^{131}I AND MIT-^{131}I IN HUMAN URINE, SALIVA AND GASTRIC JUICE: A COMPARISON BETWEEN EUTHYROID AND THYROTOXIC PATIENTS

S. MacFarlane, S. Papadopoulos, R. McG. Harden and W. D. Alexander

Gardiner Institute, Western Infirmary, Glasgow, Scotland

Iodine is secreted into the urine, saliva and gastric juice. The form in which it is present varies, both with the secretion studied and with the thyroid status of the patient. In the urine, the iodine is almost entirely in the inorganic form in euthyroid persons (*1–4*), whereas in patients with thyrotoxicosis (*1,3,5*) or dehalogenase deficiency (*6–11*) organic iodinated compounds have been found.

The iodine in saliva is said to be mainly inorganic (*5,12–14*), but some workers have claimed to find substantial amounts of organic iodine (*15,16*). In contrast to the findings in the urine, there is no evidence that the nature of the iodine in saliva depends on the thyroid status of the individual. Less is known about the form of iodine in the gastric juice in man and studies in animals have given conflicting results. Davenport (*17*) has found that iodine in the gastric juice of the dog is entirely inorganic and similar results have been obtained in other animals (*18*). However, an ability of the gastric mucosa to iodinate protein has been described in the rat (*19*) and in the dog (*16,20*).

In this study, we have investigated the form in which iodine is excreted in the urine, saliva and gastric juice in euthyroid and thyrotoxic patients after administration of radioactive iodine as inorganic ^{131}I and as L-monoidotyrosine-^{131}I (MIT-^{131}I).

MATERIALS AND METHODS

Subjects. Fifty-two patients, 28 euthyroid and 24 thyrotoxic, were studied. Their thyroid status was assessed clinically (*21*) and by estimating the serum PBI (*22*). The patients were divided into three groups:

Group I. An intravenous tracer dose of ^{131}I was given to 6 euthyroid and 6 thyrotoxic patients.

Group II. Six thyrotoxic patients were studied following a therapeutic dose of radioiodine.

Group III. MIT-^{131}I was given (1) to 6 euthyroid and 6 thyrotoxic patients intravenously, and (2) to 16 euthyroid and 6 thyrotoxic patients orally.

Materials. 3-iodo-L-tyrosine-^{131}I (MIT-^{131}I), specific activity approximately 1.5 μc/μg, and ^{131}I were obtained from the Radiochemical Centre, Amersham. A tracer dose of each isotope was diluted with saline and sterilized for intravenous injection. The purity of the MIT-^{131}I before and after sterilization was checked by chromatography and the maximum contamination with iodide was found to be 4%.

Collection of samples. In Groups I and IIIa where isotope was injected intravenously, urine was collected between 0 and 30 min and at 6 hr. A further specimen at 24 hr was collected in Group I. In Group II, urine samples were taken at 6 and 24 hr and at 4 days after the therapy dose. In patients in Group IIIb, the total urine passed was collected between 0 and 6 hr after the oral dose of MIT-^{131}I.

Mixed and parotid saliva were collected as previously described (*5*), the former between 0 and 30 min and the latter between 30 and 40 min after injection of ^{131}I or MIT-^{131}I.

In the group of patients to whom labeled substances were administered intravenously, gastric juice was collected using a Ryle's tube over the same period, 0 to 30 min, as the urine and mixed saliva collections. Precautions were taken to avoid contamination of the gastric juice with saliva.

