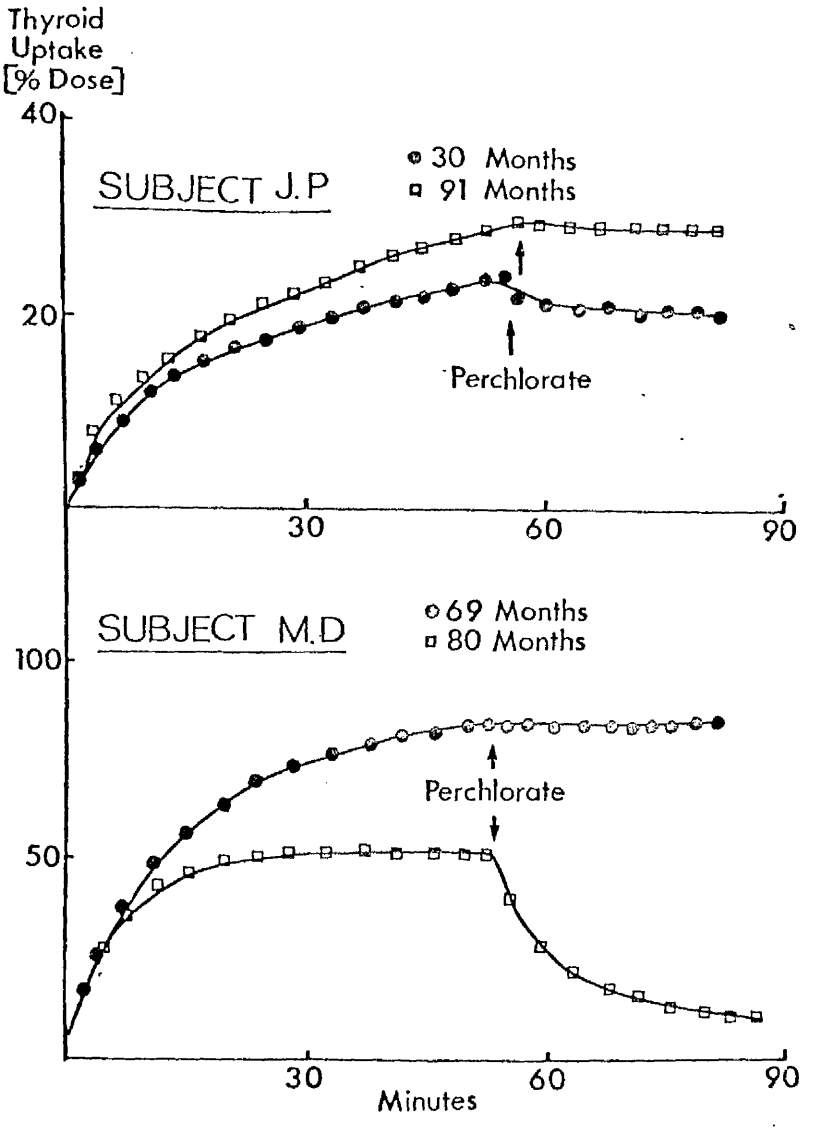


Figure 6.17 Thyroidal uptake curves of radioiodide in two thyrotoxic subjects 30-91 months after a first course of antithyroid drug therapy.

(Table 22) for the exit rate, it may be calculated that the mean effective clearance of iodide (Section 2.7) for subject J.P. is approximately 59% of the unidirectional clearance.

Unlike the previous subject, the discharge results for subject M.D. are quite variable, ranging from no detectable discharge to a maximum discharge of 80.1% of the uptake before perchlorate. The kinetic analyses reveal binding rates which are either much less than the exit rate or considerably greater; the range of estimated binding rates being 0.002->0.150 minute^{-1}. During the course of follow-up, the subject became thyrotoxic and received another course of antithyroid drug therapy. Variable discharge was still, however, observed after this further period of treatment. An answer to these problematical results was discovered unexpectedly during the last study. On that occasion, the subject inadvertently admitted to taking carbimazole surreptitiously, and to having done so in the past. Analysis of a urine sample revealed the presence of the drug.

Discussion. The results reveal no increased incidence of dischargeable radioiodide in thyrotoxic subjects who have completed antithyroid drug therapy. Earlier findings that there was a greater incidence (27) were based on less rigorous statistical testing of the observed data.

Three out of 18 drug-treated subjects, in fact, manifested significant discharge at 20-30 minutes but in 2 of these there was no evidence of a binding defect some months later. The finding of a small, but significant, "early" discharge in 1 out of 15 untreated thyrotoxic subjects has been used earlier in this thesis to provide a lower estimate of the rate of "uninhibited" binding (Section 5.5).

Follow-up studies in two-drug treated subjects who manifested dischargeable radioiodide at 50-60 minutes (85), proved to be of considerable value. In one case, it was proven that carbimazole was being taken surreptitiously, which explained the subject's euthyroid clinical state in spite of a continuing high uptake. The other subject had a relatively small but persistent binding defect. This resulted in the effective clearance of iodide being less than 60% of the unidirectional clearance, compared with a normal value of 75-100% (Section 5.5). Thus again discrepancy between an elevated uptake, yet euthyroid clinical state, was resolved. The presence of Hashimoto's thyroiditis (168) is unlikely because of the absence of circulating thyroid antibodies. A small congenital defect in binding (169) cannot be totally excluded, for there was no kinetic analysis nor discharge test performed when the presence of thyrotoxicosis was first diagnosed.

In conclusion, it is evident that significant discharge of radioiodide may be observed occasionally in untreated thyrotoxic subjects, and in subjects who have completed antithyroid drug therapy. There is no clear evidence from the results to suggest that antithyroid drugs have a long-term effect on thyroid function. Any observed discharge in untreated thyrotoxic subjects is likely to be small. This is supported by the work of Gray (28) who found no clear evidence of discharge at 10 minutes in 12 untreated thyrotoxic subjects, and of Stewart and Murray (170) who studied 11 untreated thyrotoxic subjects and found only 2 with a small discharge at 1 hour.

Observation of significant discharge, particularly when it is relatively small, may in fact indicate the presence of an underlying binding rate which is at the lower limit of the normal range. When a

large discharge is observed in the drug-treated subject, the present experience would suggest that the possibility of the subject continuing to take antithyroid drug, after the agreed course of therapy, should be thoroughly investigated.

