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THE UPTAKE OF [^{35}S]METHIMAZOLE BY SHEEP THYROID SLICES *IN VITRO*

GRAHAM G. SKELLERN and MASSOUD MAHMOUDIAN
Drug Metabolism Research Unit, Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow, G1 1XW, U.K.

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Abstract—The uptake of [^{35}S]methimazole by sheep thyroid slices has been shown to be activated in the presence of iodide. The total uptake (Q) of [^{35}S]methimazole was shown to be the sum of a saturable process and a non-saturable process. The constants Q_{max}, K_s and P in the two-term equation were determined using a published statistical method and a Fortran IV computer programme. Diiodotyrosine (DIT) at a 0.1 mM concentration stimulated the saturable uptake of [^{35}S]methimazole appreciably in the absence of iodide, whilst thyroid-stimulating hormone (TSH) inhibited uptake in the presence of iodide and was of no effect in the absence of iodide. Propylthiouracil (PTU) inhibited the saturable uptake of [^{35}S]methimazole whilst perchlorate had no effect.

It has been clearly demonstrated *in vivo* that the antithyroid drugs are concentrated in the thyroid gland in man and rat [1, 2]. Although it had been known for some time that when radio-labelled drug is administered, the radio-label accumulates in the thyroid [3–5], this accumulated radioactivity might be a metabolite of the drug.

With iodine-deficient, normal and iodine-treated rats, it has been observed [6] that there is a significant correlation between the oxidation of [^{35}S]methimazole to sulphate by the thyroid and the intrathyroidal iodine concentration. Also, the extent of accumulation of [^{35}S]methimazole is dependent on the dose of iodide.

The possibility that a drug concentration gradient could be simulated *in vitro*, using thyroid slices, has been examined with [^{35}S]thiourea and [^{35}S]thiouracil [3]. A concentration gradient was not obtained either in the presence or absence of KI in the incubation medium.

Jirousek and Cunningham [7] in their examination of the possibility that the sulphenyl iodide group might play an important role in the iodination of protein, found that the binding of [^{14}C]thiouracil to beef thyroid microsomes increased after triiodide pretreatment of the microsomes.

Pommier *et al.* [8] showed that diiodotyrosine (DIT) exerted a dual effect on protein iodination and thyroxine synthesis catalysed by thyroid peroxidase. They found that at high concentrations of free DIT protein iodination was inhibited, whilst at lower concentrations thyroxine formation was stimulated. They concluded that DIT might have some regulatory role in the synthesis of thyroxine.

The purpose of the present study was to examine the mechanisms involved in the uptake of [^{35}S]methimazole by sheep thyroid slices *in vitro* and its relationship to the presence of iodide, DIT and thyroid-stimulating hormone (TSH). The effects of perchlorate and propylthiouracil (PTU) on the uptake of [^{35}S]methimazole have also been studied, since it is known that perchlorate actively discharges iodide from the thyroid gland [9, 10], whilst PTU belongs to the same class of compounds as methimazole, in that it is an inhibitor of hormone synthesis by the thyroid gland.

MATERIALS AND METHODS

Materials

Sheep thyroid glands were obtained from a local slaughter-house, transported to the laboratory in ice and used within 2 hr of death. Blood was obtained from Sprague–Dawley male rats.

[^{35}S]methimazole (592 μCi·mg^{-1}) was a gift from Dr. W. D. Alexander, Department of Medicine, University of Glasgow. 2-[^{14}C]Methimazole was prepared according to the method of Skellern *et al.* [4]. Diiodotyrosine (DIT), thyroid stimulating hormone (TSH) and bovine serum albumin (BSA fraction *V*) were obtained from Sigma London Chemical Company Ltd. Methimazole and propylthiouracil were obtained from Aldrich Chemical Co. Haemoglobulin was obtained from Koch-Light Laboratories Ltd. All other reagents and solvents were AnalaR grade.

The [^{35}S]methimazole thyroid uptake studies were carried out in Krebs–Ringer bicarbonate buffer pH 7.5 containing glucose (8 mM). Solutions of compounds to be added to the incubation medium were prepared using this buffer.

Cellulose thin-layer plates (0.25 mm) were prepared. The developing solvents were, solvent 1, ethanol/1 M ammonium acetate (55:45 by vol.), and solvent 2, *n*-butanol–glacial–acetic acid–water (120:30:50 by vol.).