Methods. Counting samples and standards, treatment with an anion exchange resin, Amberlite IRA-400, and radiochromatography were performed as described previously (*11*). Where indicated, specimens were hydrolyzed by heating at 60°C for 30 min with half volumes of 5$\bar{N}$HCl. All specimens were chromatographed in the butanol-acetic acid system, and the urine from the patients in Group II was also chromatographed in the butanol-dioxane-ammonia system. With every batch of samples chro-

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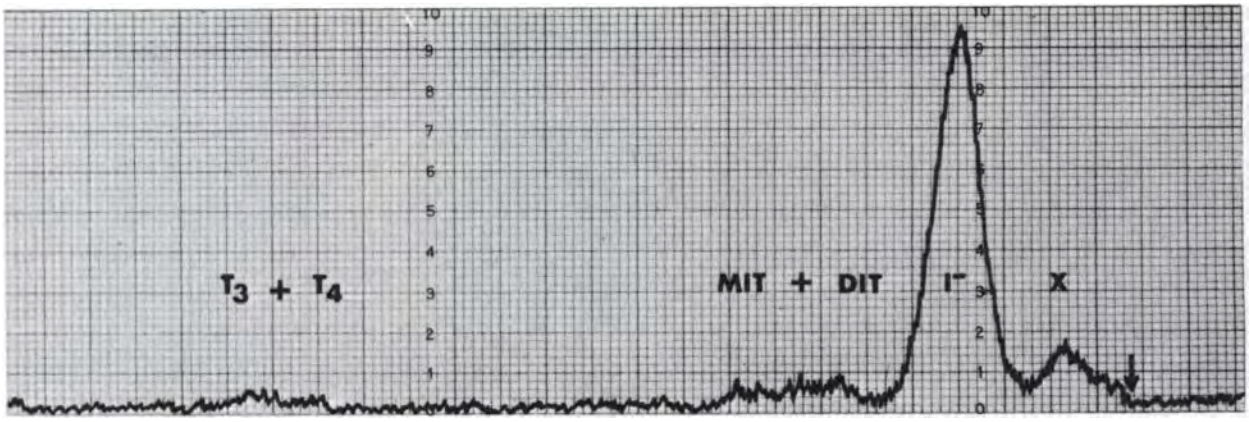

FIG. 1. Radiochromatogram of urine from thyrotoxic patient 24 hr after a therapeutic dose (10 mc) of radioiodine. Butanol-acetic acid solvent system was used. Peaks correspand to x′, iodide, iodotyrosines and iodothyronines. Arrow shows origin.

matographed control urines to which ^{131}I, MIT-^{131}I and T_4-^{131}I had been added were applied alongside as standards. No change in the chromatographic behavior of the standards was noted up to 5 days, the iodide standard showing consistently only one peak.

RESULTS

Studies after small (tracer) doses of ^{131}I administered intravenously. In the euthyroid patients radiochromatograms of urine showed activity only in the iodide region at all time intervals. Less than 1.0% of this activity remained after treatment with the anion exchange resin.

The urine from the thyrotoxic patients showed activity mainly in the iodide region, although, in all specimens, a small peak of activity was present between the origin and the iodide region. Because of the relatively low concentration of labeled iodine in the urine from the thyrotoxic patients, quantitative assessment of this unknown peak was not possible from the chromatograms. In the 0–30-min time interval after ^{131}I, an average of 0.39% of the total urinary activity passed through the anion exchange-resin column; at 6 hr, this had risen to 2.7% and at 24 hr to 5.1%. The radioactivity remaining after resin treatment was too low to be detectable on the chromatograms.

TABLE 1. DISTRIBUTION OF URINARY LABELED IODINE IN THYROTOXIC PATIENTS*

Time of urine collection	Mean percentage of total urinary activity				
	Passing through "Amberlite" resin	Present as:			
		x′	I⁻	MIT + DIT	$T_3 + T_4$
6 hr	6.9	5.4	94.6	—	—
24 hr	10.9	9.3	83.5	5.6	1.6
4 days	13.7	13.9	73.0	3.6	9.5

* At different time intervals after therapeutic dose of radioiodine.

Radiochromatograms of parotid and mixed saliva from both euthyroid and thyrotoxic patients after an intravenous tracer dose of ^{131}I showed that the activity was present entirely in the iodide zone. In all specimens of parotid saliva less than 0.5% of the total activity remained after treatment with the anion exchange resin. The corresponding values for mixed saliva were 0–3%. No difference was found between the euthyroid and thyrotoxic patients.