CHAPTER SEVEN

DIRECT QUANTITATION OF IODINE BINDING IN SUBJECTS WITH IODINATION DEFECTS

7.1 Introduction

Various defects in the synthesis of thyroid hormone have been described (91, 171, 172). One defect, however, i.e. defective iodination of tyrosine, lends itself to investigation by iodide tracer techniques (173). When that defect is present, varying proportions of the tracer remain unbound in the thyroid and so can be discharged by pharmacological doses of perchlorate. The defect may have definite genetic origins, as in forms of congenital thyroid dysfunction, including Pendred's syndrome (9, 174, 175), or may be associated with autoimmune thyroiditis (Hashimoto's thyroiditis) (168, 176). It is usual for a goitre to be present in association with clinical or subclinical hypothyroidism (9, 177). On rare occasions, the thyroid may not be enlarged if the defect is due to impaired response to thyroid stimulating hormone (9, 178), or if the gland is atrophied as the end-state of Hashimoto's thyroiditis (9). Some degree of impaired iodination has been reported in cases of simple non-toxic goitre (28, 168, 169) but the possibility exists that there may be genetic factors involved (175, 179).

Discharge tests for iodination defects vary widely in method and, consequently, in final outcome (2, 28, 81, 170). In some, the radioiodide is given orally (168, 169, 176, 180), in others it is given intravenously (28, 81, 85, 170, 181), the perchlorate being given orally or intravenously at times ranging from 10 minutes to several hours after the tracer. The results have differed widely, especially with regard to

the frequency of iodination defects in Hashimoto's thyroiditis. Some reports suggest that 30-50% of patients with the disease manifest significant discharge (91, 176, 182), while more recent studies, in which perchlorate was given intravenously at 10 minutes (28), reveal an incidence of nearly 100%.

It is customary to accept that the proportion of radioiodide discharged by perchlorate is a measure of the severity of the defect. When the iodination process has failed completely, virtually all of the tracer can be discharged; in partial failure, as in Hashimoto's thyroiditis, only 20-50% can be discharged (173). The binding rate of iodide in Hashimoto's thyroiditis and Pendred's syndrome has been estimated by Gray (28), but the methods used were indirect and probably erroneous (Section 1.10). Furthermore, the final results were not expressed in terms of the effective clearance of iodide (Section 2.7) which is a better indicator of iodine utilisation. Direct measurements of radioiodide clearance (Section 1.4) have been made in subjects with iodination defects (180, 182, 183). If these are combined with estimations of plasma inorganic and protein bound iodine, it is possible to determine an index of iodide utilisation (180, 184). However, inconsistencies do arise because the slope of the uptake curve, and therefore clearance, may change markedly with time and may even become negative during the period of observation (34, 180).

The studies reported in this chapter were performed on a group of subjects who had varying degrees of iodination defect. Observations were made of the uptake of radioiodide by the thyroid gland and of its discharge by intravenous sodium perchlorate. Data were analysed on the

basis of the open three-compartment binding model of the gland (Chapter 2). The object was to quantitate iodine binding, for the first time by direct methods, in naturally occurring defective iodination and to relate this to clinical and biochemical status. It was thought that the technique might provide some guide to suitable replacement therapy.

7.2 Patients and Methods

Ten subjects, including 6 members of one family, subjects J.S. to MD.S. (the male parent and 5 siblings) in the results table to follow, were investigated. Where necessary, thyroid hormone replacement therapy was discontinued temporarily for the purpose of the investigation.

Studies of the family first began when the only male sibling (age 12 years) presented with the characteristic features, including retarded skeletal growth, of severe thyroid deficiency. Subsequent investigations revealed that other members of the family manifested clinical or subclinical hypothyroidism, i.e. increased plasma levels of thyroid stimulating hormone. The male parent had, in fact, developed clinical hypothyroidism in adult life. Thyroid antibody tests were negative in all members of the family. None had a distinctly palpable goitre, but in one case the presence of a slightly enlarged gland was detected by radioisotope imaging. The gland was of normal size in the other members of the family.

The remaining 4 subjects consisted of one with simple non-toxic goitre, one with Hashimoto's thyroiditis (confirmed by positive thyroid antibody and perchlorate discharge tests), one with a congenital binding

defect and goitre, and one with transient pituitary failure. (These cases appear in order in the results table to follow). In the last subject, two separate studies were performed during the period of recovery to normal pituitary function.

Thyroidal uptake was observed for 60 minutes following the intravenous administration of either ^{123}I or ^{132}I-iodide. After this uptake phase, sodium perchlorate (300 mg) was administered intravenously and observations continued for a further 30 minutes (Section 3.9). Uptake measurements were made using either an uptake counter or gamma camera by the procedures detailed earlier (Sections 4.2 and 4.3). Venous blood samples were taken at appropriate times (Section 3.9) for estimation of plasma radioactivity.

A standard procedure was adopted for analysis of the data (Section 3.9). This provided estimates of the various parameters of the open three-compartment binding model of the thyroid gland. The perchlorate discharge phase was also analysed for evaluation of residual uptake (Section 2.6). This allowed a more reliable estimate of binding rate to be obtained from the proportion of dischargeable radioiodide (Section 6.3).

Statistical analysis of the results was performed using the methods described in Section 5.3.

7.3 Results

Figure 7.1 shows the variation of thyroid uptake with time in all ten subjects. Also displayed is the best-fit uptake curve and the variation of bound uptake as determined from the compartmental analysis.

