Biochemical Pharmacology, Vol. 32, No. 2, pp. 241–247, 1983.
Printed in Great Britain.

0006-2952/83/020241-07 $03.00/0
Pergamon Press Ltd.

EFFECT OF VARIATIONS IN ACUTE AND CHRONIC IODINE INTAKE ON THE ACCUMULATION AND METABOLISM OF [^{35}S]METHIMAZOLE BY THE RAT THYROID GLAND

DIFFERENCES FROM [^{35}S]PROPYLTHIOURACIL*

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(*Received* 15 *March* 1982; *accepted* 15 *July* 1982)

Abstract—Studies were performed to ascertain the effect of various levels of chronic iodine intake and varying doses of iodide [0.002–100 μmoles KI/100 g body weight (BW)] given acutely on the rat thyroid metabolism of [^{35}S]methimazole ([^{35}S]MMI, 8.76 μmoles/kg BW). Variations in both acute and chronic iodine intake were associated with as much as four-fold changes in thyroid levels of total ^{35}S and unchanged [^{35}S]MMI. Chronic low iodine intake resulted in a considerable reduction in the thyroid uptake of [^{35}S]MMI (40% decrease) from high or normal chronic iodine intake. Unlike [^{35}S]PTU studies, the effect of increasing acute iodide dosage produced a biphasic response in the thyroid uptake of [^{35}S]MMI only in low chronic iodine intake. In these animals 0.1 μmoles KI/100 g BW produced the maximum uptake of [^{35}S]MMI (300% increase) but had no effect on high or normal chronic iodine intake. In these latter groups of rats, thyroidal total ^{35}S increased to plateau levels with increasing acute iodide dosage in the range of 0.1–1 μmoles/100 g BW which were unaffected by increased iodide up to 100 μmoles/100 g BW. In low chronic iodine intake rats also, the thyroid ^{35}S level seen at 1 μmole/100 g was unaffected by increased iodide dosage up to 100 μmoles/100 g. The steady thyroid ^{35}S levels seen in this acute iodide dose range in low, normal and high chronic iodine rats were 100, 70 and 110% respectively greater than their control values. Unlike [^{35}S]PTU studies, in general, an increase in thyroid total ^{35}S achieved by varying acute or chronic iodine intake was found to be associated with a large increase in the percentage thyroid ^{35}S occurring as free inorganic sulphate with a consequent effect on thyroid unchanged [^{35}S]MMI. In chronic low iodine intake animals treated with acute radioiodide, in agreement with [^{35}S]PTU studies, no direct correlation was found between thyroid uptake or oxidation of [^{35}S]MMI and thyroidal total iodine, the accumulation or organification of acute [^{125}I]iodide, the occurrence of the Wolff–Chaikoff effect or saturation of thyroid iodide transport.

The effects of variations in chronic iodine intake and varying doses of iodide given acutely on rat thyroid uptake and metabolism of [^{35}S]PTU are presented in the preceding paper [1]. This complementary paper details the effects on [^{35}S]MMI and discusses the differences between the two drugs.

METHODS

The procedure was virtually identical to the previous [^{35}S]PTU study. Total thyroid iodine was measured by autoanalyser as previously described by Marchant *et al.* [2]. Only in the case of LID animals was [^{125}I]iodide used (0.5–20 μCi).

* Supported in part by research grant No. AM-18416 from the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institute of Health, Bethesda, MD 20205, U.S.A.

This research was presented in part at the Ninth Annual Meeting of the European Thyroid Association, West Berlin, September 1978 (abstract 10).

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RESULTS

Thyroid total ^{35}S accumulation

Variations in acute and chronic iodine intake among the several groups was associated with thyroid total ^{35}S levels that lay between 12.5 ± 0.95 (mean ± S.E.M.) and 52.0 ± 3.88 nmoles/g thyroid (Fig. 1).

Effect of chronic iodine intake. Comparing control animals from each chronic iodine intake group, there was no difference between NID and HII rats [21.7 ± 3.78 (mean ± S.E.M.) and 22.8 ± 2.85 nmoles ^{35}S/g thyroid respectively]. However, thyroid total ^{35}S level in LID rats was reduced by 40% to 12.9 ± 2.57 nmoles/g thyroid.