Methods

Binding of methimazole to blood components. Equilibrium dialysis was used for the determination of binding of 2-[^{14}C]methimazole to BSA, haemoglobulin and rat plasma proteins in the absence of trichloracetic acid (TCA). The method of Giri and Combs [11] was adopted for the study of the binding of methimazole to rat blood constituents in the presence of TCA. Methimazole was determined either spectrophotometrically at 252 nm or by liquid scintillation

counting. The spectrophotometric method was suitable only for higher concentrations of methimazole (1 mM) though it was still necessary to correct for endogenous 252 nm absorbing material by measuring the absorbance of the test solution against a blank containing the proteins or cells without the inclusion of methimazole. 2-[^{14}C]Methimazole was used to measure the extent of binding at lower concentrations of drug.

Incubation procedure. The sheep thyroids were sliced, weighed into vials (0.1–0.2 g) and pre-incubated with the Krebs–Ringer bicarbonate buffer, containing KI in the concentration range 0.01–1 mM, for 30 min at 37°. To this was added an appropriate aliquot of [^{35}S]methimazole solution to give a final concentration of 1 μm in a 10 ml volume. The thyroid slices were incubated for a further 2 hr. Control incubations were carried out, omitting KI.

After incubation, the vials were put on ice, the thyroid slices removed and surplus adhering buffer removed. The slices were weighed into liquid scintillation vials, and dissolved in 0.5 M NaOH (4 ml) by being left overnight in a water bath at 37°. A commercial scintillator (11 ml, PCS Amersham/Searle Corporation) was added to the thyroid digests.

A toluene-based scintillator (10 ml, 2-(4′-*t*-butyl phenyl)-5-(4″-biphenylyl)1,3,4-oxadiazole) was added to aliquots of the incubation medium and standard [^{35}S]methimazole solutions. All samples were counted in a liquid scintillation counter (Nuclear, Chicago), and measurements corrected by using a channels-ratio quench correction method.

A preliminary study was performed using the above conditions and varying the incubation time between 15 min and 2.5 hr, and 2 hr shown to be sufficient time to establish equilibrium.

Identification of the nature of the radioactivity and binding in the thyroid slices. (i) The initial conditions were as described under Incubation Procedure, using KI over a concentration range of 0–1 mM, and 2 μM [^{35}S]methimazole. The slices, instead of being digested, were homogenised with 3 vols. of water. Aliquots of the thyroid homogenate and incubation medium were applied to cellulose thin-layer plates by evaporating the solvent off using a stream of oxygen-free nitrogen and these were developed in Solvents 1 and 2 as described under Materials. The [^{35}S]-containing compounds were located on the plates using a radio-chromatogram scanner (Panax Ltd.). (ii) Thyroid slices were incubated with 0.1 mM KI and 2 μM [^{35}S]methimazole, as described under Incubation Procedure. The slices were then homogenised with 5 vols. of water. The thyroid homogenate was centrifuged (600 *g*, 10 min) and the resulting supernatant ultrafiltered (4 ml) using Diaflo membranes (mol. wt cut-off, 2500, Amicon). Duplicate samples of 600 g-pellet, high mol. wt fraction and ultrafiltrate were taken for liquid scintillation counting.

Effect of KI *on the uptake of* [^{35}S]*methimazole by thyroid slices.* Conditions were as described under Incubation Procedure, pre-treating the thyroid slices with KI over a concentration range of 0–1 mM. For a given KI concentration a final [^{35}S]methimazole concentration of 0.1–10 μM was achieved in the incubation medium.

Effect of DIT, PTU, TSH *and perchlorate on the uptake of* [^{35}S]*methimazole by thyroid slices.* The possible interaction of DIT and PTU on the uptake of [^{35}S]methimazole by thyroid slices was studied using the conditions described under Incubation Procedure with KI and [^{35}S]methimazole concentrations up to 0.1 mM and 10 μM respectively. The slices were pre-incubated either with DIT over a concentration range of 0–1 mM, or with 1 μM or 10 μM PTU.

The effect of TSH and perchlorate was examined by pre-incubating the slices either in the absence or presence of 1 mM KI and TSH (1 and 10 mU ml^{-1}). Alternatively, the slices were pre-incubated either in the absence or presence of 0.1 mM and 1 mM $KClO_4$.