In both the euthyroid and thyrotoxic patients, radiochromatograms of gastric juice after an intravenous tracer dose of ^{131}I showed only labeled iodide. The percentage of the total activity remaining after treatment with the anion exchange resin was 0–1.5%.

Studies after large (therapy) doses of ^{131}I administered orally. Table 1 shows the distribution of urinary labeled iodine in thyrotoxic patients at different time intervals after a therapeutic dose of ^{131}I. Radiochromatograms of urine passed 6 hr after the dose showed two distinct zones of labeled iodine. These were iodide and a zone of activity between the origin and the iodide region found after an intravenous tracer dose of ^{131}I. Hydrolysis of the urine specimens did not affect the distribution of labeled iodine. Resin treatment of the urine removed the iodide completely but left the zone near the origin unchanged. This zone, although it had a chromatographic mobility similar to zone x found after MIT-^{131}I (see results in next section), differed in its behavior to the anion resin treatment and

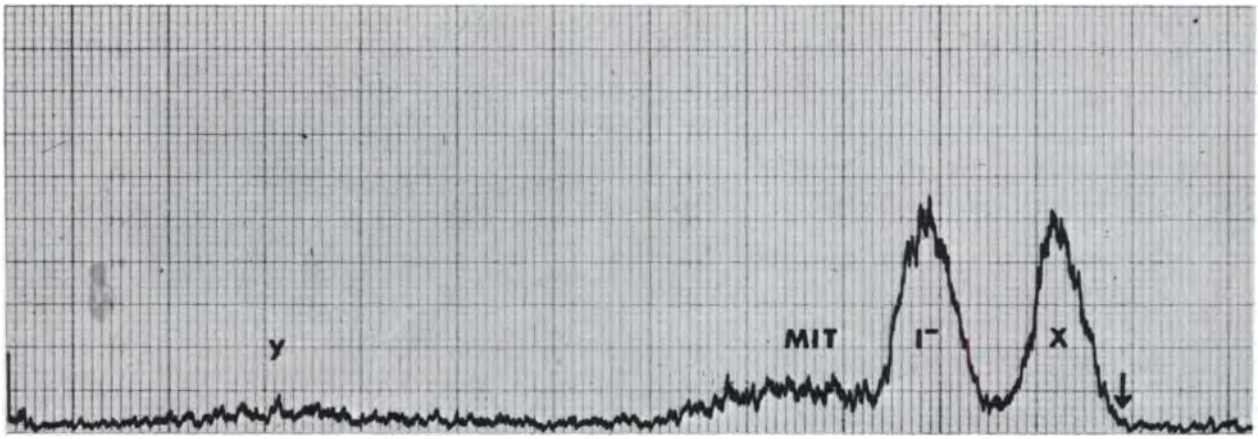

FIG. 2. Radiochromatogram of urine from euthyroid patient 0–6 hr after oral administration of MIT-^{131}I. Butanol-acetic acid solvent system was used. Zones of labeled iodine correspond to x, iodide, MIT and y are shown. Arrow shows origin.

hydrolysis; thus, we shall refer to the compound found after administration of ^{131}I as x′.

Specimens of urine at 24 hr and at 4 days were chromatographed both in the butanol-acetic acid and the butanol-dioxane-ammonia systems. In the urine specimen at 24 hr, the mean percentage of the total labeled iodine present as x′ had increased and zones of activity corresponding to the iodotyrosine and iodothyronine standards were detectable (Fig. 1). The urine specimen at 4 days showed a further increase in x′ and the iodothyronine zone and a decrease in the iodotyrosine region. In each case the activity remaining after resin treatment corresponded to zone x′, and the distribution of labeled iodine remained unchanged after hydrolysis.

