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Normal and Abnormal Di-iodo-L-Tyrosine Metabolism in Human Subjects

By CHRISTINA CAMERON

Medical Research Council, Department of Clinical Research, University College Hospital Medical School, London, W.C. 1

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Three human subjects who had been treated for myxoedema rapidly deiodinated intravenously administered $[3:5\text{-}^{131}\text{I}_2]$ di-iodotyrosine, $[^{131}\text{I}]$ iodide being the main radioiodine metabolite found in the urine after 3 hr. (Albert & Keating, 1951). Subsequently, normal human subjects also were shown

to deiodinate $[^{131}\text{I}_2]$ di-iodotyrosine rapidly (Ruegamer & Chodos, 1956, 1958; Stanbury, Kassenaar, Meijer & Terpstra, 1956). $[^{131}\text{I}_2]$ Di-iodotyrosine has been found in the plasma (Costa *et al.* 1953) and in the urine (McGirr, Hutchison & Clement, 1956) after administration of ^{131}I in patients with goitrous

cretinism; after intravenous administration of [$^{131}\text{I}_2$]di-iodotyrosine in such patients, Stanbury, Meijer & Kassenaar (1956) recovered labelled di-iodotyrosine from the urine. These findings indicate a defect in the peripheral deiodinating system of some goitrous cretins.

In the course of some investigations of di-iodotyrosine metabolism in human beings in whom thyroid abnormality had been suggested by clinical examination, it became apparent that further information about normal di-iodotyrosine metabolism might be useful. In some of these subjects, for which the results are presented here, the rate of deiodination was apparently within the normal range reported by Stanbury, Kassenaar, Meijer & Terpstra (1956), yet small amounts of ^{131}I -labelled compounds of unknown structure were found in their urine. As it seemed important to know which of these compounds could arise from normal metabolism, further investigation of di-iodotyrosine metabolism in normal subjects was undertaken.

A secondary object was to compare the metabolism of [$^{131}\text{I}_2$]di-iodotyrosine administered by intravenous and by oral routes. If the rate of metabolism of an oral dose was similar to that of an intravenous dose, measurement of the rate of metabolism of [$^{131}\text{I}_2$]di-iodotyrosine as a clinical test would be simplified, particularly in children. Fletcher, Litvak & Stanbury (1958) found that doses of orally administered di-iodotyrosine were more rapidly deiodinated than intravenous doses, although amounts used were about a thousand times those used here.

An investigation was therefore made of the metabolism of [$^{131}\text{I}_2$]di-iodotyrosine in a group of six subjects whose peripheral iodine metabolism could be considered normal. The fate of [$^{131}\text{I}_2$]di-iodotyrosine administered orally and intravenously to the same subject was compared. Three of the subjects were normal adults whose thyroids were 'blocked' before di-iodotyrosine was given, and four were patients without any functioning thyroid tissue, maintained in a euthyroid state by thyroxine administration. The latter group had previously received therapeutic doses of ^{131}I for destruction of normal thyroid and subsequently of thyroid carcinoma. No concentration of ^{131}I by any tissue had been detected in any member of this group for at least 2 years before the investigation of di-iodotyrosine metabolism.

EXPERIMENTAL

[$^{131}\text{I}_2$]Di-iodo-L-tyrosine was prepared by exchange as described by Tong, Taurog & Chaikoff (1954). The exchange solution was made alkaline with aq. 2N-NH₃ soln., concentrated to a few drops under reduced pressure at 60° and applied in a narrow band to a large sheet (6 in. × 18 in.) of

Whatman no. 3 MM paper for chromatography in butanol-dioxan-aq. 2N-NH₃ soln. (4:1:5, by vol.). The labelled di-iodotyrosine was located by means of a radioautograph, and eluted from the chromatogram in ethanol-aq. 2N-NH₃ soln. (1:1, v/v) solution which was evaporated to dryness under reduced pressure at 50°. The residue was taken up in fresh sterile 0.9% NaCl soln. and, after sterilization by passage through a bacterial filter, the solution was dispensed into 5 ml. ampoules which were stored in the cold until used. Chromatographic analysis showed the preparation to contain 90–98% of [$^{131}\text{I}_2$]di-iodotyrosine.