The binding of methimazole to various blood constituents in the presence or absence of TCA is shown in Table 1. TCA induced binding significantly except in the case of BSA. In the absence of TCA, binding was not significant. [^{35}S]Methimazole was shown to be the major detectable compound in the thyroid homogenate and medium at various KI concentrations, after a 2 hr incubation of [^{35}S]methimazole and thyroid slices (Fig. 1). Solvents 1 and 2 (Fig. 1) are both capable of separating [^{35}S]methimazole from sulphate. Some radioactivity was detected at the origin. This may be artefactual due to irreversible binding due to the application of a whole homogenate to the t.l.c. plate.

The results of the incubation of [^{35}S]methimazole with thyroid slices, and subsequent ultrafiltration of the homogenate are shown in Table 2. Most of the radioactivity is associated with the high mol. wt material.

The optimum incubation time required for the maximum equilibrium uptake of [^{35}S]methimazole,

Table 1. The percentage of methimazole bound to various blood components and fractions in either the presence or absence of TCA

Blood components and fractions	% of methimazole bound	
	Absence of TCA	Presence of TCA*
Plasma	NS (20 μM)	18.4
Erythrocytes	NS (18 μM)	96.3
Erythrocytes (ghosts)	—	99.3
Erythrocyte cytosol	—	75.8
Haemoglobulin	NS (35 μM)	45.5
BSA	NS (35 μM)	5.0

Figures in parentheses are methimazole concentrations.
* 1 mM methimazole used in all TCA experiments. NS—not significant.

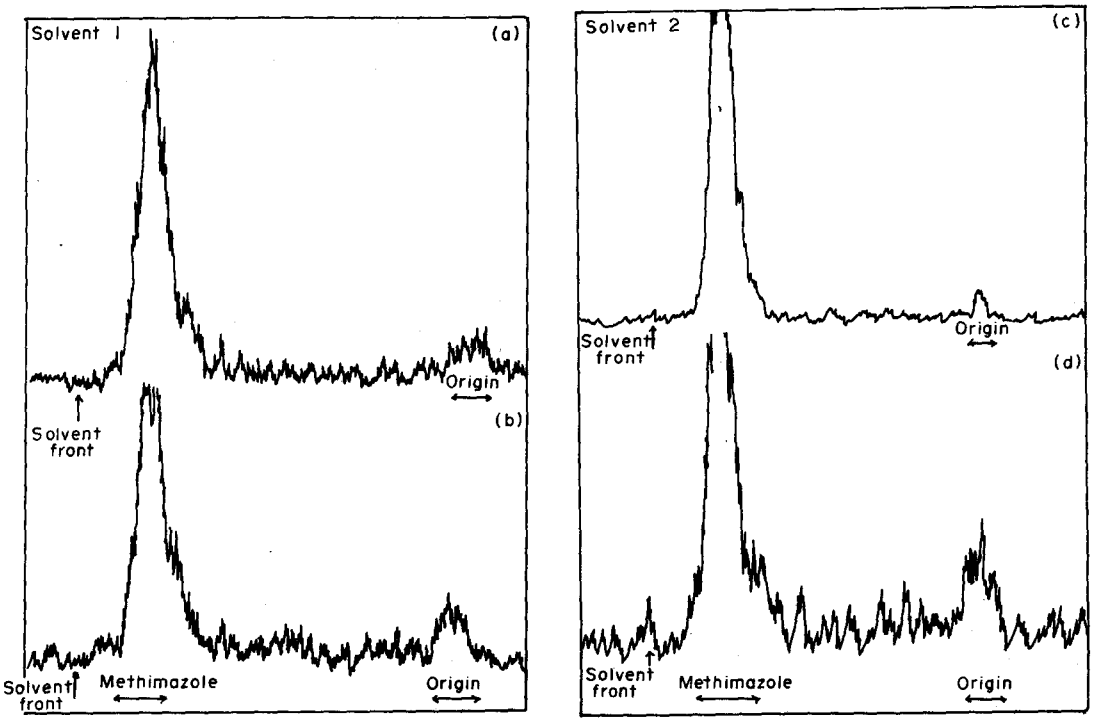

Fig. 1. Typical radiochromatograms of medium (A and C) and thyroid homogenate (B and D). Incubations were as described in Methods using 1 mM KI. Solvent 1, ethanol–1 M ammonium acetate (55:45 by vol). Solvent 2, *n*-butanol–glacial acetic acid–water (120:30:50 by vol.).

