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IODINE EXCRETION IN URINE, SALIVA, GASTRIC JUICE AND SWEAT IN DEHALOGENASE DEFICIENCY

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SUMMARY

The excretion of iodine in urine, saliva, gastric juice and sweat has been studied by using ^{131}I-labelled monoiodotyrosine in a patient with the dehalogenase type of dyshormonogenesis. Iodinated components '*x*', iodide, monoiodotyrosine and '*y*' were found in the urine. A previously undescribed component (compound '*u*') accounted for a large fraction of the urinary radioactive iodine. Organic iodinated compounds were not excreted in the saliva. Only inorganic iodide was found in the gastric juice. No organic iodine was detected in the sweat. The plasma inorganic iodine (PII) derived from salivary iodine measurements gave low values indicative of iodine deficiency. The PII values obtained from the urinary iodine were falsely high due to the presence of organic iodinated compounds.

PII = Plasma Inorganic Iodine

INTRODUCTION

In one type of congenital goitre dehalogenase deficiency has been demonstrated. This defect is associated with circulating monoiodotyrosine (MIT) and di-iodotyrosine (DIT) and the excretion of significant amounts of organic iodine in the urine. A diagnostic test in which labelled compounds in the urine are measured after administration of [^{131}I]monoiodotyrosine ([^{131}I]MIT) has been described (McGirr, Hutchison & Clement, 1959*b*). Iodine is also excreted in man in the saliva, the gastric juice and the sweat (Brown-Grant, 1961). Although there have been thorough studies of urinary iodine excretion in such patients after administration of ^{131}I or [^{131}I]MIT (McGirr, Hutchison & Clement, 1956; Stanbury, Meijer & Kassenaar, 1956; McGirr, Clement, Currie & Kennedy, 1959*a*; McGirr *et al.* 1959*b*; Choufoer, Kassenaar & Querido, 1960; Murray, Thomson, McGirr & Wallace, 1965), there has been no work on the form in which iodine occurs in saliva, gastric juice or sweat. This has now been investigated in a patient.

Case report

The patient, a 29-yr.-old woman, had a goitre from early childhood. She was first seen at the Endocrine Clinic, Western Infirmary, in 1963. Her physical development was normal, but she was mentally retarded. Her skin was coarse and cold and she had

a large diffuse goitre extending retrosternally. At that time the serum PBI was less than 1 μg./100 ml., the serum cholesterol level was 316 mg./100 ml. and an E.C.G. was typical of hypothyroidism. Tests for thyroid auto-antibodies were negative. After an intravenous tracer dose of ^{131}I there was a rapid thyroidal uptake followed by rapid discharge. Column chromatography indicated the presence of iodotyrosines in the urine. It was concluded that goitre and hypothyroidism were due to dehalogenase deficiency and treatment with thyroxine (T_4) was started. The goitre decreased markedly in size and the patient became more alert. The serum cholesterol concentration returned to normal. Maintenance therapy was continued with 60 μg./day tri-iodothyronine (T_3). Therapy with T_3 was temporarily discontinued 3 days before the present studies.

MATERIALS AND METHODS

[^{131}I]3-Iodo-L-tyrosine in 50 % propylene glycol solution was obtained from the Radiochemical Centre, Amersham; 30 μC of [^{131}I]MIT containing approximately 20 μg. stable MIT was diluted with water for oral administration. For intravenous injection the MIT was diluted with 0·9 % NaCl solution and sterilized at a pressure of 15 lb./sq.in. for 20 min. at a temperature of 120°. In each test an aliquot was retained for use as standard. The purity of the preparation was checked by chromatography before and after sterilization. Non-sterile [^{131}I]MIT contained 1 % as iodide and sterilized [^{131}I]MIT 4 % as iodide.

Collection of samples

Urine. After the oral administration of [^{131}I]MIT, urine was collected from 0 to 1 hr., from 1 to 2 hr. and a sample at 24 hr. On another occasion [^{131}I]MIT was given intravenously and urine was collected from 0 to 35 min. after injection.