The ^{131}I content of solutions used for injection and of the urine subsequently collected was measured in a liquid-counting apparatus employing a Geiger tube of 10 ml. capacity which gave 12 500 counts/min./μC of ^{131}I with a background of 12–15 counts/min. (M6, 20th Century Electronics Ltd.). Doses (15–100 μC) of the labelled material were given either intravenously or orally. The dose contained 10–75 μg. of di-iodotyrosine, as calculated from the amount of stable di-iodotyrosine used in the exchange. Complete collections of all urine passed were made, specimens being usually obtained 1, 2, 4 and sometimes 6 hr. after administration of the labelled material.

After measurement of the volume of urine and the radioiodine content of a sample, 10 ml. of each specimen was taken for analysis. This sample was mixed with 10 ml. of ethanol and adjusted to pH 8.0–9.0 with 2N-NaOH. A few milligrams of carrier NaI were added to the solution, which was passed through a packed column of AgCl (1 cm. × 7 cm.) to remove inorganic iodide (Fletcher, 1957). The effluent, which contained the organic iodine compounds, was neutralized and concentrated under reduced pressure at 60° to 2–5 ml. The concentrated solution was taken to pH 1.0–2.0 with 9% (w/v) HCl and extracted three times with an equal volume of butan-1-ol, a procedure which usually removed an estimated 85–110% of the ^{131}I in organic combination. In cases where the activity was low (less than 0.002 μC of organic radioiodine/ml. of urine), for example, in samples at 4 hr. after administration of a small dose (15 μC), a portion of the butanol solution was extracted once with an equal volume of 2N-NaOH. The proportion of activity in the aqueous phase was taken as an approximate measure of the proportion of the total organic activity which still behaved as iodotyrosine, as the estimate made by chromatographic analysis was not always satisfactory in urines of low activity.

In samples of higher activity, a portion of the first butanol extract equivalent to 0.5–3.0 ml. of urine was made alkaline with ethanol-aq. 2N-NH₃ soln. (1:1, v/v), concentrated under reduced pressure at 60° and applied as a narrow band 1 in. long to a strip of Whatman no. 3 MM chromatography paper (1.5 in. × 18 in.). Micro amounts of carrier substances (iodide, 3-mono- and 3,5-di-iodotyrosine and 3,5-di-iodo-4-hydroxyphenyl-lactic acid) were applied to the band in ethanol-aq. 2N-NH₃ soln. (1:1, v/v). Duplicate chromatograms were usually run in butanol-dioxan-aq. 2N-NH₃ soln. (4:1:5, by vol.) and in butan-1-ol–2N-acetic acid (1:1, v/v).

The radioiodine content of the separated components was usually measured by means of an automatic scanning device employing a mica end-window Geiger tube (EW 3 H, 20th Century Electronics Ltd.), which gave approx. 60 000 counts/min./μC of ^{131}I with a background of 20 counts/min. The mica window was shielded by a brass

plate with a 2 mm. slit through which the chromatogram was viewed. The chromatogram was drawn through mechanically at 1 in./hr., the counts being recorded automatically at the same time. No attempt was made to calculate the absolute amount of ^{131}I in any component. Radioautographs of all chromatograms were made on Ilford Industrial X-ray film, which required an exposure time of 2 weeks.

The carrier substances were located by staining with a solution of equal parts of ceric sulphate (1% in $\text{N-H}_2\text{SO}_4$) and sodium arsenite (5% in $\text{N-H}_2\text{SO}_4$) mixed immediately beforehand. The chromatograms were sprayed with a solution of 1% *o*-phenylenediamine in acetone (Fletcher & Stanley, 1955). In the latter part of this work the chromatograms were then sprayed with concentrated aq. NH_3 , which neutralized the $\text{N-H}_2\text{SO}_4$ and thereby prolonged the life of the paper. The position of blackened areas on the film was compared with that of carrier substances on the chromatograms. The R_F values of any ^{131}I -labelled compounds of unknown structure were noted.

RESULTS

Table 1 presents the quantitative results of the excretion of ^{131}I after the administration of [$^{131}\text{I}_2$]di-iodotyrosine to nine control subjects. Five of the subjects were healthy volunteers (nos. 1-5), of whose thyroids three had been 'blocked' by prior administration of 50 mg. of sodium iodide (nos. 1-3). Four others (nos. 6-9), who were patients who had been treated with large doses of ^{131}I for thyroid carcinoma, were without any functioning thyroid tissue and were being maintained in a euthyroid state by administration of thyroxine. Subject no. 9 also had urinary tuberculosis.