Effect of acute iodide. The effect of acute iodide pre-treatment prior to [^{35}S]MMI administration on the thyroid level of total ^{35}S varied considerably depending on the chronic iodine intake (Fig. 1).

Effect of iodine intake on thyroid metabolism of [^{35}S]MMI

In TLC of thyroid homogenates on cellulose plates in an ethanol/1 M ammonium acetate solvent system

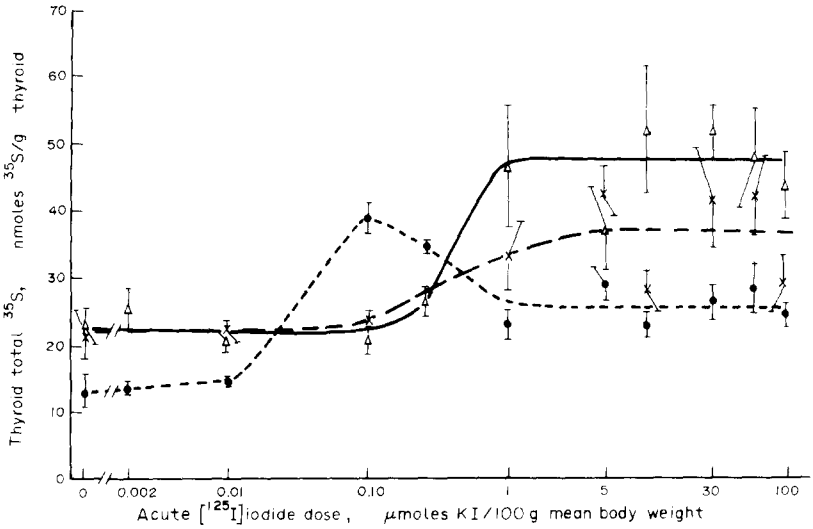

Fig. 1. The effect of chronic iodine intake (●-----●, LID; ×—×, NID; △——△, HII) and acute iodide dose given 1 hour prior to [^{35}S]MMI administration on rat thyroid total ^{35}S level. In general each point is the mean ± S.E.M. for six thyroid glands. Only the acute iodide given to LID rats was ^{125}I-labelled.

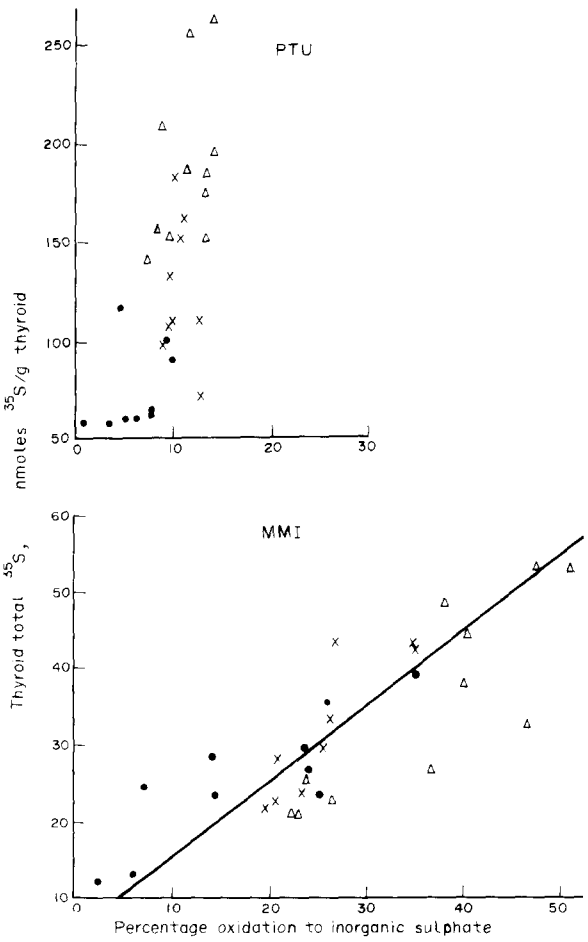

Fig. 2. The thyroid total ^{35}S level, 1 hour after [^{35}S]MMI (lower panel) or [^{35}S]PTU (upper panel, previously unpublished) administration obtained after various acute iodide doses and LID (●), NID (×) or HII (△) plotted in relation to the percentage of thyroid ^{35}S occurring as free inorganic sulphate. Each point is the mean for 2–18 thyroid glands.