Saliva. Parotid saliva was obtained by using a modified Carlson–Crittenden cup, placed over the parotid duct orifice (Mason, Harden, Rowan & Alexander, 1966); mixed saliva was collected by having the patient spit into a wide-necked bottle. The parotid saliva was collected at high flow rates induced by lemon juice stimulation between 65 and 80 min. and at low flow rates (resting conditions) between 90 and 120 min. after the oral dose of [^{131}I]MIT. Mixed saliva was collected from 130 to 145 min.

Gastric juice. Gastric juice was collected with a Ryle's tube. The patient was asked not to swallow, and the head rest of the dental chair was tilted forward to facilitate the simultaneous collection of mixed saliva and to minimize the passage of saliva into the pharynx. Gastric juice was collected from 0 to 30 min. after the intravenous injection of 30 μC [^{131}I]MIT; it was filtered under suction in a Buchner flask to remove mucus before measuring the radioactive isotope. The activity in the mucus had previously been found to be entirely in the inorganic form.

Sweat. Sweat from all four limbs was collected in polythene bags (Harden & Alexander, 1963) 30–60 min. after 30 μC [^{131}I]MIT had been given orally.

Measurement of radioactivity

All specimens were counted in an 'Ekco' well-type scintillation counter immediately after collection. An aliquot of the standard was counted at the same time and

the radioactivity of each sample expressed as a percentage of the dose. Samples of urine, saliva and gastric juice were retained for chromatography.

To investigate further the labelled iodine compounds on chromatograms of crude specimens, hydrolysis and an anion exchange resin were employed. Samples of urine and gastric juice were hydrolysed by heating at 60° with half the volume of 5 N-HCl for 30 min. The hydrolysates were neutralized with concentrated NH_4OH and a sample applied to chromatography paper.

Samples of crude urine, saliva and gastric juice were passed through 100×3 mm. columns containing an anion exchange resin (Amberlite, IRA-400 in the chloride form) which is known to remove inorganic iodide and free iodotyrosines, and the effluent was counted against the crude specimen. The radioactivity remaining after the treatment with Amberlite was expressed as a percentage of that in the crude specimen. The Amberlite-treated urines were chromatographed. Specimens of hydrolysed urine were also passed through Amberlite columns and chromatographed. A further sample of urine which had been first treated with Amberlite was hydrolysed as described previously and chromatographed.

Fifty to two hundred μl. of each specimen was applied as a spot to Whatman No. 1 chromatography paper. ^{131}I-labelled standards of iodide, MIT, DIT, T_3 and T_4 were applied alongside as markers. Ascending chromatography at room temperature in the solvent system *n*-butanol:acetic acid:water (78:10:12) was used throughout. When the solvent front had progressed 30–35 cm., the paper was removed from the tank, dried and cut into 4 cm. wide strips. The strips were scanned in a Tracerlab 4π chromatogram scanner and the labelled compounds in the specimen identified by comparison with scans of the standard strips. The relative amount of each labelled compound on the chromatograms was determined by planimetry and expressed as a percentage of the total radioactivity on the strip. After the scanning, autoradiograms of the strips were prepared.

Measurements of plasma inorganic iodine (PII)

After a dose of 50 μc ^{132}I had been administered the PII was calculated both from the specific activity of the urinary and from that of the salivary iodine (Harden, Mason & Buchanan, 1965).

RESULTS

Distribution of radioactivity in chromatograms

Urine

Chromatograms of urine specimens taken at all time intervals after administration of [^{131}I]MIT showed five distinct zones of radioactive iodine. Iodide and a peak corresponding to MIT were present. In addition, zones of activity corresponding to '*x*' and '*y*', as designated by Stanbury *et al.* (1956), were prominently displayed. A further zone of radioactive iodine, termed '*u*' in the Plate (fig. 1), developed between iodide and MIT.