Table 2. The comparison of the values found experimentally from the ultrafiltration experiment with theoretical values for the saturable term, $Q_{max}/1 + (K_s/[S])$ and non-saturable term $P[S]$

Experimental results Per cent of [^{35}S]radioactivity in tissue		Theoretical results* Per cent of the total uptake (Q)	
Large particle	17.5		
High mol. wt fraction	68.4	Calculated saturable term	77.7
Ultrafiltrate	14.1	Calculated non-saturable term	23.3
Tissue/medium	1.84	$Q/[S]$	2.66

Conditions, KI = 0.1 mM and a final [^{35}S]methimazole concentration of 1.76 nmoles ml^{-1}. Each value is the mean of two experiments.

* Calculated by applying the [^{35}S]methimazole concentration into the equation of the KI 0.1 mM curve (Fig. 3, curve C).

giving a concentration gradient between thyroid and medium, was between 1.5–2.0 hr, after the initial 30 min pre-incubation with KI. Thereafter, the ratio of the concentration of [^{35}S]methimazole in the thyroid slice to that in the medium began to decline. The concentration of KI which gave the maximum thyroid to medium ratio of [^{35}S]methimazole was 1 mM (Fig. 2).

Examination of the total uptake over the concentration range of 0.1–10 μM for [^{35}S]methimazole showed that uptake was stimulated by increasing the KI concentration (Fig. 3). It could be demonstrated that the curves fitted an equation, containing a saturable term and a non-saturable term, of the form

$$Q = Q_{max}/[1 + (K_s/[S])] + P[S] \quad \text{Equation 1.}$$

Where Q = the total uptake of [^{35}S]methimazole by the thyroid slices.

Q_{max} = the maximum uptake by the thyroid slices.

K_s = the [^{35}S]methimazole concentration at half the maximum value.

$[S]$ = the concentration of [^{35}S]methimazole in the incubation medium.

P = non-saturable term constant.

The data could not be fitted by equations containing only a saturable term or only a non-saturable term.

With the aid of a published statistical method [12] and a Fortran IV computer programme, the constants Q_{max}, K_s and P were determined (Table 3). This method was unsuitable for the 1 mM KI data and the constants Q_{max} and K_s were determined using a published graphical method [13] of calculating the best straight line fitted to Equation 1 rearranged to:

$$[S]/Q = [S]/Q_{max} + K_s/Q_{max}.$$

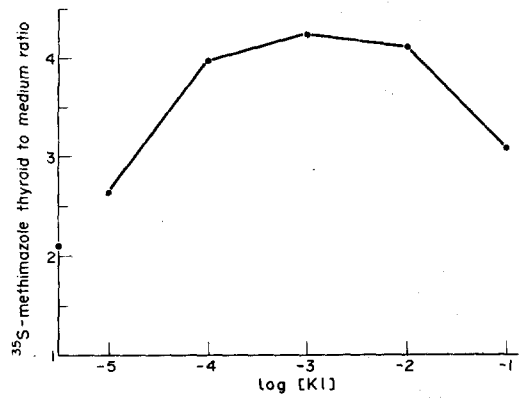

Fig. 2. The effect of varying the KI concentration on the uptake of [^{35}S]methimazole by thyroid slices. Each value is the mean of three determinations.

A comparison of the theoretical values for the saturable and non-saturable terms in Equation 1, at fixed KI and [^{35}S]methimazole concentrations, was made with the experimental data obtained from the ultrafiltrate binding experiment (Table 2).

The effect of various concentrations of DIT in the presence of 1 mM KI on the accumulation of [^{35}S]methimazole was to suppress it, whilst in the absence of KI, DIT stimulated [^{35}S]methimazole uptake (Fig. 4).