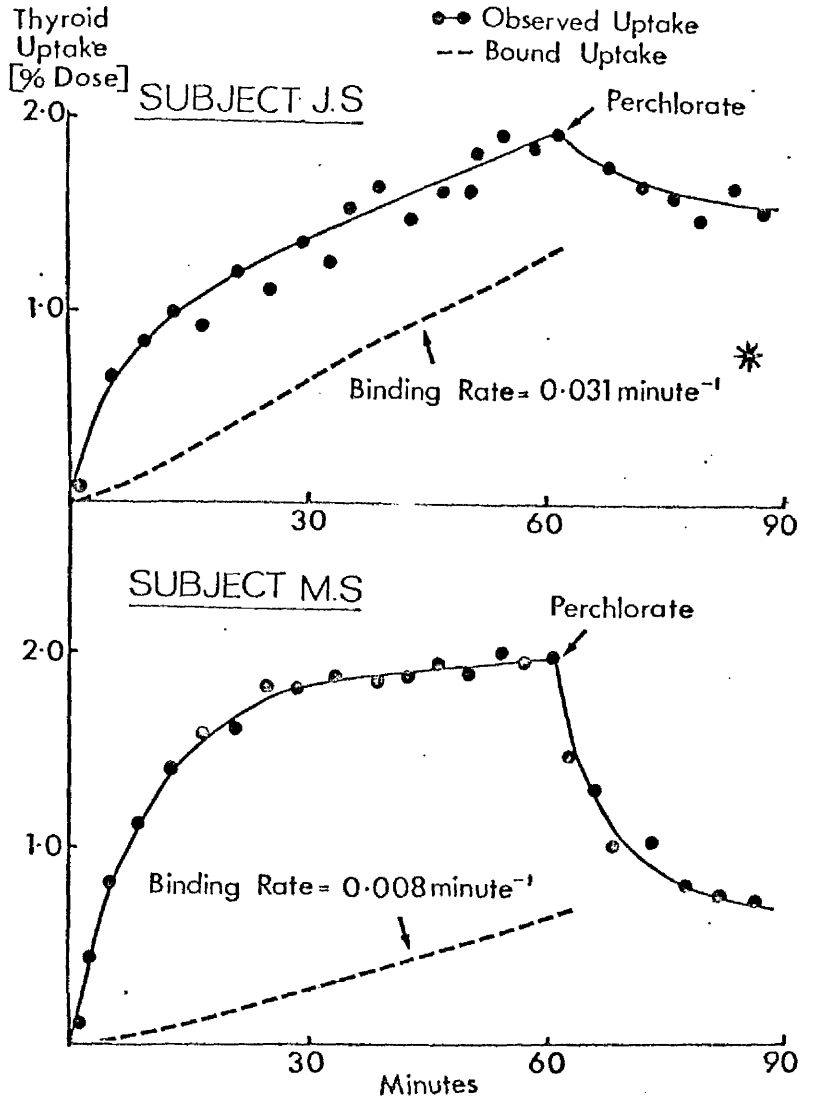


Figure 7.1 Thyroidal uptake of radioiodide and its discharge by perchlorate in subjects with iodination defects. Except where indicated, the curve of bound uptake with time is derived from the binding rate estimate from analysis of the uptake phase.

* estimated from the perchlorate discharge phase.