It was observed that when the volume of the butanol extract used for chromatography was derived from more than 1 ml. of urine, some activity was found at or near the origin of chromatograms developed in butan-1-ol-acetic acid, and sometimes in butan-1-ol-dioxan-aq. NH_3 soln. In analyses where an extract derived from more than 1 ml. of urine was used the average amount of this fraction was 25% of the ^{131}I in organic combination for oral doses, and 15% for intravenous doses of [$^{131}\text{I}_2$]di-iodotyrosine. It seems probable that the fraction is an artifact arising during chromatography. When more than 1 ml. of urine must be extracted to concentrate sufficient of ^{131}I -labelled compounds for detection on the chromatogram by means of a scan or a radioautograph, the salt which is extracted from the urine and carried on to the paper with the iodine compound is enough to cause artifact formation. Small amounts of other components, representing from 1 to 5% of the activity other than iodide, were observed near di-iodotyrosine on the scans and radioautographs of chromatograms developed in butan-1-ol-acetic acid. Their chromatographic behaviour was not the same as that of either 3-moniodotyrosine or 3:5-

di-iodo-4-hydroxyphenyl-lactic acid. The degree of separation achieved in chromatograms developed in butan-1-ol-dioxan-aq. NH_3 soln. was not usually sufficient to resolve iodotyrosines and the fraction of ^{131}I found near the origin of the chromatograms which was regarded as being an artifact.

The quantitative results of the metabolism of [$^{131}\text{I}_2$]di-iodotyrosine in nine patients with thyroid abnormality are presented in Table 2. The status of thyroxine administration at the time of investigation is noted in each case. One subject, no. 6, was investigated when thyroxine administration had been stopped for 4 weeks and again when administration had been resumed. The results differ markedly on the two occasions: 20.3% of the label was excreted during the first 4 hr. in organic combination when thyroxine was not being administered, a value much greater than the normal mean of 4.7 (± 0.4 S.E.; S.D. 1.1). This organic fraction was only 50-70% soluble in butanol. Heating the urine at 40° at pH 4.5 or 5.4 improved the butanol solubility of the ^{131}I in organic combination, but the components which were rendered soluble by such treatment were of unknown structure. A short period of heating produced a component immobile in butanol-dioxan-aq. NH_3 soln., but longer periods of 6 or 7 hr. produced a component running between the origin and di-iodotyrosine in butan-1-ol-dioxan-aq. NH_3 soln. When this subject was receiving thyroxine, the organic fraction of ^{131}I in the urine was soluble in butanol, and the proportion of the fraction appearing on radioautographs of the chromatograms as components of unknown structure was no greater than that observed in normal subjects, and so may have consisted only of artifacts.

In another case (no. 5, Table 2) an important amount of an unknown component was observed on the chromatogram developed in butan-1-ol-acetic acid to run between the origin and iodide. However, the quantitative results did not suggest an abnormality of di-iodotyrosine metabolism, as the amount of radioactivity appearing in organic form in the urine was little, if any, above the normal range.

DISCUSSION

The mean value for the percentage of the intravenous dose excreted in organic form during the first 4 hr. was 4.7 (± 0.4 S.E.; S.D. 1.1). This is greater than the value which may be calculated from Stanbury, Kassenaar, Meijer & Terpstra (1956) of 2.31 (± 0.17 S.E.; S.D. 0.99). The difference is probably explainable by the difference in the method of analysis. In the work presented here the organic fraction of ^{131}I was taken as all that remaining after the removal of [^{131}I]iodide on a

Table 1. *Metabolism of [3:5-¹³¹I]₂di-iodotyrosine in normal subjects*
 DIT, 3:5-Di-iodotyrosine; DITlac, 3:5-di-iodo-4-hydroxyphenyl-lactic acid.