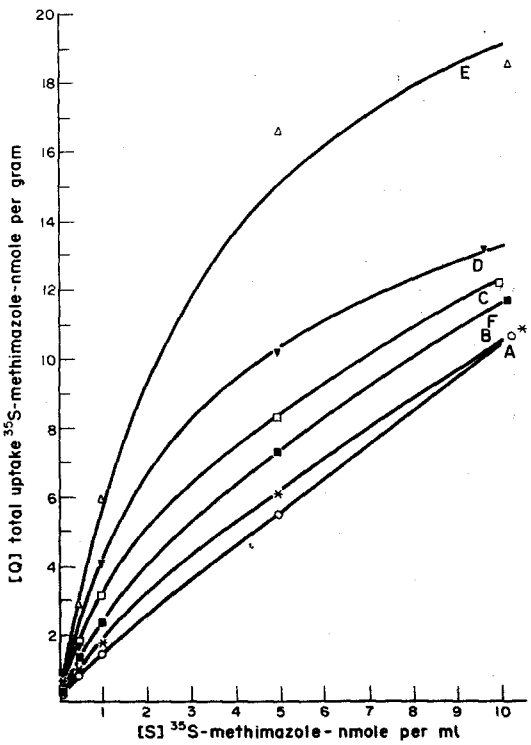

Fig. 3. The total uptake (Q) of [^{35}S]methimazole by thyroid slices, depending on the concentration of free [^{35}S]methimazole [S] in the medium at equilibrium. The concentration range of [^{35}S]methimazole was 0.1–10 μM. Each value is the mean of four determinations. Curves obtained using the computer programme. Curve (a) KI = 0; (b) KI = 0.01 mM; (c) KI = 0.1 mM; (d) KI = 0.5 mM; (e) KI = 1.0 mM; (f) DIT = 0.1 mM, KI = 0.

Table 3. Calculated constants for Equation 1, describing the uptake of [^{35}S]methimazole by thyroid slices, with varying KI and DIT concentrations

KI mM	DIT mM	Q_{max} nmoles·g^{-1}	K_s nmoles·ml^{-1}	P ml·g^{-1}
0	0	0.75	0.58	0.97
0.01	0	2.87	1.73	0.81
0.10	0	7.17	1.75	0.62
0.50	0	14.09	2.52	0.20
*1.00	0	25.91	3.61	0
0	0.1	5.60	2.35	0.71

Data from Fig. 3.
* Determined using a graphical method.

Pre-incubation of the thyroid slices with 0.1 mM DIT instead of KI and examination of the total uptake of [^{35}S]methimazole over the wide concentration range of [^{35}S]methimazole, showed that the results also fitted a model of the form of Equation 1 (Fig. 3). The calculated constants K_s and Q_{max} are comparable with the results obtained with thyroid slices pre-incubated either with 0.1 mM or 0.01 mM KI (Table 3).

Propylthiouracil exerts its effect in the thyroid in a similar manner to methimazole and therefore would possibly act as an inhibitor of [^{35}S]methimazole uptake. Pre-incubation of the thyroid slices with 0.1 mM KI and with either 1 or 10 μM PTU showed that the inhibitory action of PTU on the uptake of [^{35}S]methimazole increased in magnitude with increasing PTU concentrations (Fig. 5). The constants Q_{max}, K_s and P were determined using the Fortran IV computer programme (Table 4).

The perchlorate ion is capable of discharging iodide from the thyroid gland [9], but had no effect on the uptake of methimazole (Table 5).

Table 6 shows the effect of TSH on the [^{35}S]methimazole uptake. In the presence of 1 mM KI, uptake

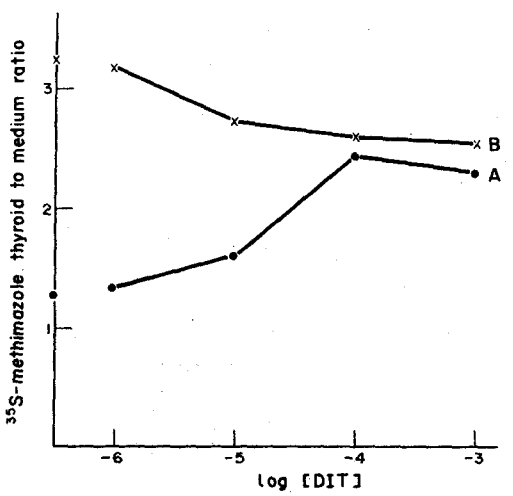

Fig. 4. The effect of various DIT concentrations on the uptake of [^{35}S]methimazole by thyroid slices. Each value is the mean of four determinations. Curve (a) KI = 0; (b) KI = 1 mM.