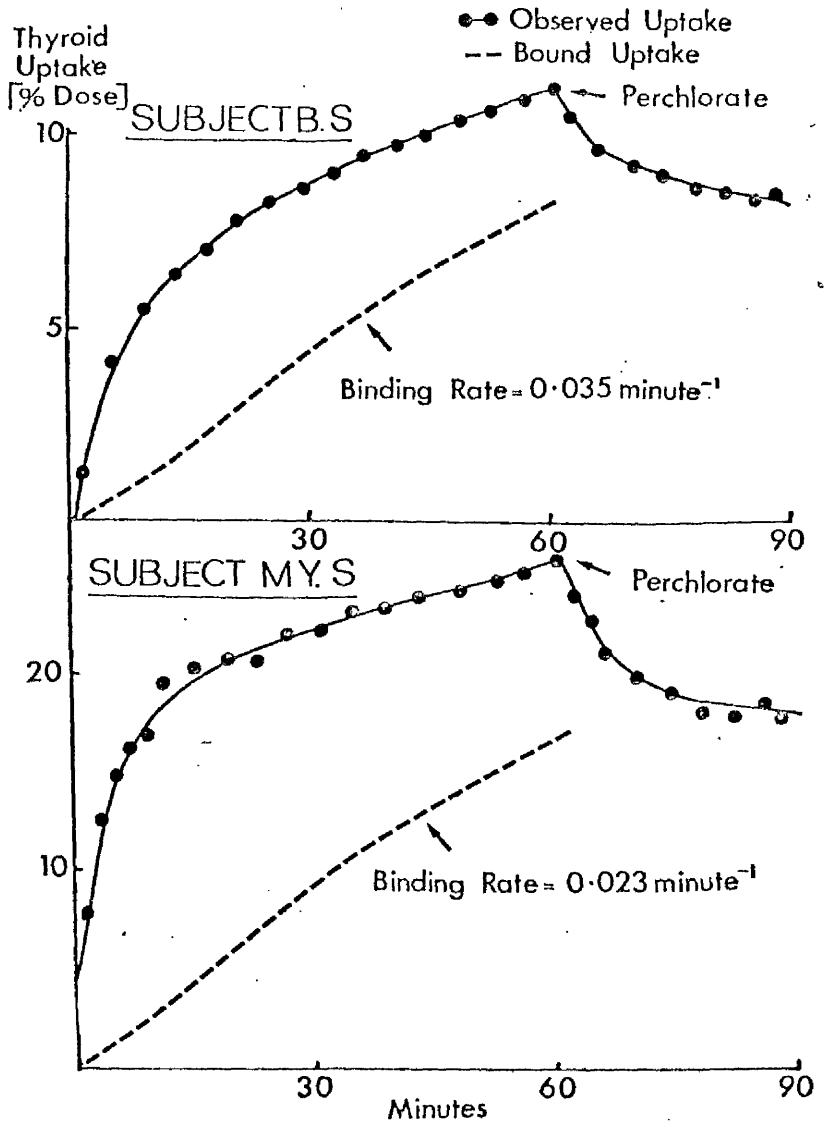


Figure 7.1 cont'd

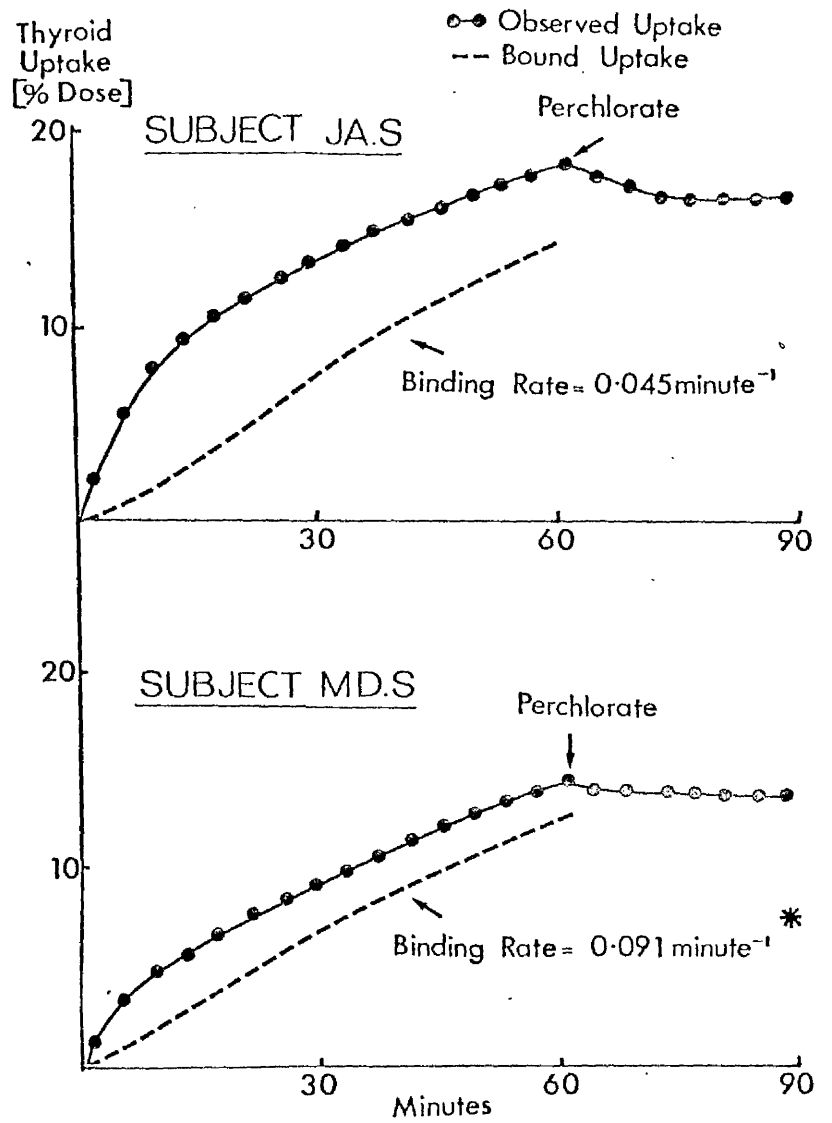


Figure 7.1 cont'd

* estimated from the perchlorate discharge phase.

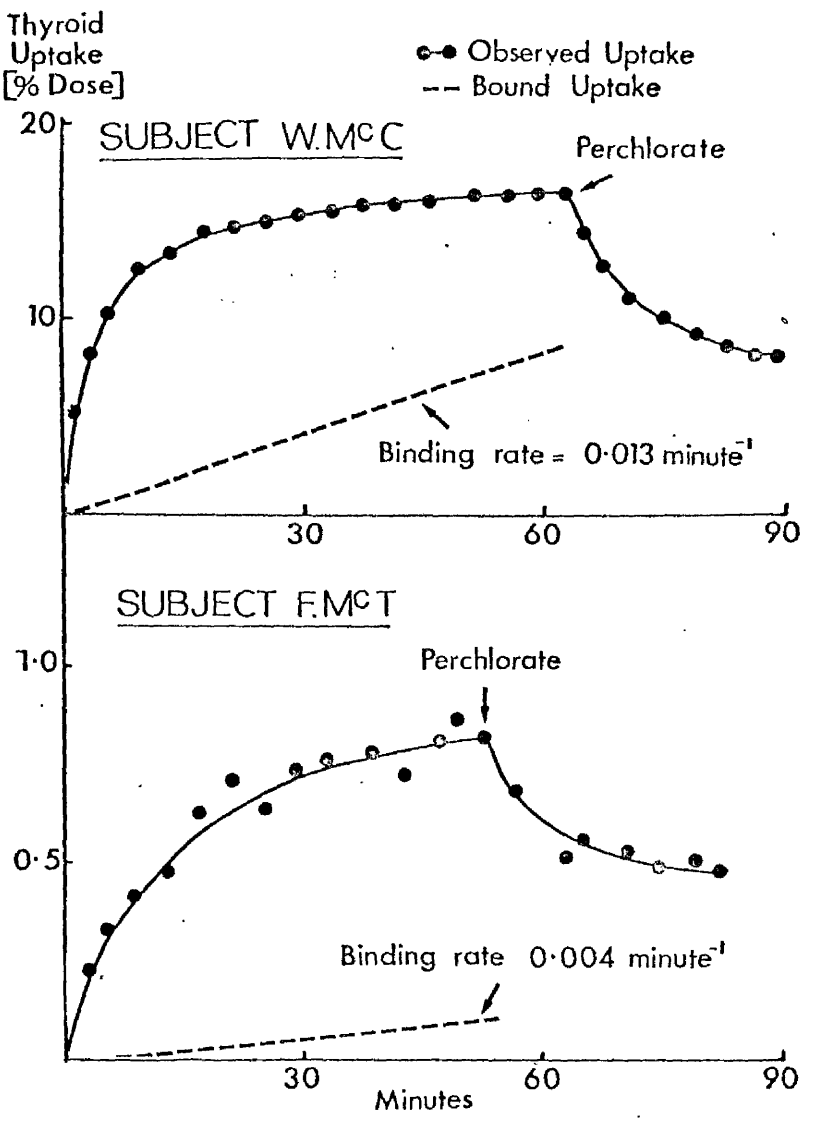


Figure 7.1 cont'd.