No.	Intravenous administration					Oral administration				
	Wt. of DIT (μ g.)	Time after dose (hr.)	Cumulative percentage of dose	Cumulative percentage of dose in organic form	DIT/DITlac	Wt. of DIT (μ g.)	Time after dose (hr.)	Cumulative percentage of dose	Cumulative percentage of dose in organic form	DIT/DITlac
1	13.5	1	19.8	3.2	2.8	30	1	6.1	0.6	—
		2	31.2	4.3	1.6		2	15.2	1.7	1.1
		4	44.8	5.3	1.1		4	29.7	2.7	0.3
		—	—	—	—		6	40.2	3.1	0.2
2	13.5	1	13.5	3.6	5.6	30	1	7.8	0.7	1.6
		2	19.7	4.0	2.8		2	16.6	1.6	0.7
		4	36.3	5.3	3.5		4	29.7	2.4	0.8
		—	—	—	—		6	40.6	2.7	—
3	21	1	18.0	3.8	5.1	35	1	5.7	0.8	0.8
		2	26.1	4.8	1.3		2	15.8	2.0	1.5
		3	32.6	5.2	—		4	28.9	2.9	0.9
		4	36.8	5.5	0.7		6	37.9	3.4	—
4	25	1	14.5	4.4	—	—	—	—	—	—
		2	22.2	5.4	—					
		3	27.9	6.0	—					
5	25	1	10.4	2.7	—	—	—	—	—	—
		2	17.5	3.7	—					
		3	23.2	4.3	—					
		7	37.3	4.4	—					
6	42	1	13.4	1.8	10.2	60	1	6.5	0.6	0.9
		2	20.4	2.7	—		2	12.7	1.3	0.8
		4	31.6	3.5	1.4		4	23.9	2.5	0.9
7	45	1	12.7	1.9	8.1	60	1	3.7	0.4	0.7
		2	20.3	2.5	1.6		2	10.1	1.1	0.9
		4	33.3	3.1	1.3		4	20.8	1.9	0.7
8	45	1	6.6	1.4*	6.1	60	1	5.6	0.7	1.2
		4	(10.9)	(2.3)	2.6		2	14.7	1.8	0.6
		—	—	—	—		4	27.7	2.8	0.6
9	—	—	—	—	—	28	1	4.6	—	—
		—	—	—	—		2	13.1	1.3	—
		—	—	—	—		4	31.8	1.9	—
		—	—	—	—		6	43.7	2.0	—
				Mean 4 hr. value						
				4.7 $\pm$ 0.4, s.d. 1.1						
										Mean 4 hr. value
										2.4 $\pm$ 0.2, s.d. 0.4

* Some urine was lost.

Table 2. *Metabolism of [3:5-¹³¹I]₂di-iodotyrosine in subjects with thyroid abnormality*

Doses of di-iodotyrosine were administered intravenously except to patient no. 9, who was given the dose orally.

No.	Age	Sex	Diagnosis	Thyroid medication at time of investigation	Dose of DIT (μg.)	Time after dose (hr.)	Cumulative percentage of dose	Cumulative percentage of dose in organic form
1	14	F	Goitre, hypothyroidism	Thyroid 'block' with 1 g. of KI	25	1	8.8	1.9
						2	15.8	3.4
						3	21.1	4.1
						4	27.1	4.5
2	13	M	Goitre, hypothyroidism	Thyroid 'block' with 1 g. of KI	25	0.75	7.5	1.1
						5.25	33.2	5.2
3	11	F	Goitre	None	10	2	24.7	4.8
						4.5	38.4	6.7
						5.5	40.6	6.9
4	4	F	Goitre?	None	5	1	6.5	1.3
						2	11.7	2.0
						6	14.6	2.4
5	9	F	Goitre	None	10	1	14.1	4.2
						2	22.4	5.7
						4	32.9	7.3
						6	41.1	8.0
6	16	F	Hypothyroidism	Thyroxine stopped for 4 weeks	75	1	17.5	13.8
						2	23.1	18.7
						4	25.0	20.3
						6	26.6	21.9
			Thyroxine resumed	25	25	8	27.6	22.6
						1	13.0	3.3
						2	16.9	4.2
						4	20.0	5.2
7	12	M	Hypothyroidism	Thyroxine stopped for few days	40	0.6	4	0.1
						3.1	13.5	1.75
						9.25	17.8	1.77
8	17	M	Goitre	Iodopyrin	25	1	8.3	2.7
						2	15.2	4.6
						3	18.4	5.5
						4	20.2	6.0
9	4	M	Goitre	50 mg. of thyroxine daily	25 (orally)	1	0.1	0
						3	14.8	1.2
						4	22.2	1.6
						6	31.3	2.0
10	10	M	Goitre	30 mg. of thyroxine daily	25	0.75	13.6	4.2
						3	23.6	7.6
						5	25.4	8.2

column of silver chloride. An appreciable proportion of this organic fraction is not in fact di-iodotyrosine. Stanbury, Kassenaar, Meijer & Terpstra (1956) calculated the amount of [¹³¹I]₂di-iodotyrosine from a scan of a chromatogram either of the whole urine, or of a butanol extract of the whole urine. Their value of 2.31% is therefore the amount of [¹³¹I]₂di-iodotyrosine which has not been metabolized in any way during the first 4 hr., whereas the mean value of 4.7% obtained here is the total of di-iodotyrosine and any of its metabolites which have not been deiodinated.