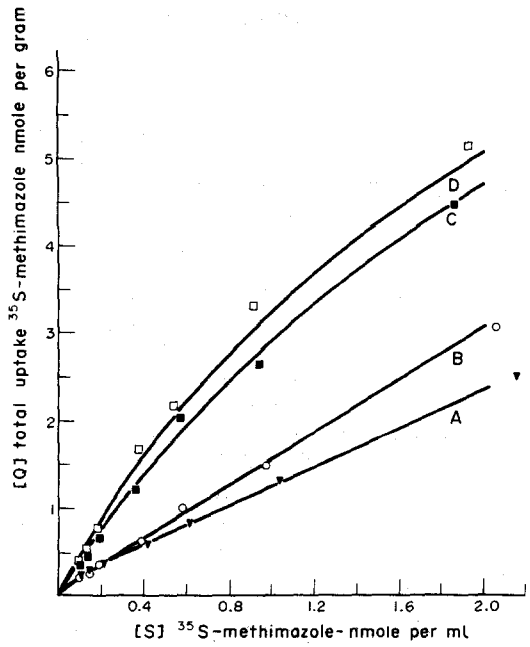

Fig. 5. The effect of PTU on the total uptake (Q) of [^{35}S]methimazole by thyroid slices, depending on the concentration of free [^{35}S]methimazole [S] in the medium at equilibrium. The concentration range of [^{35}S]methimazole was 0.1–2 μM. Each value is the mean of three determinations. Curves obtained using the computer programme. Curve (a) KI = 0 mM, PTU = 0; (b) KI = 0.1 mM, PTU = 10 μM; (c) KI = 0.1 mM, PTU = 1 μM; (d) KI = 0.1 mM, PTU = 0.

was significantly reduced, whilst in the absence of KI it had no effect. It was observed that there was some variability from one tissue preparation to another, as is reflected in the standard deviation values (Tables 5 and 6).

DISCUSSION

TCA has been shown to induce the binding of methimazole to erythrocytes and cellular components, resulting in 10–100 per cent binding in some cases (Table 1). TCA-induced binding has also been demonstrated with phenylthiourea and erythrocytes [14]. Therefore, since reagents such as TCA might cause artefactual induced binding of [^{35}S]methimazole to thyroid tissue, no such reagents were used in subsequent experiments.

Table 4. Calculated constants for Equation 1 describing the uptake of [^{35}S]methimazole by thyroid slices in the presence of PTU

KI mM	PTU μM	Q_{max} nmoles·g^{-1}	K_s nmoles·ml^{-1}	P ml·g^{-1}
0.1	0	7.17	1.75	0.62
0.1	1	6.60	2.01	0.70
0.1	10	0.05	0.04	1.50
0	0	0.17	0.09	1.08

Data from Fig. 5.

Table 5. Effect of the perchlorate ion on the uptake of [^{35}S]methimazole by thyroid slices

KI mM	$KClO_4$ mM	[^{35}S]methimazole thyroid/medium Mean	S.D.
0	0	1.48	0.24
0	1	1.39*	0.40
0.1	0	3.45	1.24
0.1	1	4.07*	1.80

Each value is the mean of four experiments. S.D. = Standard deviation.
* No significant difference from the controls.

The uptake of [^{35}S]methimazole by thyroid slices has been clearly demonstrated *in vitro*, and has been shown to be dependent on the concentration of iodide in the incubation medium (Fig. 2). Other workers examining the binding of [^{14}C]thiouracil to either β-lactoglobulin [15] or beef liver microsomes [7], have found that binding was negligible at low iodide concentrations, and increased with increasing triiodide concentration although TCA was used to stop the reaction after the incubation.

It is difficult to explain why Maloof and Soodak [3] did not find a concentration gradient between sheep thyroid slices and medium with [^{35}S]thiourea and [^{35}S]thiouracil even when KI was present in their incubation medium. No indication was given as to whether the thyroid slices were pre-incubated with the iodide. This may be important, since it is possible that the binding sites have to be activated prior to binding the thiocarbamides.