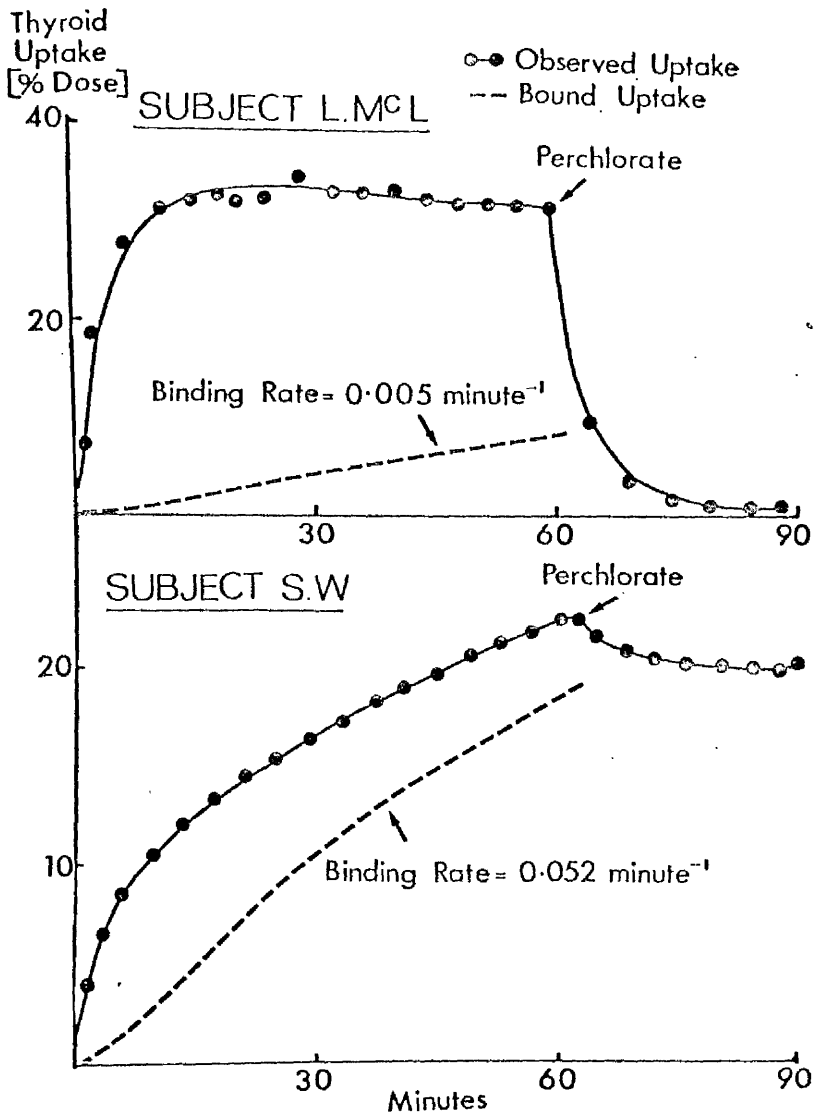


Figure 7.1 cont'd

Considerable variation can be seen, from subject to subject, in the final slope of the curve during the 60 minute uptake phase. This is evidently the result of varying degrees of binding defect, the lower the binding rate, the lower the final slope of the uptake curve. In most cases there appears to be reasonable agreement between the observed residual uptake after perchlorate and the bound uptake estimated by the compartmental analysis.

Results of the digital analysis of the uptake and perchlorate discharge phases, along with estimated errors, are presented in Table 27. Included also are details of the biochemical status of each subject and the 20 minute uptake of radioiodide. Two estimates of binding rate are given, one from analysis of the uptake phase and the other from knowledge of the dischargeable fraction at 60 minutes (Figure 6.14). Effective clearance of iodide is expressed as a percentage of the unidirectional clearance (Section 2.7) and also in absolute terms (ml $minute^{-1}$). The estimate of binding rate from the discharge phase was used in the calculation of effective clearance, since it is generally more reliable (Section 6.3). This is particularly true in the case of patients J.S. and MD.S. when the analysis failed to produce a least sum of squares solution that was significantly better than the solution with the exit rate equal to zero (Section 3.7).

The unidirectional clearance in the group ranges from 5.6-1352.0 ml $minute^{-1}$, reflecting a varying response to circulating thyroid stimulating hormone (TSH), which is clearly elevated in 7 cases and at the upper limit of the normal range in 2 further cases. Only two members (MY.S. and JA.S.) of the family under study showed any positive response to elevated TSH output. In each case the unidirectional

TABLE 27

RADIOIODIDE KINETICS IN SUBJECTS WITH IODINATION DEFECTS

PATIENT, SEX	20 MIN. UPTAKE % dose	PLASMA HORMONE nmol l^{-1} T_4	PLASMA HORMONE nmol l^{-1} T_3	PLASMA TSH mU l^{-1}	ANALYSIS OF UPTAKE PHASE: UNIDIRECTL CLEARANCE SE) ml min^{-1}	ANALYSIS OF UPTAKE PHASE: EXIT RATE $_{-1}$SE) min^{-1}	ANALYSIS OF UPTAKE PHASE: BINDING RATE $_{-1}$SE) min^{-1}	ANALYSIS OF DISCHARGE PHASE: FRACTIONAL DISCHARGE AT 60 min (±se) %	ANALYSIS OF DISCHARGE PHASE: BINDING+ RATE (95% RANGE) min^{-1}	EFFECTIVE * IODIDE CLEARANCE (95 % RANGE) %	EFFECTIVE IODIDE CLEARANCE (95% RANGE) ml min^{-1}
J.S., M	1.1	10.0	0.77	>52	5.6±0.3	–	>0.15	21.5±3.6	0.031±0.017	40.3 ± 13.2	2.2 ± 0.7
M.S., M	1.7	6.0	0.46	24.5	10.1±0.6	0.060 ± 0.007	0.008 ± 0.002	64.8±2.5	0.006±0.002	9.1 ± 3.4	0.9 ± 0.3
B.S., F	7.5	30.1	1.84	>49	44.4±2.0	0.053 ± 0.005	0.035 ± 0.002	25.2±0.7	0.027±0.007	33.8 ± 7.2	15.0 ± 3.2
MY.S., F	20.7	40.2	1.90	>52	201.2±15.1	0.069 ± 0.006	0.023 ± 0.001	29.5±0.9	0.023±0.007	25.0 ± 6.6	50.3 ± 13.3
JA.S., F	11.0	60.1	1.94	9.8	59.0±1.5	0.027 ± 0.003	0.045 ± 0.003	11.0±0.4	0.057±0.018	67.9 ± 8.4	40.1 ± 5.0
MD.S., F	7.1	61.3	1.95	<1.2	31.6±0.2		>0.15	5.5±0.4	$0.091^{+0.02}_{-0.03}$	66.4 ± 6.1	31.6 ± 0.2
W.McC., F	14.4	63.0	2.15	48.0	135.2±6.7	0.077 ± 0.006	0.013 ± 0.001	52.3±0.6	0.010±0.003	11.5 ± 3.4	15.5 ± 4.6
F.McT., F	0.6	–	–	>50	7.5±1.2	0.054 ± 0.018	0.004 ± 0.007	39.0±4.4	0.016±0.004	22.9 ± 12.6	1.7 ± 0.9
L.McL., F	42.0	<14	<0.5	>50	1352 ± 146	0.121 ± 0.016	0.005 ± 0.001	98.1±0.5	0.000±0.001	0.0 ± 0.8	0.0 ± 10.8
S.W., F	–	27.0	1.06	10.0	–	–	–	–	–	–	–
	7.6	64.1	2.60	10.7	36.4±4.2	0.011 ± 0.007	0.002 ± 0.016	26.9±1.2	0.025±0.007	69.4 ± 27.7	25.3 ± 10.1
	14.1	96.2	2.69	10.7	84.9±4.0	0.040 ± 0.005	0.052 ± 0.003	11.0±0.5	0.057±0.018	58.8 ± 9.8	49.9 ± 8.3
	–	101.0	3.19	8.3	–	–	–	–	–	–	–
Normal range	<8	59-174	1.3-3.3	<10							