A specimen of urine collected from 0 to 35 min. after i.v. injection of [^{131}I]MIT was hydrolysed and treated with Amberlite resin. In the crude sample (Plate, fig. 1*a*), zone '*x*' contained 8·6% of the total urinary activity and iodide, 5·9%. MIT accounted for 20·2%, zone '*u*', 44·5% and '*y*', 20·8% of the total radioactive iodine.

A chromatogram of the hydrolysed urine showed that zone '*x*' had been completely removed; there was an increase in the size of the MIT zone which now accounted for 31·7 % of the total radioactive iodine. The size of zone '*u*' remained approximately the same (42·2 %) and there was no significant change in the size of the iodide or '*y*' zones.

Amberlite treatment of the urine specimen removed 60 % of the urinary activity. Chromatography showed that the remaining 40 % of the radioactive iodine was entirely contained in zone '*u*' (Plate, fig. 1*b*). This zone of activity remained completely unchanged when the Amberlite-treated urine was hydrolysed. When urine was first hydrolysed and then treated with Amberlite, the activity remaining was again 40 % and was all present in zone '*u*'.

In the period 0–2 hr. after an oral dose of [^{131}I]MIT the patient excreted 21·5 % of the dose in the urine; '*x*', MIT, '*u*' and '*y*' accounted for 19·7 % of the dose. The amount of radioactive iodide excreted during this period was 1·8 % of the dose which was slightly more than that contaminating the original preparation (1 %). The radioactivity of the urine at 24 hr. was insufficient to allow further investigation.

Saliva

After administration of [^{131}I]MIT the concentration of radioactive iodine present in both parotid and mixed saliva was low. Chromatograms of parotid and mixed saliva showed no other zones of activity apart from iodide. Amberlite treatment of saliva entirely removed the activity. A chromatogram of mixed saliva is shown in the Plate (fig. 2).

Gastric juice

Chromatograms of gastric juice after intravenous injection of [^{131}I]MIT showed two zones of radioactivity. These were iodide and a peak of activity near the origin. The slow-moving peak was converted entirely to iodide by hydrolysis. Furthermore, when smaller quantities of gastric juice were applied, only the iodide peak was detectable (Plate, fig. 3). It therefore appears that the origin material is merely an artifact caused by the high viscosity of the gastric juice. The radioactivity in the gastric juice was entirely removed by treatment with Amberlite resin.

Sweat

In the 3 ml. of sweat collected from 30 to 60 min. after oral administration of [^{131}I]MIT there was no radioactive iodine.

Measurements of inorganic iodine in plasma

The PII value derived from the specific activity of the urinary iodine was 0·28 μg./100 ml. and the corresponding PII value calculated from the specific activity of the salivary iodine was 0·04 μg./100 ml. On another occasion the values obtained were 0·28 μg./100 ml. and 0·06 μg./100 ml., respectively.

DISCUSSION

Chromatograms of the urine of this patient are characteristic of dyshormonogenesis caused by dehalogenase deficiency. After oral or i.v. administration of [^{131}I]MIT, the urine contained very little radioactive iodine in the inorganic form in comparison with normal subjects. However, some deiodination must have occurred since the amount of iodide excreted in the urine, saliva and gastric juice exceeded that contaminating the administered [^{131}I]MIT.