Studies after an intravenous tracer dose of MIT-^{131}I. Chromatographic analysis of all urine specimens collected between 0 and 30 min showed peaks of activity corresponding to the iodide and the iodotyrosine standards and two other zones of activity, x and y, in both euthyroid and thyrotoxic patients. Hydrolysis of urine removed zone x almost completely with most of the activity going to the MIT zone. The mean percentage of the total urinary activity remaining after anion exchange treatment was 29.3 in the euthyroid patients and 36.6 in the thyrotoxic patients. This residual activity was all present as a fraction of the original MIT zone. As the anion resin was found to remove 98% of MIT-^{131}I added to urine, the activity remaining after resin treatment cannot be free MIT but is presumably an MIT-conjugate. This peak of activity had an R_f value similar to compound "u" which we have previously described (*11*). Thus, we shall subsequently refer to the activity remaining after resin treatment as u, and the zone of activity on our chromatograms which contains u and free MIT as the "MIT-fraction."

Table 2 shows the labeled iodine corresponding to each zone in crude, hydrolyzed and resin-treated urine expressed as a percentage of the total labeled iodine in the specimens of urine collected between 0 and 30 min.

Table 3 shows the distribution of urinary labeled iodinated constituents in euthyroid and thyrotoxic patients at 0–30 min and at 6 hr after intravenous injection of MIT-^{131}I. In euthyroid persons, the mean percentage of the total urinary iodine present as x and iodide at 6 hr had increased compared with that in the specimen at 0–30 min while that of the MIT compound u and y decreased.

The urine of the thyrotoxic patients collected between 0 and 30 min and at 6 hr presented a pattern similar to that of the euthyroid patients, although, in each case, labeled iodide accounted for a smaller percentage of the total urinary activity.

In both euthyroid and thyrotoxic patients after administration of MIT-^{131}I, radiochromatography

TABLE 2. DISTRIBUTION OF LABELED IODINE IN CRUDE, HYDROLYZED AND "AMBERLITE" RESIN-TREATED URINE*

Patients	Treatment of urine sample	Mean percentage labeled iodine present as: x	I⁻	MIT-fraction	y
Euthyroid	Crude	3.4	40.5	46.8	9.3
	Hydrolysis	0.9	40.9	48.6	9.6
	"Amberlite"	—	—	29.3	—
Thyratoxic	Crude	4.8	25.2	60.2	9.8
	Hydrolysis	0.9	25.0	64.1	10.0
	"Amberlite"	—	—	36.6	—

* In the period 0–30 min after intravenous administration of tracer dose of MIT-^{131}I to 6 euthyroid and 6 thyrotoxic patients.

TABLE 3. DISTRIBUTION OF LABELED IODINE IN CRUDE URINE*

Patients	Time of collection	Mean % total urinary activity as: x	I^-	u	MIT	y
Euthyroid	0–30 min	3.4	40.5	29.3	17.5	9.3
	6 hr	20.7	62.1	6.1	4.0	7.1
Thyrotoxic	0–30 min	4.8	25.2	36.6	23.6	9.8
	6 hr	30.0	46.6	10.6	4.8	8.0

* At two different time intervals after intravenous administration of MIT-^{131}I to 6 euthyroid and 6 thyrotoxic patients. Mean percentage of labeled iodine in each zone is shown, expressed as percentage of mean total labeled iodine on chromatograms.

showed that the activity in the gastric juice, parotid and mixed saliva was entirely in the iodide zone. No activity was detectable after treatment with the anion exchange resin. In all specimens, the concentration of radioactivity was low compared with that found after ^{131}I administration; it was even lower in the thyrotoxic patients than in the euthyroid patients.

Studies after an oral dose of MIT-^{131}I. In both euthyroid and thyrotoxic patients after intravenous injection of MIT-^{131}I, the proportion of urinary labeled iodine present as MIT or its conjugates was greater than that expected on the basis of previous studies (*8,23*). To ascertain whether the route of administration of the isotope was important, a further group of euthyroid and thyrotoxic patients was studied after oral administration of MIT-^{131}I.