(55/45 v/v) three ^{35}S peaks were consistently found: protein-bound ^{35}S, inorganic sulphate and unchanged MMI as previously reported by Marchant and Alexander [3]. In NID and HII rats the effect of a different acute iodide dose was mainly a change in the percentage of thyroid ^{35}S occurring as free inorganic sulphate and unchanged MMI, but in LID rats considerable changes in all three components were observed (Table 1). A general correlation was found between the thyroid total ^{35}S level and the percentage of thyroid ^{35}S occurring as free inorganic sulphate for [^{35}S]MMI but not for [^{35}S]PTU (Fig. 2). The effect of acute and chronic iodine intake on the actual thyroid level of unchanged [^{35}S]MMI (a combination of thyroid uptake and metabolism) is shown in Table 2. In contrast to observations for [^{35}S]PTU, variations in acute and chronic iodine intake did not have the same effect on the thyroid level of unchanged [^{35}S]MMI as on the thyroid total ^{35}S level. This was mainly due to variation in the percentage of thyroid ^{35}S occurring as unchanged [^{35}S]MMI.

Serum ^{35}S data

Neither the level of total ^{35}S in serum nor the percentage of this ^{35}S occurring as unchanged [^{35}S]MMI (where measured, LID and HII rats only) was affected by the range of acute iodide doses administered. Therefore the effect of variations in acute iodide intake on the changes in the thyroid accumulation of total ^{35}S and unchanged [^{35}S]MMI was not due to changes in the serum level of [^{35}S]MMI. Overall mean serum ^{35}S levels (± S.E.M.) for LID, NID and HII rats were 11.1 ± 0.15, 8.9 ± 0.11 and 11.0 ± 0.16 nmoles/ml. In LID and HII rats respectively the overall mean percentage of serum total ^{35}S as unchanged [^{35}S]MMI (± S.E.M.) was 60.1 ± 1.74 and 64.0 ± 1.42%.

Thyroid iodine metabolism

The acute iodide administered was only ^{125}I-labelled for LID rats and so for these animals only

are detailed thyroid iodine data presented. Table 3 shows the thyroid accumulation of acute radioiodine and the percentage of that accumulated which is protein-bound. Figs 3–5 show in the same LID rats at the time of death the thyroid total ^{35}S level in comparison with thyroid total iodine, thyroid organified [^{125}I]iodine and the T/S*I^- ratio respectively.

DISCUSSION

Variations in iodine intake/acute administration affected the thyroid uptake and metabolism of [^{35}S]MMI differently from [^{35}S]PTU although some similarities were apparent.