The pattern of labelled compounds in chromatograms of urine was similar to that found by Stanbury *et al.* (1956), and McGirr *et al.* (1959*b*), after administration of [^{131}I]MIT. Peaks of activity corresponding to '*x*', iodide, MIT and '*y*' were observed. In addition, a fifth zone of activity, '*u*', with a mobility slightly less than that of MIT was present in our chromatograms. Choufoer *et al.* (1960) have described several unidentified zones of radioactive iodine in chromatograms of urine from a patient with the dehalogenase type of dyshormonogenesis after administration of ^{131}I. The identity of the fifth zone of activity, zone '*u*', found in our chromatograms remains unknown. It does not correspond to zone '*z*' described by Choufoer *et al.* (1960), which has an R_F value greater than that of MIT. The radioactive iodine remaining after Amberlite resin treatment, 40 % of the total urinary activity, was entirely contained in the '*u*' zone. This zone was unaffected by hydrolysis. Our patient excreted only 20 % of the total urinary activity as free [^{131}I]MIT. The remainder of the radioactive iodine was excreted as iodide and as organic compounds, presumably conjugates of [^{131}I]MIT. Of these, compound '*u*' contributed most to the total radioactive iodine in the urine.

No organic iodinated compounds were found in either parotid or mixed saliva. The low concentration of radioactive iodide found in the saliva suggests that no deiodination of [^{131}I]MIT had occurred in the salivary glands. The fact that the salivary iodine was entirely in the inorganic form, while the urine contained significant amounts of organic iodine is of clinical importance, as in measurements of PII (Wayne, Koutras & Alexander, 1964), it is assumed that all the urinary and salivary iodine is derived from the plasma inorganic iodide. Falsely high PII values are therefore obtained when PII is derived from the specific activity of urinary iodine. In contrast, measurement of PII from the specific activity of the salivary iodine gives correct values. A similar situation occurs in thyrotoxic patients who excrete organic iodinated compounds in the urine but not in the saliva (Alexander, Papadopoulos, Harden, MacFarlane, Mason & Wayne, 1966).

Inorganic iodide was the only radioactive component found in the gastric juice after administration of [^{131}I]MIT. As in the saliva, the concentration of radioactivity was low suggesting that all the radioactive iodide in the gastric juice was derived from radioactive inorganic iodide in the plasma and that no significant deiodination of [^{131}I]MIT had occurred in the gastric glands.

No labelled iodine was detectable in the sweat of the patient after oral administration of [^{131}I]MIT. Chromatograms of urine collected during the same period showed that deiodination of [^{131}I]MIT had occurred to a very limited extent. It seems, therefore, that in this patient, [^{131}I]MIT does not contribute to the iodine in sweat and there was not sufficient free ^{131}I in the circulation for its presence in sweat to be

detectable. It therefore appears that although there may be some similarities between the sweat glands and the kidneys (Kuno, 1956), they differ in their handling of organic iodinated compounds.

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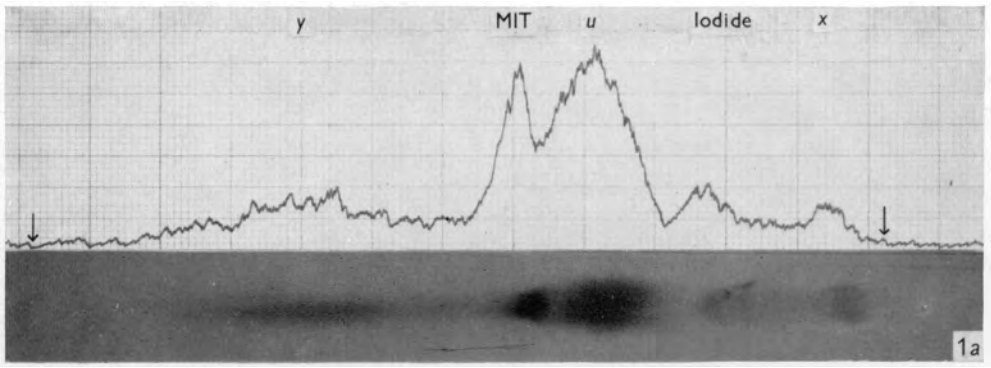
DESCRIPTION OF PLATE