It would appear that most of the [^{35}S]methimazole binds to high mol. wt components of thyroid tissue (Table 2). Some radioactivity remained at the origin in the t.l.c. experiments (Fig. 1), but most migrated as free methimazole and must therefore have been bound reversibly since dissociation occurs during the development of the t.l.c. plate, but did not occur during ultrafiltration. In studies with [^{14}C]thiouracil [7] and [^{35}S]thiourea [16, 17] using microsomal preparations appreciable amounts of the radioactivity were bound to the protein irreversibly.

That the [^{35}S]methimazole uptake was dependent on the iodide concentration (Fig. 2) is consistent with results found by other researchers. Marchant *et al.* [6] demonstrated *in vivo* that rats on a low iodide

Table 6. Effect of varying TSH concentrations on the uptake of [^{35}S]methimazole by thyroid slices

KI mM	TSH mUml^{-1}	[^{35}S]methimazole thyroid/medium mean	S.D.
1	0	3.71	1.24
1	1	3.08*	1.20
1	10	2.63*	0.60
0	0	1.34	0.25
0	1	1.27†	0.10
0	10	1.18†	0.03

Each value is the mean of six experiments. S.D. = Standard deviation.
* Significantly different from the control ($P < 0.025$).
† No significant difference from the control.

diet had a lower ability to accumulate or metabolise [^{35}S]methimazole in the thyroid than animals on a normal diet. The thyroid to plasma ratio was 2.96 in controls compared to 1.2 in iodide-deficient rats. Further to this, rats receiving an acute dose of iodide after being maintained on a low iodide diet showed an increased ability to accumulate and metabolise the drug, giving a thyroid to plasma ratio of 4.2 for [^{35}S]methimazole.

The total uptake of drug can best be described by Equation 1 which consists of two terms, one being a term describing a saturable process and the other describing a non-saturable process. A single term equation could not describe the data.

An *in vivo* examination [2] of the accumulation of [^{35}S]thiouracil by the rat thyroid showed that it was dose-dependent, in that at low concentrations there was rapid uptake of the drug and a greater percentage of the dose administered was accumulated by the thyroid, whilst a lesser percentage was accumulated at higher drug concentrations. In this study, the authors felt that this could be explained in terms of a carrier transport mechanism being saturated at high drug concentration.

When iodide was not added to the incubation mixture, the non-saturable terms contributed significantly to the total uptake (Q) although some saturable binding was seen (Fig. 3) probably because of the presence of endogenous iodide in the thyroid.

With increasing KI concentration the non-saturable term (P) became less significant; at 1 mM KI, $P = 0$ and the total uptake (Q) was solely due to the saturable process (Table 3). Q_{max} is a measure of the number of binding sites per gram of thyroid tissue and is seen to increase with increasing KI concentration (Table 3). The reciprocal of K_s can be considered as a constant describing the overall affinity of [^{35}S]methimazole for binding sites. A 10-fold increase in KI, i.e. 0.01 mM to 0.1 mM, produced little change in K_s, whilst Q_{max} was increased by over a factor of two. A 100-fold increase in KI, i.e. 0.01–1 mM gave a 2-fold increase in K_s, indicating a decrease by a factor of two in the overall affinity of [^{35}S]methimazole for the thyroid cellular components. The concentration of KI in these studies would not be sufficiently high to have changed the water content of the tissue. Assuming that it is constant, the non-saturable term constant (P) is a measure of the diffusional processes taking place.

As shown in Table 2 for one set of experimental conditions the fraction of the drug taken up by the saturable process is similar to the fraction bound to high mol. wt components of the tissue while the fraction taken up by the non-saturable process is similar to the fraction located in the 'tissue water' (ultrafiltrate). It is proposed that the saturable uptake is the result of an active uptake associated with reversible binding to cell components while the non-saturable uptake is the result of passive diffusion.

The inhibitors of thyroxine synthesis fall into two groups, those which inhibit iodide uptake by the thyroid and those which block the synthesis of thyroxine and triiodothyronine. Perchlorate belongs to the first class of compounds in that it actively discharges iodide from the thyroid and competitively inhibits iodide uptake by the thyroid [9, 10]. If the concentration of free iodide ions in the cell were important for the uptake of methimazole, it would have been anticipated that perchlorate would have blocked [^{35}S]methimazole uptake. This was not the case since perchlorate did not affect the accumulation of [^{35}S]methimazole (Table 5), suggesting that the iodine must be bound in some form to activate methimazole uptake.