Table 4 shows the urinary excretion of labeled MIT and MIT-conjugates in the period 0–6 hr after an oral dose of MIT-^{131}I to euthyroid and thyrotoxic patients. The distribution of urinary labeled iodine in a typical euthyroid patient is shown in Fig. 2. In this period the percentage of the dose excreted by the euthyroid patients was 27.3 ± 2.43. In the thyrotoxic patients there was a tendency for the isotope excretion to be higher, 33.1 ± 3.10 ($0.05 < p < 0.10$). The mean percentage of the total urinary ^{131}I present as MIT or MIT-conjugates was 36.4 in the euthyroid patients and 46.8 in the thyrotoxic patients. These figures are significantly different ($p < 0.025$). The mean percentage of the dose excreted as MIT or MIT-conjugates was 9.8 for the euthyroid patients which was significantly lower than the 14.9% excreted by the thyrotoxic patients ($p < 0.005$).

DISCUSSION

Organic iodine compounds have been found in the urine of thyrotoxic patients following a tracer dose of ^{131}I. In contrast, in euthyroid patients all the urinary iodine was found to be inorganic. These results confirm previous reports (*1–5*). We have demonstrated that the organic fraction in the thyrotoxic patients appeared on the chromatograms between the origin and the iodide zone in the butanol-acetic acid solvent system. An identical zone of organic iodine, x′, was found on radiochromatograms of urine from thyrotoxic patients after a therapeutic dose of radioiodine. The finding of compound x′ in the urine of thyrotoxic patients but not of euthyroid patients and the fact that the proportion of urinary labeled iodine present to x′ increases with time suggests that this compound is thyroidal in origin. That compound x′ is probably a conjugate formed from one of the intermediates in thyroid hormone synthesis is indicated by its early appearance in the urine. Compound x′ does not behave chromatographically like any of the known thyroid hormones or hormone precursors; moreover, it also differs in its behavior to resin treatment. Further attempts to determine the nature of compound x′ by hydrolysis yielded no results.

Small amounts of iodotyrosines and iodothyronines were found in addition to iodide and compound x′ in later urine samples from the thyrotoxic patients. These findings suggest that in thyrotoxic patients, in addition to the loss of nonhormonal organic iodine in the urine, there is a leak of iodotyrosines from the thyroid gland. The appearance of iodothyronines in the urine 4 days after a therapeutic dose of ^{131}I may be the result of damage to the thyroid causing abnormal release of thyroid hormone from the gland.

Following administration of MIT-^{131}I intravenously, a large proportion of the total urinary labeled iodine was excreted in the form of MIT or MIT-conjugates in both the euthyroid and thyrotoxic patients during the first 30 min. This contrasts with the findings of Stanbury *et al* (*23*) who found that

TABLE 4. EXCRETION OF LABELED MIT AND MIT-CONJUGATES IN THE URINE OF EUTHYROID AND THYROTOXIC PATIENTS*

Patients	% dose excreted	% ^{131}I in urine as MIT†	% dose in urine as MIT†
EUTHYROID			
No. of patients	16	16	16
Range	7.5–38.6	12.3–53.1	3.2–17.2
Mean	27.3	36.4	9.8
S.E. of mean	2.43	2.76	1.17
THYROTOXIC			
No. of patients	6	6	6
Range	23.6–41.8	36.7–53.8	12.5–17.5
Mean	33.1	46.8	14.9
S.E. of mean	3.10	2.90	0.73

* In period 0–6 hr after oral administration of MIT-^{131}I.
† Includes free MIT and MIT-conjugates.