The mean value of 4.7 (± 0.4 s.e.; s.d. 1.1)% of the dose excreted in organic form during the first 4 hr. after an intravenous dose is significantly

greater than the mean value of 2.4 (± 0.2 s.e.; s.d. 0.4)% excreted during the first 4 hr. after an oral dose. The difference may be due in part to the time taken for absorption of an oral dose of di-iodotyrosine from the intestine, but also suggests a more rapid deiodination of oral doses. In three subjects (nos. 1, 2 and 3, Table 1) collections were continued until 6 hr. after an oral dose, but even with the addition of this increment the total of the organic radioiodine excreted was less than that excreted during the first 4 hr. after an intravenous dose in the same subject. Moreover, the amount of organic radioiodine excreted during the 4–6 hr. period after an oral dose was less than that excreted during the preceding 2 hr. period, which

indicates therefore that the cumulative total of organic radioiodine over longer periods will always be smaller after oral doses than after intravenous doses.

The ratio of [3:5- $^{131}\text{I}_2$]di-iodotyrosine to [3:5- $^{131}\text{I}_2$]di-iodo-4-hydroxyphenyl-lactic acid in the urine is greater during the first 2 hr. after an intravenous dose than at any time after an oral dose. Thus it appears that deiodination and deamination may both be occurring in the intestine. However, the difference between the mean 4 hr. values of organic radioiodine excreted by normal subjects after intravenous and after oral doses (Table 1) is sufficiently constant to suggest that abnormal metabolism of di-iodotyrosine could be detected after an oral dose.

A highly significant difference was observed between the results obtained in one subject (no. 6, Table 2) for metabolism of an intravenous dose after thyroxine administration had been stopped for 4 weeks, and those after administration had been resumed. In the first test 20.3% of the dose appeared in organic form during the first 4 hr., and in the second test 5.2%, a value which is within the normal range. It is unlikely that the difference in rate of deiodination is the result of myxoedema itself. Stanbury & Litvak (1957) have shown that myxoedematous patients can deiodinate di-iodotyrosine at a rate which is nearly normal. Moreover, the organic fraction of radioactivity which appeared in the urine was apparently quite normal when the subject was taking thyroxine, but grossly abnormal after administration had been stopped. An inability to deiodinate di-iodotyrosine peripherally has been ascribed to an enzyme defect reflecting the lack of the deiodinase enzyme in the gland (Stanbury, Meijer & Kassenaar 1956). In the subject investigated here, the peripheral defect is apparently reversible by administration of thyroxine.

The proportion of the dose appearing in organic form in the urine during the first 4 hr. is 8.2% in subject no. 10 (Table 2), which is higher than normal although no abnormal metabolites were detected by chromatography. The subject therefore exhibits only the quantitative aspect of the abnormality, but it should be noted that thyroxine was being administered at the time of the investigation.

In another subject (no. 5, Table 2) the proportion of the dose excreted in organic combination during the first 4 hr. is 7.3%, which is greater than the normal mean, plus two standard deviations ($4.7 + 2.2$), but the normal standard deviation has not been established with accuracy sufficient to show if this difference is significant. In this subject a ^{131}I -labelled compound was detected on radioautographs of chromatograms run in butan-1-ol-

acetic acid which did not coincide with either 3:5-di-iodotyrosine or 3:5-di-iodo-4-hydroxyphenyl-lactic acid. In normal subjects the ^{131}I -labelled artifacts detected on chromatograms run in butan-1-ol-acetic acid occurred at the origin, but the compound of unknown structure detected in the urine of this subject had R_f 0.1. This compound may therefore be an abnormal di-iodotyrosine metabolite but the results of the test are not conclusive in this case.

In conclusion it would seem that some hypothyroid subjects do not deiodinate di-iodotyrosine normally but that treatment with thyroxine may restore deiodination to normal (no. 6, Table 2). This finding confirms that of Mosier, Blizzard & Wilkins (1958), who investigated two goitrous cretins whose rates of deiodination of di-iodotyrosine were slow and found they were able to deiodinate at a normal rate after treatment with thyroid extract. However, other hypothyroid subjects may deiodinate normally before treatment with thyroxine (nos. 1 and 2, Table 2). This finding is in agreement with that of Stanbury & Litvak (1957), who found that myxoedema itself does not cause a defect in peripheral deiodination of di-iodotyrosine.