+ estimated from the fraction discharged by perchlorate (Figure 6.14)

* determined using the observed exit rate or the mean value, 0.046 min^{-1} (Table 22) and the second estimate of binding rate.

clearance is above the normal range, 14.0-40.6 ml minute^{-1} (Section 5.4), and in the case of MY.S. the thyroid gland was just detectably enlarged. Exit rates in the group are similar to those found previously in normal and thyrotoxic subjects (Sections 5.4 and 6.2), and have been included in an estimate of the mean value in all patients studied (Table 22).

Observed discharges by perchlorate range widely, from 5.5% to 98.1% of the uptake at 60 minutes. Binding rates range from 0.000 to 0.091 minute^{-1}. There is reasonable agreement overall between the two estimates of binding rate; the observed differences are not significant ($p>0.1$).

The effective clearance of iodide, when expressed as a percentage of the unidirectional clearance, ranges from 0.0 to 69.4%. In those cases where the effective clearance exceeds 60% there is no clear biochemical evidence of thyroid disease. In absolute terms, the range of effective clearance is 0.0-50.3 ml minute^{-1}. Three of the 4 subjects with effective clearance at the lower end of the latter range (J.S., M.S. and L.McL.) manifest abnormally low levels of both T3 and T4. Hormone levels were not measured at the time of investigation in the fourth subject (F.McT.) but these were known to be markedly reduced several months previously. All four subjects were clinically hypothyroid.

The biochemical data shown for patient S.W., who had transient pituitary failure, reveal that the radioiodide investigations were performed during a time of improving thyroid function, related to the restoration of normal pituitary function. This is borne out by the results of the kinetic analysis, where both unidirectional clearance

and binding rate increased significantly with time ($p<0.01$).

Figure 7.2 is a plot of effective iodide clearance (ml minute^{-1}) against residual uptake (% dose) after perchlorate. There is highly significant linear correlation between these two parameters ($p<0.001$).

7.4 Discussion

The fundamental parameters of the iodide accumulation and utilisation process have been measured for the first time in subjects with widely varying degrees of iodination defect. This has provided quantitative assessment of the binding of iodide and of the extent to which the thyroid gland may compensate for the defect. An incidental feature of the study was that it provided a further check on the reliability of the compartmental analysis technique developed in this thesis, there being good agreement between the predictions of the analysis and observations after perchlorate.

Binding rates ranged widely, from 0.000 to 0.091 minute^{-1}, but in all cases the value was less than the lower limit, 0.150 minute^{-1}, of the normal range (Section 5.5). The present estimates are considerably less than those found by Gray in subjects with iodination defects (28). However his method was indirect and was based on the doubtful assumption that perchlorate affects binding (Section 1.10 and 3.8). Furthermore, if the binding were of the order of 0.2 minute^{-1}, as his results suggest (Table 1), there would have been problems with the digital analysis in the present cases. In these circumstances, the binding rate would have been considerably greater than the exit rate, leading to computational difficulties (Section 3.7).

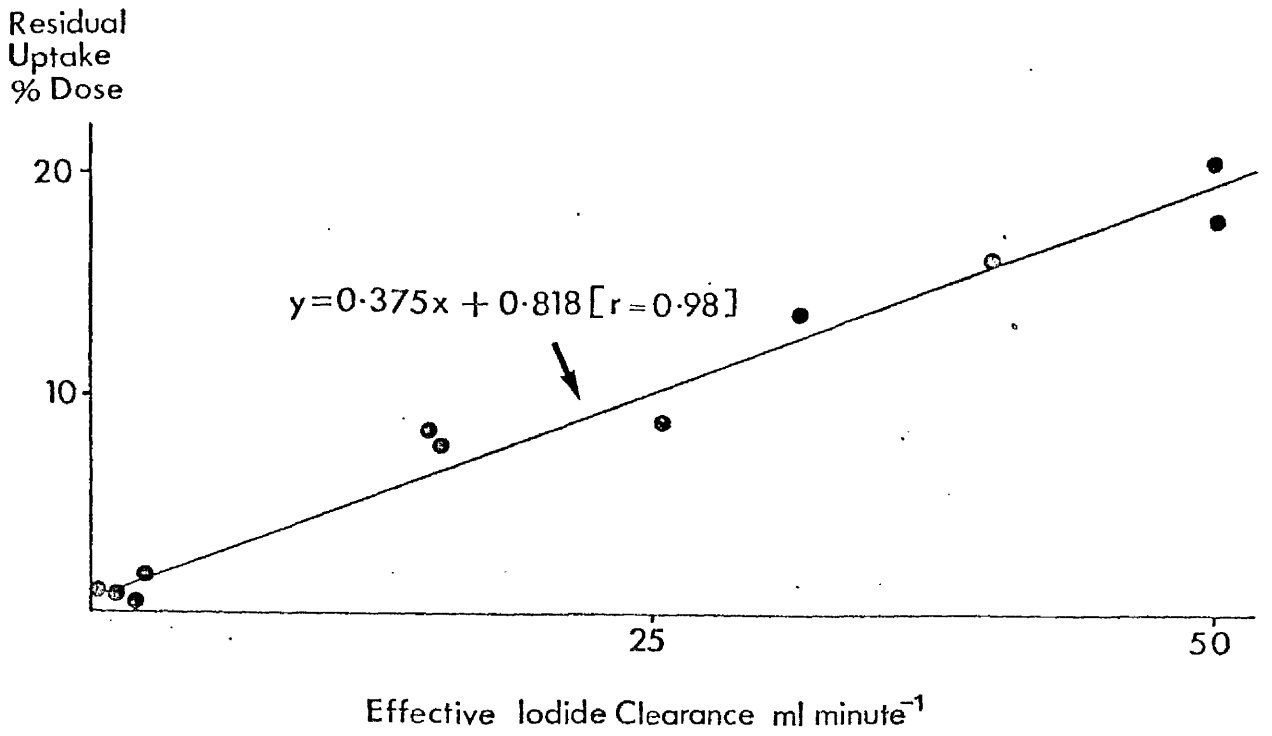


Figure 7.2 Correlation between the residual uptake of radioiodide, after perchlorate at 60 minutes, and the effective clearance of iodide in subjects with iodination defects.