For both drugs as much as a four-fold difference was observed in thyroid total ^{35}S radioactivity and unchanged drug with variations in acute and chronic iodine intake. Thyroid accumulation of [^{35}S] MMI and [^{35}S]PTU in control LID rats was considerably less than in NID or HII rats. Maximum thyroid uptake of [^{35}S]MMI and [^{35}S]PTU in LID rats was achieved with an acute iodide dose of 0.1 μmoles/100 g BW administered prior to the antithyroid drug. In LID animals it was most clearly shown that thyroid accumulation of [^{35}S]MMI and [^{35}S]PTU appeared independent of the occurrence of the Wolff–Chaikoff effect and saturation of the thyroid transport system for iodide and not directly related to the thyroid total iodine level, or thyroid accumulation or organification of acute iodide. For both [^{35}S]MMI and [^{35}S]PTU, thyroid accumulation of ^{35}S radioactivity after acute iodide administration in the range of 5–100 μmoles KI/100 g BW was greatest for HII rats and least for LID rats. Here the similarity between [^{35}S]MMI and [^{35}S]PTU ends. For [^{35}S]PTU, in LID, NID or HII rats increasing acute iodide dose throughout the full range was not associated with marked changes in the proportions of thyroid total ^{35}S occurring as unchanged drug, metabolites or protein-bound ^{35}S and no trend was obvious. In contrast for [^{35}S]MMI increasing doses of acute iodide throughout the full range did have a marked effect on the relative proportions of protein-bound ^{35}S, inorganic sulphate and unchanged [^{35}S]MMI contributing to the thyroid total ^{35}S and the effect was different with different chronic iodine intakes. Therefore, unlike the [^{35}S]PTU study, the thyroid level of unchanged [^{35}S]MMI did not follow changes in the thyroidal total ^{35}S level. In the [^{35}S]MMI but not the [^{35}S]PTU study an increase in thyroidal total ^{35}S was associated with a marked increase in the percentage of ^{35}S occurring as free inorganic sulphate. Examination of previously published data for [^{35}S]MMI, where increases in thyroid accumulation of ^{35}S were achieved by factors other than variation in iodine intake, generally shows simultaneous increases in the percentage occurring as free inorganic sulphate and vice versa [9–13]. The implication is that under these conditions the thyroid reacts to an increased uptake of MMI by accelerating the oxidation of the drug to inorganic sulphate.

The effect of increasing doses of acute iodide on thyroid accumulation of total ^{35}S and unchanged [^{35}S]MMI was considerably different to what was seen in the [^{35}S]PTU study. In the latter in LID, NID and HII rats, increasing the iodide dose up to

Table 1. Effect of acute and chronic iodine intake on thyroid metabolism of [^{35}S]MMI (8.76 μmoles/kg)

Acute iodide (μmoles KI/100 g mean BW)	% thyroid ^{35}S, LID rats*			% thyroid ^{35}S, NID rats*			% thyroid ^{35}S, HII rats*		
	PB-^{35}S	Sulfate	MMI	PB-^{35}S	Sulfate	MMI	PB-^{35}S	Sulfate	MMI
Control	61.5 ± 4.89	5.7 ± 2.01	24.9 ± 2.62 (6)	17.0 ± 0.81	19.3 ± 3.78	53.2 ± 4.00 (6)	14.8 ± 1.39	26.2 ± 4.39	55.7 ± 4.48 (6)
0.002	60.1 ± 6.03	2.5 ± 1.13	23.4 ± 5.80 (6)	—	—	—	14.2 ± 2.07	24.0 ± 3.41	47.3 ± 3.98 (4)
	NS	NS	NS				NS	NS	NS
0.01	43.5 ± 5.93	3.7 ± 1.00	39.0 ± 4.57 (6)	22.3 ± 2.20	20.6 ± 3.27	48.4 ± 1.70 (6)	18.8 ± 2.28	23.0 ± 2.22	40.4 ± 5.27 (6)
	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.10	17.0 ± 2.73	35.2 ± 2.66	36.8 ± 4.80 (6)	21.1 ± 0.98	23.4 ± 1.33	50.3 ± 1.97 (6)	24.7 ± 4.79	22.9 ± 4.01	38.4 ± 2.12 (5)
	P < 0.001	P < 0.001	NS	P < 0.01		NS	NS	NS	P < 0.01
0.25	17.5 ± 1.86	30.0 ± 2.76	44.2 ± 3.44 (5)	—	—	—	23.7 ± 2.67	36.9 ± 2.69	30.9 ± 2.77 (6)
	P < 0.001	P < 0.001	P < 0.002				P < 0.01	NS	P < 0.001
1	31.5 ± 8.08	25.0 ± 4.41	32.3 ± 8.17 (4)	20.3 ± 1.55	26.3 ± 3.62	49.0 ± 2.99 (6)	20.0 ± 2.97	31.8 ± 4.61	36.2 ± 4.07 (6)
	P < 0.01	P < 0.005	NS	NS	NS	NS	NS	NS	P < 0.01
5	32.4	23.8	31.2 (1)	17.9 ± 3.86	26.8 ± 0.39	50.4 ± 0.63 (2)	19.0 ± 1.52	39.5 ± 4.41	32.4 ± 4.19 (5)
				NS	NS	NS	NS	NS	P < 0.005
10	43.7 ± 1.44	14.4 ± 4.14	35.0 ± 7.37 (3)	21.1 ± 2.42	21.1 ± 3.35	45.9 ± 2.07 (6)	21.7 ± 3.25	47.1 ± 3.74	26.4 ± 2.77 (6)
	NS	NS	NS	NS	NS	NS	NS	P < 0.005	P < 0.001
30	39.8 ± 6.28	24.1 ± 4.56	27.3 ± 5.10 (3)	24.7 ± 4.40	35.2 ± 5.84	33.6 ± 2.59 (6)	16.8 ± 0.74	50.7 ± 1.71	26.5 ± 2.67 (5)
	P < 0.002	P < 0.005	NS	NS	NS	P < 0.005	NS	*P < 0.001*	P < 0.001
60	41.8 ± 2.25	14.9 ± 2.53	29.9 ± 2.35 (4)	20.0 ± 1.65	34.9 ± 3.61	36.8 ± 1.73 (5)	19.5 ± 1.23	38.2 ± 3.15	32.9 ± 3.66 (5)
	P < 0.02	NS	NS	NS	*P < 0.02*	P < 0.01	NS	NS	P < 0.005
100	34.0 ± 7.35	7.1 ± 1.35	43.3 ± 3.00 (2)	26.0 ± 3.15	25.6 ± 5.03	33.6 ± 3.00 (5)	18.8 ± 1.87	40.4 ± 3.47	29.0 ± 1.35 (6)
	NS	NS	P < 0.02	P < 0.02	NS	P < 0.005	NS	NS	P < 0.001