SUMMARY

1. Di-iodotyrosine metabolism in normal adults has been investigated by analysis of urinary iodine compounds after intravenous or oral administration of [3:5- $^{131}\text{I}_2$]di-iodotyrosine.

2. The proportion of the label appearing in the urine as iodide is greater during the first 4 hr. after an oral dose than after an intravenous dose.

3. Results of similar investigations in a group of patients with goitre and hypothyroidism are presented.

4. In one hypothyroid patient, an abnormally slow rate of deiodination of di-iodotyrosine became normal when the subject was receiving thyroxine.

5. Two subjects who were regarded as hypothyroid had a normal rate of deiodination of di-iodotyrosine.

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Radioiodine Compounds of Human Urine after ^{131}I Therapy

1. FRACTIONATION

By CHRISTINA CAMERON

Medical Research Council, Department of Clinical Research, University College Hospital Medical School, London, W.C. 1

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After administration of radioiodine (^{131}I) in therapeutic amounts for the treatment of thyrotoxicosis and thyroid carcinoma, organic ^{131}I -labelled compounds appear in the urine, due in part presumably to the destruction of thyroid tissue by radiation from ^{131}I taken up by the gland. Concentration of these compounds for identification is difficult, since the total quantity of iodine excreted per day as iodide and as organic iodine compounds is small in comparison with the other constituents of urine.

Rall (1950) made quantitative estimates of the organic radioiodine compounds in such urine by precipitation with zinc hydroxide according to the Somogyi technique. The organic iodine fraction was also concentrated by butanol extraction and fractionated by phenol-water chromatography. Horst & Heuwieser (1957) employed butanol extraction followed by chromatography in butanol-dioxan-ammonia for the identification and quantitation of radioiodine compounds in urine.

Such methods were found by Fletcher (1956, 1957*a*) to be of limited use as the degree of separation achieved was poor, and in most cases too little of any one organic compound could be isolated to permit a detailed study and conclusive identification. A method was therefore developed in which the organic iodine fraction was concentrated on ion-exchange resin (Zeo-Karb 215) after removal of inorganic iodide on a column of silver chloride. Extraction of the organic fraction into butanol and chromatography of the extract on large sheets of chromatography paper (6 in. $\times$ 18 in., Whatman no. 3MM) effected partial separation of the mixture of iodine compounds. Each component of the original mixture thus fractionated had then to be subjected to further paper chromatography to complete the separation of radioiodine compounds.

The method was applied to the study of the urines of the fifth day after administration of 80 mc of ^{131}I for destruction of normal thyroid tissue and of 150 mc for treatment of thyroid carcinoma, at which time the proportion of radioiodine in organic combination in the urine was maximal, comprising 5–10% of the total. The major components of the organic radioiodine fraction were identified as 3:5-di-iodotyrosine and 3:5-di-iodo-4-hydroxy-phenyl-lactic acid (Fletcher, 1957*b*).

In order to study the other minor components observed by Fletcher (1957*b*) and to isolate the organic iodine fraction from urines excreted 24–72 hr. after therapy, in which the proportion of radioactivity in organic combination is usually only 1–2%, a modification was introduced to avoid the losses caused by deiodination and by decay of activity during the stages of paper chromatography. Kieselguhr-column chromatography was employed (Gross & Pitt-Rivers, 1952, 1953) in the isolation of 3:5:3'-tri-iodothyronine from rat thyroid and in the study of the ^{131}I -labelled compounds excreted in the urine after administration of $[3':5':^{131}\text{I}]_2$ thyroxine and $[3':^{131}\text{I}]$ -3:5-tri-iodothyronine to dogs (Flock, Bollman, Grindlay & Orvis, 1956; Flock, Bollman & Grindlay, 1957). The methods described were applied to the separation of mixtures of iodine compounds of known structure, but the recovery of the iodotyrosines was poor. Similarly, the recovery of organic radioiodine compounds was poor when the methods were used to fractionate the mixture concentrated from urine after ^{131}I therapy. The method described here, which gave an improved recovery of organic radioiodine compounds, was developed by fractionation of aqueous mixtures of iodine compounds of known structure on kieselguhr columns packed in aqueous solvents.