PTU belongs to the second class of compounds, as does methimazole, in that it is an inhibitor of the synthesis of thyroxine and triiodothyronine by the thyroid gland [22]. In incubation experiments in which the concentration of KI was 0.1 mM, the total [^{35}S]methimazole uptake was decreased by PTU. Thus when the concentration of PTU was 10 μM the plot of methimazole uptake against methimazole concentration (Fig. 5) was similar to that obtained for uptake in the absence of KI (and PTU). In both these curves, the saturable process makes only a very small contribution to total uptake (Table 4). Thus PTU appears to inhibit the saturable uptake process, possibly by competing with [^{35}S]methimazole for intracellular binding sites.

Some investigators [18–20] postulate that the iodide undergoes a two electron oxidation, resulting in a protein-bound iodinium ion (I^+) which is active in the iodination of protein in the thyroid gland, and it is with this protein bound iodinium ion that methimazole is thought to react forming a mixed disulphide and liberating I^- [20]. Alternatively, it has been postulated that the iodide undergoes a one electron oxidation, giving a free radical active iodinating species (E–I) which reacts with a tyrosine radical [21]. Either of these iodination species may be the activator of the uptake of [^{35}S]methimazole.

Taurog [23] demonstrated that the extent of metabolism of [^{35}S]methimazole and [^{35}S]propylthiouracil was dependent on the concentration of substrate present in the incubation medium containing the thyroid peroxidase isolated from pig. At low concentrations of drug (10 μM), [^{35}S]methimazole was metabolised to sulphate, as shown by t.l.c. Doubling the [^{35}S]methimazole concentration resulted in no metabolism, as measured by the absence of sulphate. Similar results were obtained with PTU. This observation could explain why in this present study only intact [^{35}S]methimazole was found in the thyroid homogenate by t.l.c. (Fig. 1), indicating that metabolism had not occurred and that methimazole was not covalently bound, although reversible binding was demonstrated (Table 2).

DIT can, to some extent, substitute for KI in stimulating saturable uptake. The effect of DIT depends on its concentration (Fig. 4). Thus 0.1 mM DIT results in similar uptake to 0.1 mM KI in the experiments studied (Fig. 3). Q_{max} with 0.1 mM DIT is similar to that found with 0.1 mM KI (Table 3). However, DIT appears to be a less efficient stimulator than KI. Inclusion of DIT (0.1 mM or 1.0 mM) in incubations containing 1.0 mM KI significantly reduces ($P < 0.05$) methimazole uptake (Fig. 4). DIT is appreciably deiodinated when incubated with sheep thyroid slices [24] and the possibility that free iodide produced in this way contributed to methimazole uptake in the present experiments cannot be overlooked.

Perchlorate in either the presence or absence of KI

did not affect the uptake of [^{35}S]methimazole by thyroid slices (Table 5).

TSH significantly reduced the uptake of [^{35}S]methimazole in the presence of KI, but not in the absence of KI (Table 6). This is in partial agreement with the findings of other investigators [6, 18, 25] who found that TSH in the presence of KI decreased the uptake and metabolism of thiourea, but contrary to our findings, they found that TSH alone increased the uptake and metabolism of thiourea. Bobek [26] found that *in vivo* and *in vitro* in rat the uptake of [^{35}S]thiocyanate was increased in the presence of TSH, although the metabolism of thiocyanate to sulphate was decreased.

The results presented in this study demonstrate the uptake of [^{35}S]methimazole by thyroid slices. The [^{35}S]methimazole concentration used in these studies are comparable to the levels found in plasma from thyrotoxic patients receiving the drug (unpublished results). It is of interest to observe that the thyroid to medium ratios for [^{35}S]methimazole in this study (Fig. 2) are of the same order of magnitude as those found in rat [6] and human [1] *in vivo* studies. The results would indicate that we are observing the transport of [^{35}S]methimazole into the cell, although the possibility of surface binding to the thyroid slice cannot be ignored. It cannot be stated with any certainty whether iodide is activating a saturable transport process or activating sites on the iodinating enzyme. From our present knowledge of thyroid peroxidase, and the effect of perchlorate, PTU and DIT in this study on the uptake of [^{35}S]methimazole, we would presume that iodide is activating sites on the thyroid peroxidase. Further studies with isolated thyroid enzymes are being carried out.

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