* All results presented as means ± S.E.M. Values in parentheses indicate the number of rats in each group. P values derived from Student's *t* quoted relative to control data.

NS = no statistically significant difference from control ($P \geq 0.02$). TLC on cellulose plates in solvent system ethanol/1 M ammonium acetate (55/45 v/v).

Table 2. Effect of acute and chronic iodine intake on thyroid accumulation of [^{35}S]MMI

Acute iodide (μmoles KI/100 g mean BW)	nmoles unchanged MMI/g thyroid		
	LID	NID	HII
Control	3.2 ± 0.68 (6)	11.5 ± 1.94 (6)	12.1 ± 1.00 (6)
0.002	3.0 ± 0.81 (6)	—	13.1 ± 1.13 (4)
	NS		NS
0.01	5.7 ± 0.58 (6)	10.6 ± 0.63 (6)	8.4 ± 1.39 (6)
	NS	NS	NS
0.10	14.7 ± 2.80 (6)	11.7 ± 0.60 (6)	8.1 ± 1.09 (5)
	$P < 0.005$†	NS	NS
0.25	15.5 ± 1.11 (5)	—	8.0 ± 0.66 (6)
	$P < 0.001$		$P < 0.01$
1	8.5 ± 2.01 (4)	15.8 ± 1.93 (6)	16.3 ± 3.33 (6)
	$P < 0.02$	NS	NS
5	7.6 (1)	21.7 ± 1.48 (2)	11.8 ± 1.51 (5)
		(NS)	NS
10	7.9 ± 2.08 (3)	12.7 ± 1.20 (6)	14.1 ± 2.73 (6)
	NS	NS	NS
30	8.6 ± 2.87 (3)	13.7 ± 2.10 (6)	14.0 ± 1.97 (5)
	NS	NS	NS
60	9.1 ± 2.06 (4)	15.4 ± 1.79 (5)	15.9 ± 2.85 (5)
	$P < 0.02$	NS	NS
100	10.4 ± 0.33 (2)	9.99 ± 1.32 (5)	12.6 ± 1.24 (6)
	$P < 0.002$	NS	NS

* Results are presented as means ± S.E.M. Values in parentheses indicate the number of rats in each group.