injected L-monoiodotyrosine was excreted almost entirely as iodide. At all time intervals studied most of the labeled MIT in the urine was in the form of conjugates, x, u and y. In the euthyroid and thyrotoxic patients, the proportion of urinary labeled iodine present as x increased with time, indicating that the incorporation of MIT-^{131}I into this complex is fairly slow or that it is excreted at a slow rate. Its early appearance in the urine suggested that this complex is peripheral in origin. In contrast to that of x the proportion of u and y decreased with time. These compounds probably formed rapidly in the periphery and either excreted unchanged or de-iodinated slowly. The percentage of urinary labeled iodine present as free MIT decreased with time and was at a maximum in the first specimen. At all times the proportion of urinary labeled iodine present as MIT or MIT-conjugates was greater in the thyrotoxic than in the euthyroid patients.

The excretory pattern of MIT-^{131}I suggests the following fate of injected MIT-^{131}I. In euthyroid and thyrotoxic persons injected MIT-^{131}I is mostly de-iodinated while part of it is incorporated into complexes, rapidly in the case of u and y and more slowly into x. Once in the form of a complex the MIT-^{131}I appears to be more resistant to de-iodination.

In the 6-hr period following an oral dose of MIT-^{131}I we found that the euthyroid patients excreted 3.2–17.2% of the dose in the urine as MIT or MIT-conjugates. This range was wider than that of 0–6% previously found in a smaller group of subjects by McGirr *et al* (*8*). The range obtained for the thyrotoxic patients, 12.5–17.5% was significantly higher than that for the euthyroid patients. This might be due to an impaired ability to de-iodinate MIT or to a difference in the renal handling in thyrotoxicosis. Owing to the high specific activity of the preparation available to us it proved impossible to measure the specific rotation of the MIT-^{131}I by conventional means. Evidence that the MIT-^{131}I was in the L-form is the fact that several of the euthyroid subjects excreted only small amounts of the oral dose of MIT-^{131}I in the form of MIT or MIT-conjugates in the period 0–6 hr. In the study of Stanbury *et al* (*23*) between 12.4 and 15.5% of a dose of DL-MIT-^{131}I appeared in the urine as free MIT. These values are far in excess of the values found in any of our patients for free MIT alone. In our study we found a mean of 9.8% of the dose for free MIT, plus MIT-conjugates and as can be seen in Fig. 2 only a small proportion of this was present as free MIT. Moreover, in our study the same batch of isotope was used for both thyrotoxic and euthyroid subjects.

In saliva and gastric juice only small amounts of organic iodine were demonstrated after administration of ^{131}I. No difference was found between the amount of organic iodine in the saliva and gastric juice in the euthyroid patients and that of the thyrotoxic patients. In mixed saliva from 0–3% was organic, while in parotid saliva the organic fraction was less than 0.5%. The organic iodine in gastric juice accounted for 0–1.5%. The nature of this organic iodine has not been investigated further.

Studies with MIT-^{131}I have shown no evidence to suggest that MIT appears unchanged in saliva or gastric juice.

SUMMARY

The excretion of ^{131}I and MIT-^{131}I in the urine, saliva and gastric juice has been studied in euthyroid and thyrotoxic patients. In euthyroid patients, the administered ^{131}I appeared in the urine almost entirely as inorganic iodide. In contrast, the urine of the thyrotoxic patients contained both organic and inorganic iodine. On chromatography, the organic fraction appeared as a single peak, x′, near the origin. After therapeutic doses of radioiodine given to thyrotoxic patients, iodotyrosines and iodothyronines were found in addition in the urine. Following administration of MIT-^{131}I, appreciable amounts of radioactive MIT and MIT-conjugates were found in the urine of both euthyroid and thyrotoxic patients. The percentage of the administered MIT-^{131}I excreted in 6 hr as MIT or MIT-conjugates by the thyrotoxic patients was found to be significantly higher than that excreted by the euthyroid patients. In saliva and gastric juice after administration of ^{131}I or MIT-^{131}I, the labeled iodine was almost entirely inorganic in both euthyroid and thyrotoxic patients.

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