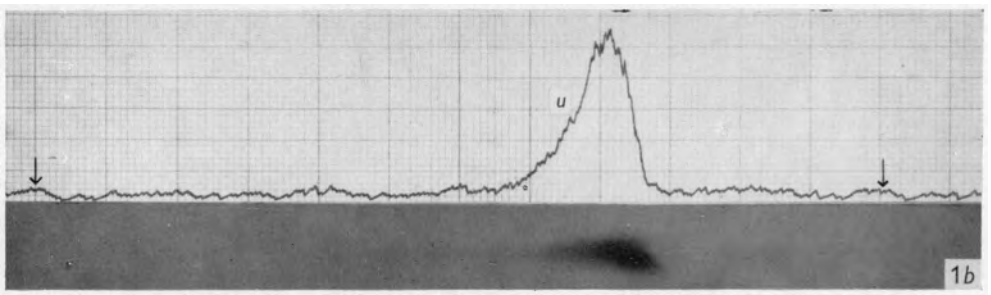
The origin is designated by an arrow on the right of each chromatogram.

Fig. 1. Chromatograms of urine of a patient with dehalogenase deficiency, after administration of [^{131}I]-monoiodotyrosine (MIT): (*a*) zones corresponding to '*x*', iodide, '*u*', MIT and '*y*' are shown; (*b*) after treatment with Amberlite resin: only zone '*u*' is present.

Fig. 2. Chromatogram of mixed saliva from a patient with dehalogenase deficiency after administration of [^{131}I]MIT. Only iodide is demonstrated.

Fig. 3. Chromatogram of gastric juice from a patient with dehalogenase deficiency after administration of [^{131}I]MIT. Only iodide is present.


y
MIT
u
Iodide
x
1a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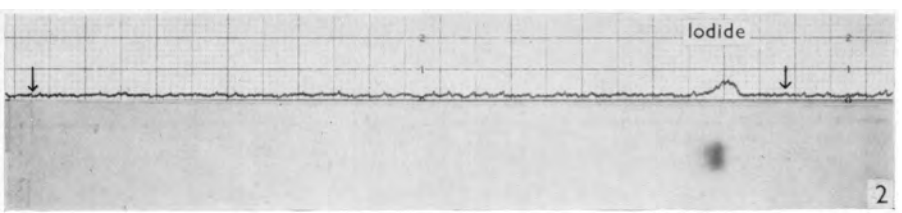
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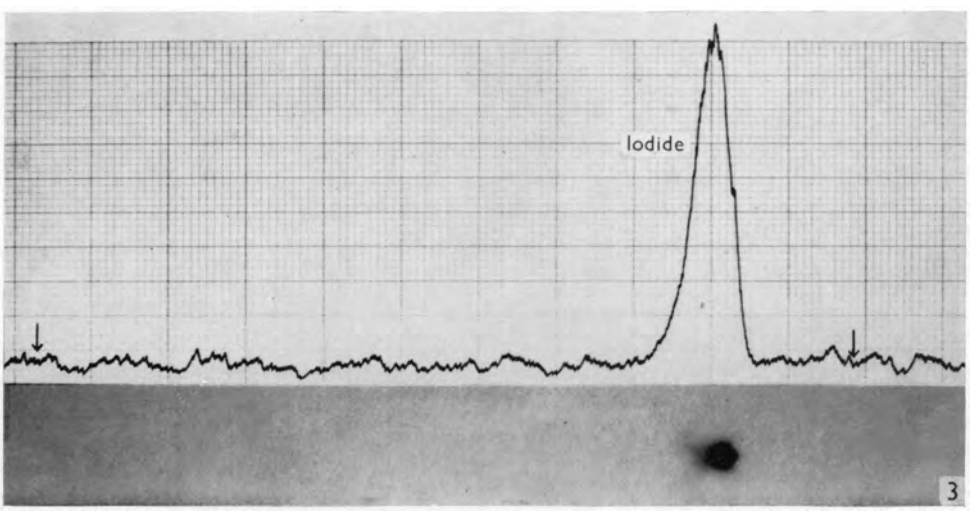
2


Iodide
3