† P values derived from Student's *t* quoted relative to control data.

NS = no statistically significant difference from control ($P \geq 0.02$)

0.1 μmoles KI/100 g BW was associated with increasing thyroid levels of ^{35}S and unchanged [^{35}S]PTU. At iodide doses between 0.1 and 5 μmoles KI/100 g BW the thyroid level of ^{35}S and unchanged [^{35}S]PTU fell from peak accumulation to a level lower than control values in LID and NID rats but higher than control values in HII rats. Generally, irrespective of the acute iodide dose, the thyroid level of ^{35}S and unchanged [^{35}S]PTU was highest in the highest chronic iodine intake rats. In this [^{35}S]MMI study increasing acute iodide up to 0.1 μmoles KI/100 g BW had no affect on the thyroid ^{35}S level in NID or HII rats or on unchanged [^{35}S]MMI in NID rats but decreased [^{35}S]MMI in HII rats. In LID rats, both the thyroid ^{35}S and [^{35}S]MMI levels increased more than threefold over this iodide range and both of these parameters were considerably higher in the LID rats at 0.1 μmoles KI/100 g BW than comparable levels in

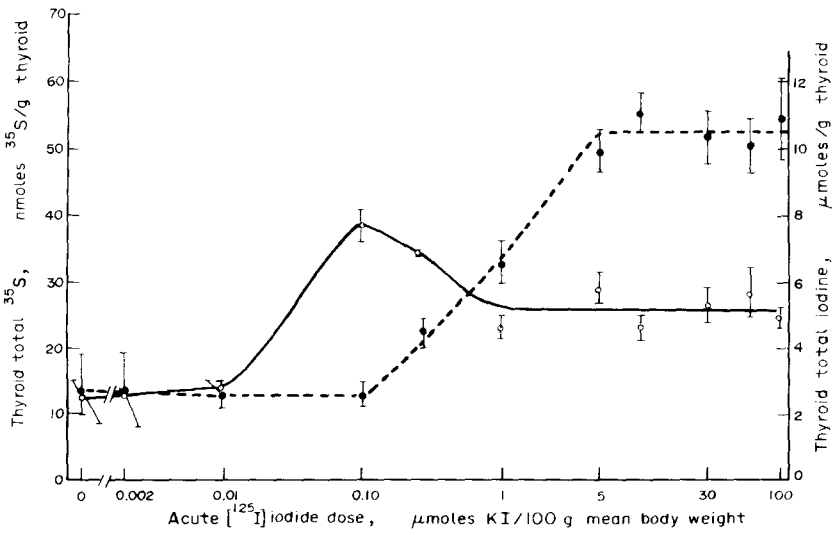

Fig. 3. In LID rats, the effect of acute [^{125}I]iodide dose prior to [^{35}S]MMI administration on thyroid total ^{35}S (○——○) and thyroid total iodine (●-----●). Each point is the mean ± S.E.M. for six thyroid glands.

NID or HII rats. However increasing iodide between 0.1 and 1 μmoles KI/100 g BW in LID rats resulted in a fall in both of these parameters from peak values to a steady level of at least twice the control values. In NID and HII rats increasing iodide in this range resulted in an increase in both parameters up to plateau levels, higher than control values.