The effective clearance of iodide, as a percentage of the unidirectional clearance, was always less than 70%, whereas the normal range is 75-100% (Section 5.5). There was, however, no convincing biochemical evidence of thyroid disease in those cases where the effective clearance was 60% or greater. In fact, the only evidence of defective iodination was the presence of dischargeable uptake which is not usually found unless there is a binding defect (Section 6.4). This would suggest that compensatory mechanisms, leading to increased output of TSH, are manifestly activated only when the effective clearance falls below 60%. Only then, as a rule, will thyroid hormone replacement therapy be deemed necessary.

If, under increased stimulation of the gland, the effective clearance of iodide (in ml $minute^{-1}$) fails to achieve a value within the normal range, then the output of thyroid hormone is likely to be seriously impaired. This was clearly evident in a number of cases where the effective clearance was well below the lower limit, 14.0 ml $minute^{-1}$ (Section 5.4), of the normal range. The achievement of a normal value suggests a state of compensated hypothyroidism.

In practice, the effective clearance of iodide may be estimated without digital analysis by making use of the correlation between that parameter and the residual uptake after perchlorate (Figure 7.2). This enables the severity of the defect, and therefore appropriate replacement therapy, to be determined on the basis of a perchlorate discharge test.

The absence of response to endogenous to TSH stimulation, such as increased unidirectional clearance and enlargement of the thyroid gland,

in most of the family included in the study, is unusual. In spite of the absence of thyroid antibodies, Hashimoto's thyroiditis cannot be totally excluded because final histological evidence (9, 28) was not available at the time of writing of this thesis. Alternatively, the basic defect may be one of lack of response to endogenous TSH (178). A more complete study of the family is currently being undertaken.

APPENDIX ONE

FIRST PARTIAL DERIVATIVES OF THE SUM OF SQUARES AND FITTING FUNCTIONS

Derivative of S with respect to K_{TP}

From Section 2.2, the sum of squares function is

$$S = \sum_j (U_0(t_j) - U(t_j))^2 / V_j$$

where the fitting function is given by

$$U(t) = C\ F(t) + U_F(T)\ H(t) + U_B(T)$$

Thus

$$\partial S/\partial K_{TP} = -2 \sum_j (U_0(t_j) - U(t_j))\ (\partial U(t_j)/\partial K_{TP})/V_j$$

Differentiation of the fitting function yields

$$\partial U(t)/\partial K_{TP} =$$

$$\sum_{i=1}^{3} \frac{C\ A_i e^{-K_i t}\ (K_B - K_i)}{(K_{TP} + K_B - K_i)^2\ K_i}$$

$$+ \sum_{i=1}^{3} \frac{C\ A_i e^{-K_i T} K_B}{(K_{TP} + K_B - K_i)} \left[\frac{1}{(K_{TP} + K_B)(K_{TP} + K_B - K_i)} \right.$$

$$\left. + \frac{1}{(K_{TP} + K_B)^2} - \frac{1}{K_i (K_{TP} + K_B - K_i)} \right]$$

$$+ \sum_{i=1}^{3} \frac{C\ A_i e^{-(K_i T + (K_{TP} + K_B)(t-T))}}{(K_{TP} + K_B - K_i)} \left[(t - T) \right.$$

$$+ \frac{1}{(K_{TP} + K_B - K_i)} - \frac{K_B}{(K_{TP} + K_B)^2} - \frac{K_B (t - T)}{K_{TP} + K_B}$$

$$\left. - \frac{K_B}{(K_{TP} + K_B)(K_{TP} + K_B - K_i)} \right]$$

$$+ U_F(T) e^{-(K_{TP} + K_B)(t-T)} \left[\frac{K_B}{(K_{TP} + K_B)^2} \right.$$

$$\left. + \frac{K_B (t - T)}{K_{TP} + K_B} - (t - T) \right]$$

$$- U_F(T) \frac{K_B}{(K_{TP} + K_B)^2}$$

Derivative of S with respect to K_B

The procedure is the same as that for the parameter K_{TP} except that

$$\partial U(t) / \partial K_B =$$

$$\sum_{i=1}^{3} \frac{C\ A_i e^{-K_i t}}{(K_{TP} + K_B - K_i)} \left[\frac{K_B}{K_i (K_{TP} + K_B - K_i)} - \frac{1}{(K_{TP} + K_B - K_i)} \right.$$

$$- \frac{1}{K_i} \Bigg]$$

$$+ \sum_{i=1}^{3} \frac{C\, A_i e^{-K_i T}}{(K_{TP} + K_B - K_i)} \Bigg[\frac{1}{K_i} - \frac{K_B}{K_i (K_{TP} + K_B - K_i)}$$

$$- \frac{1}{(K_{TP} + K_B)} + \frac{K_B}{(K_{TP} + K_B)(K_{TP} + K_B - K_i)}$$

$$+ \frac{K_B}{(K_{TP} + K_B)^2} \Bigg]$$

$$+ \sum_{i=1}^{3} \frac{C\, A_i e^{-K_i T + (K_{TP} + K_B)(t-T)}}{(K_{TP} + K_B - K_i)} \Bigg[(t-T)$$

$$- \frac{1}{(K_{TP} + K_B - K_i)} + \frac{1}{(K_{TP} + K_B)} - \frac{K_B}{(K_{TP} + K_B)^2}$$

$$- \frac{K_B t}{(K_{TP} + K_B)} + \frac{K_B T}{(K_{TP} + K_B)} - \frac{K_B}{(K_{TP} + K_B)(K_{TP} + K_B - K_i)} \Bigg]$$