The fragmentary data of other studies on the effect of iodine intake on thyroid accumulation and metabolism of MMI generally agrees well with the detailed overall picture described in this extensive [^{35}S]MMI study (see Table 3). In comparison with control 21-day LID rats given saline instead of [^{35}S]antithyroid drug ([^{35}S]PTU study), like [^{35}S]PTU, the [^{35}S]MMI dose administered to LID rats in the present study had only a minor inhibitory effect on organification of the acute iodide administered over the time period studied. It seems relevant to refer to results obtained by Taurog [14] using a purified thyroid peroxidase system *in vitro* which could iodinate thyroglobulin. He found that the inhibitory potency of PTU and MMI on this reaction was proportional to the drug/iodide ratio. If the ratio was low, inhibition was transient and reversible and the drug was extensively oxidised. If the ratio was high then the inhibition was long-lasting and irreversible with little or no drug oxidation. A mechanism was proposed suggesting competition between drug and iodide for the peroxidase, iodide being required to induce peroxidase oxidation of the drug. Neither in the present [^{35}S]MMI studies nor in the previous [^{35}S]PTU studies was there a significant trend of increasing oxidation of drug in the thyroid with a rapidly decreasing drug/iodide level ratio in the thyroid. There would appear to be no agreement between the present *in vivo* results and those of Taurog *in vitro*. However, the drug/iodide ratio measured for the whole rat thyroid is not necessarily the same as the ratio at the site of peroxidase in the thyroid. Due to compartmentation within the thyroid gland there are possible differences in the localised concentrations of drug and iodide in different compartments. In addition, the thyroid and serum levels of drug and iodide *in vivo* are liable to be changing rapidly with time whereas *in vitro* their concentrations in the incubation medium are kept relatively constant. In the present *in vivo* studies a single low dose of each drug has been studied whereas *in vitro* a wide variety of concentrations were investigated. For these reasons, comparison of the present results *in vivo* with those of Taurog *in vitro* should be cautious and any conclusions drawn should be tentative.

Recent data obtained *in vitro* with isolated thyroid gland or thyroid slices showed that the concentration of iodide in the incubation medium could affect the thyroid accumulation of antithyroid drugs. Skellern and Mahmoudian [15] using sheep thyroid slices and [^{35}S]MMI found that when the medium iodide concentration was increased over the range 0.01–100 mM the maximum accumulation of ^{35}S was seen at a concentration of 1 mM KI. This was approximately double the accumulation seen in iodide-free medium. A more reliable *in vitro* method was developed by Lang and Alexander [16] using whole thyroid glands from very young rats. They found

Table 3. Results reported by previous investigators of effects of acute iodide treatment and/or iodine deficiency on thyroid uptake and oxidation of MMI in the rat

Ref. No.	Chronic iodine intake	Acute iodide (i.p.) (μmoles/100 g)	[^{35}S]MMI dose (i.p.) (μmoles/kg)	% change in		
				Thyroid MMI level (%)	Thyroid total ^{35}S (or ^{14}C) (%)	% of thyroid ^{35}S as sulphate (%)
4	NID	2	5.83	130 ↑	285 ↑	150 ↑
5	LID, 10 days	–	4.37	↔	25 ↓	23 ↓
2	NID	30	8.76	40 ↑	95 ↑	60 ↑
	LID, 10 days	–	8.76	65 ↓	55 ↓	40 ↓
	LID, 21 days	–	8.76	50 ↓	55 ↓	80 ↓
	LID, 21 days	2	8.76	10 ↓	35 ↑	30 ↑
6	NID	0.00–0.1	8.76	↔	↔	↔
	NID	1–15	8.76	↔ or ↑	↑	↑
7	LID	–	5.50	35 ↓	60 ↓	80 ↓
8	LID	–	[^{14}C]MMI	–	50 ↓	–
	LID	0.1	[^{14}C]MMI		100 ↑	

LID = low-iodine diet. NID = normal-iodine diet. ↑ = increase. – = not studied. ↓ = decrease. ↔ = no change.