$$+ U_F(T) e^{-(K_{TP} + K_B)(t-T)} \Bigg[\frac{K_B (t-T)}{(K_{TP} + K_B)}$$

$$+ \frac{K_B}{(K_{TP} + K_B)^2} - \frac{1}{(K_{TP} + K_B)} - (t-T) \Bigg]$$

$$+ U_F(T) \frac{K_{TP}}{(K_{TP} + K_B)^2}$$

Derivatives of U(t) with respect to C, $U_F(T)$ and $U_B(T)$

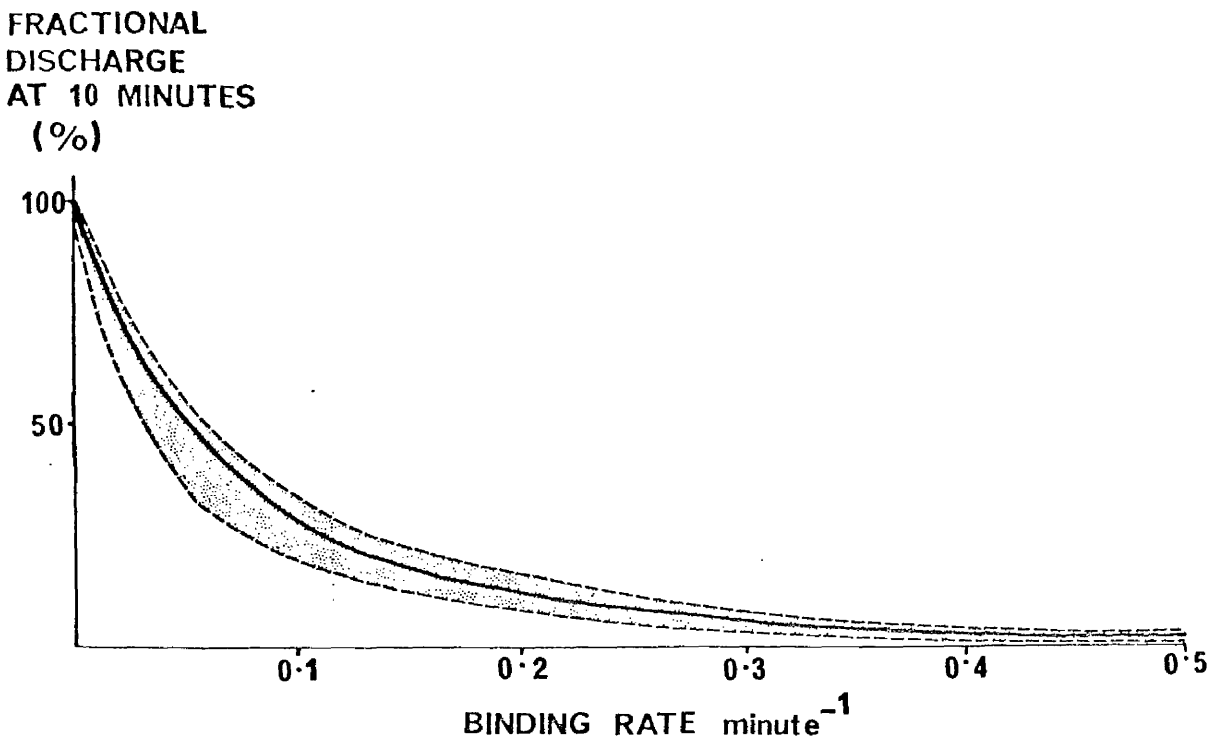
$$\partial U(t)/\partial C = F(t)$$

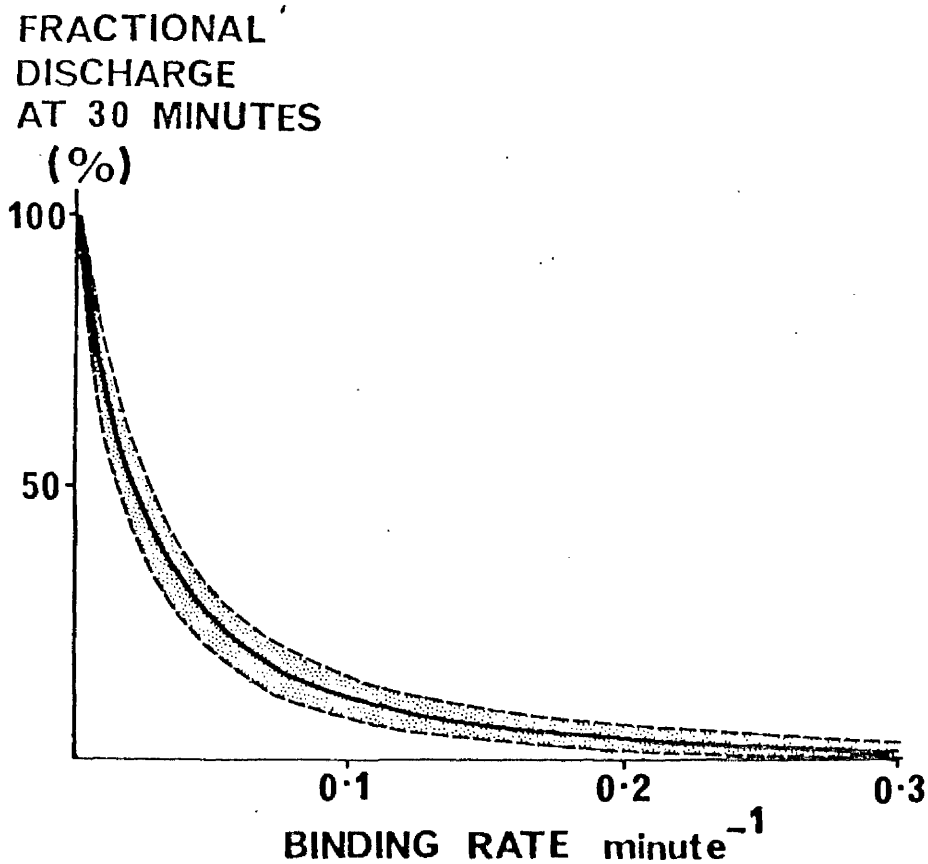
$$\partial U(t)/\partial U_F(T) = H(t)$$

$$\partial U(t)/\partial U_B(T) = 1$$

APPENDIX TWO

Figures showing the relationship between binding rate and the fractional discharge of radioiodide at 10 and 30 minutes (Section 6.3).





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