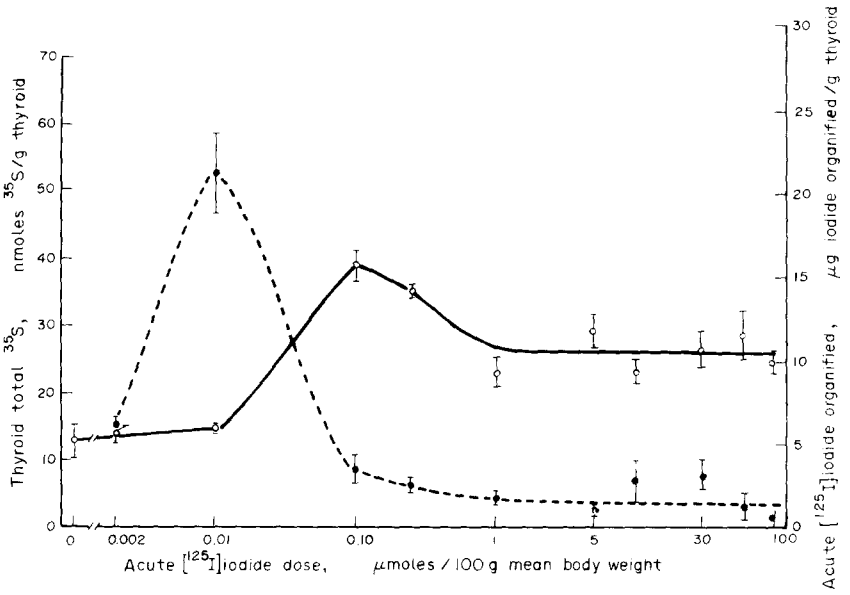

Fig. 4. In LID rats, the effect of acute [^{125}I]iodide dose prior to [^{35}S]MMI administration on thyroid total ^{35}S (○——○) and the thyroid organified acute [^{125}I]iodide (●-----●). Each point is the mean ± S.E.M. for six thyroid glands.

that increasing the concentration of inorganic iodide in the incubation medium in the range 1–1300 μM was associated with increasing thyroid uptake of [^{35}S]PTU and [^{35}S]MMI; at 1300 μM iodide the increase was four-fold. Using a double-label procedure they were able to show that concentrations of 1 mM ouabain, 0.5 mM 2,4-dinitrophenol or 2 mM sodium percholate which strongly inhibited thyroid accumulation of free inorganic [^{125}I]iodide had no significant inhibition on simultaneous thyroid accumulation of unchanged [^{35}S]PTU in the same thyroid glands.

Variation in iodine intake, acutely and chronically, has been shown to have considerable influence on the thyroid accumulation and oxidation of [^{35}S]PTU and [^{35}S]MMI in the rat. However, the particular aspect of thyroid iodine metabolism which is directly related to thyroid accumulation and oxidation of antithyroid drugs remains to be clarified. Studies are being carried out to find whether the level of acute and chronic iodine intake influences accumulation and oxidation of antithyroid drugs by the human thyroid. If so, the effects may be clinically relevant in treating hyperthyroidism in individuals with different chronic iodine intakes or when antithyroid drugs and iodine are being administered together, e.g. in preparation for partial thyroidectomy and treatment of thyrotoxicosis or thyroid crisis.

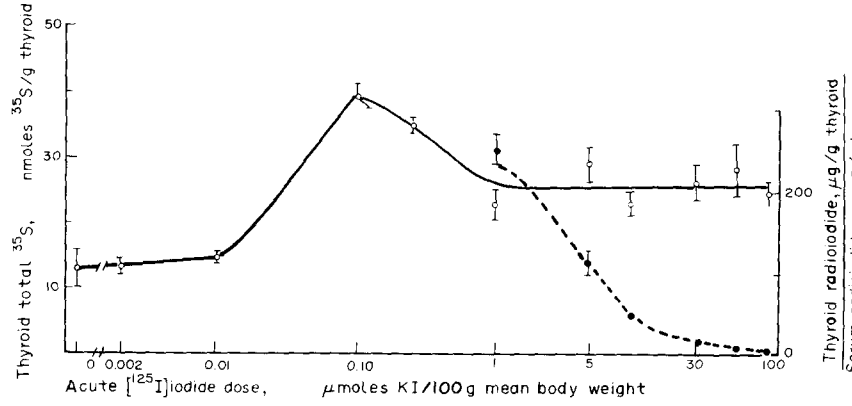

Fig. 5. The effect in LID rats of acute [^{125}I]iodide dose prior to [^{35}S]MMI administration on thyroid total ^{35}S (○——○) and the thyroid/serum ratio of free radioiodide (●------●). Each point is the mean ± S.E.M. for six thyroid glands.

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