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IODINE-CONTAINING COMPOUNDS OF EXTRATHYROIDAL TISSUES *

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Several methods, giving values essentially in agreement, are now available to determine the iodine content of plasma. Attempts to quantitate the iodine content of tissues have not yielded consistent results. The purpose of our paper is to report the application to this problem of a method based on isotopic equilibrium and using I^{125} as a radioactive tracer. With this procedure, it is possible to measure the concentration of total iodine, iodide, thyroxine, triiodothyronine, and some unidentified iodine-containing substances in plasma, selected tissues, and excreta of the rat.

METHODS

Diets. Rats were fed two separate diets differing in their iodine content. The first, designated "high-iodine," consisted of a standard laboratory diet [1] containing 1.7 μg of iodine [2] per g of ration; the second, designated "low-iodine," was a special low-iodine ration [3] containing 0.06 μg of iodine [2] per g. To avoid the possibility of producing iodine deficiency (1), we added enough potassium iodide to raise the iodine content to 0.22 μg per g. The amount of iodine added to the diets as I^{125} was negligible. The two diets, therefore, differed approximately tenfold in iodine concentration. Enough feed was obtained from the manufacturers to complete all the experiments with one lot, avoiding variations in the stable-iodine content.

I^{125} was obtained carrier free from a commercial source.[4] Its purity was confirmed by chromatography and by decay analysis. Uniform distribution of I^{125} within the diet simply by mixing the feed with the isotope as iodide proved impossible, even with extensive mixing. It was necessary to regrind the mixture and mix it again before satisfactory agreement between radioactivity of samples was obtained (Table I, batches no. 1 and 2). With the following procedure, we obtained excellent results. An appropriate amount of radioactive iodine (usually 10 mc) was diluted to 150 to 200 ml with distilled water, added to several hundred g of ground feed, and thoroughly mixed. The mixture was dried in an oven and passed through a grinding mill to obtain a finely pulverized material. This was added to the remaining feed, which also had been finely ground, and the entire amount was mixed in a single bulk feed mixer for 18 to 24 hours. Fifteen 0.5-g portions were randomly sampled from the mixture, and the radioactivity was measured in a well-type scintillation detector to check the completeness of mixing and to establish the I^{125}: I^{127} ratio. The improved dispersion of the label is clear from the data presented in Table I (batches no. 3–9).

Preparation of tissue. Male albino rats (Holtzman) weighing 200 to 600 g were used in all experiments. To avoid an abrupt change in total iodine intake when the feeding of labeled diet began, we first maintained the rats on the appropriate test diet, but without the radioactive iodine, for 4 to 8 weeks.

After the initiation with labeled diet, the animals were sacrificed at intervals by exsanguination under ether anesthesia. The tissues were immediately excised, blotted free of residual blood, rinsed with water, and chilled on ice. Either whole small organs or 1 to 2 g of tissues were weighed to ± 10 mg and counted in a well-type scintillation counter. A sample of the diet was counted at the same time to correct for physical decay and instrument variation. For 24 hours before sacri-

TABLE I

Radioactivity variation of 15 samples of test diets after addition of I^{125}

Batch no.	Coefficient of variation
	SD/mean × *100%*
1	19.0
2	14.8
3	5.5
4	7.9
5	6.5
6	6.8
7	4.2
8	4.8
9	5.1

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[1] Rockland rat ration, New City, N. Y.

[2] Assayed by Albert L. Chaney Laboratories, Glendale, Cal.

[3] General Biochemical Corp., Chagrin Falls, Ohio.

[4] Oak Ridge National Laboratory, Oak Ridge, Tenn.

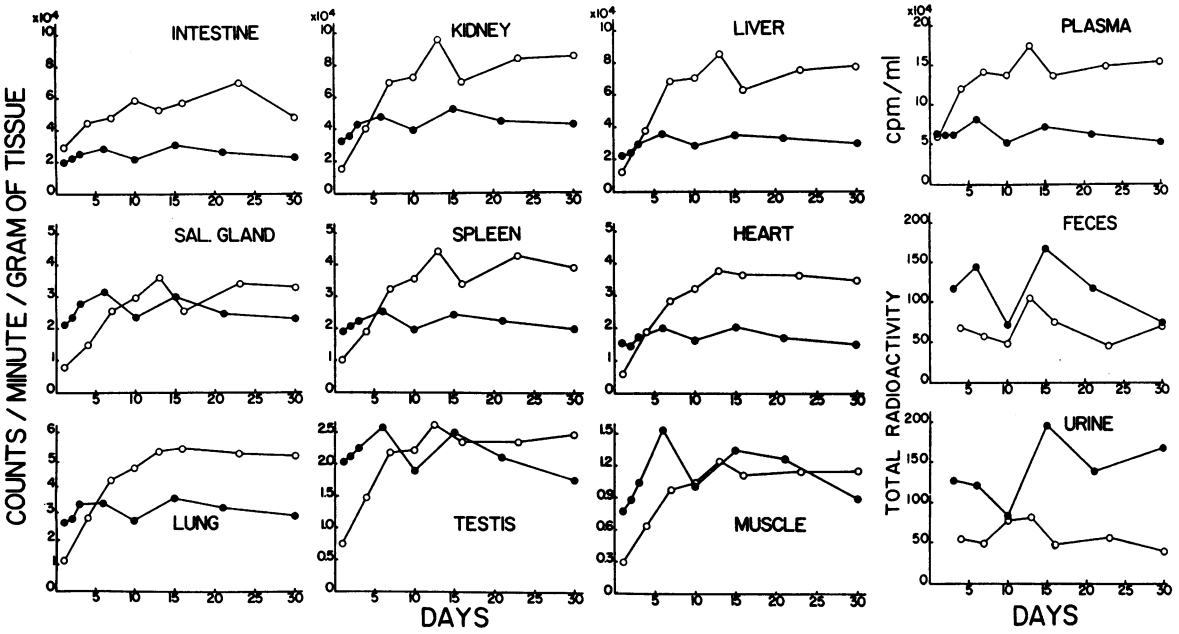

FIG. 1. CUMULATION OF I^{131} IN VARIOUS TISSUES, PLASMA, AND EXCRETA. The ordinate reflects the radioactivity corrected for physical decay expressed as counts per minute per gram of tissue, or per milliliter of plasma, and for total 24-hour urine and feces. No. animals = 3 per sample day. ●——● = high-iodine diet; ○——○ = low-iodine diet.

fice, the animals were placed in metabolic cages that permitted separate collection of urine and feces.

Extraction and separation of products. The tissues and feces were homogenized with a Teflon-glass homogenizer in 2 vol of water. The homogenates were extracted with *n*-butanol followed by the addition of chloroform and back-extraction with 2 N NH_4OH as described by Flock and Bollman (2). More than 90% of the radioactivity was extracted from all tissues, whereas the amount extracted from feces varied from 30 to 60%. A small quantity of thiouracil was added to the extract as a precaution against instability of iodinated organic compounds during evaporation and chromatography. The ammoniacal extracts were concentrated *in vacuo* and chromatographed in descent for 48 hours with a tertiary-amyl alcohol system (3). Some samples were also chromatographed in a butanol-ethanol-ammonia system (4). Urine was chromatographed directly. Colorimetrically identifiable quantities of marker compounds were added to the extract before chromatography. The position of the thyronine compounds was determined with 4-amino-antipyrine reagent (5) and iodide with palladium chloride reagent (4). The radioactivity was localized on the chromatograms by scanning with a 4-pi anticoincidence gas-flow counter equipped with a continuously recording system.[5] Argon was used as the counting gas and isobutane as the internal quencher.

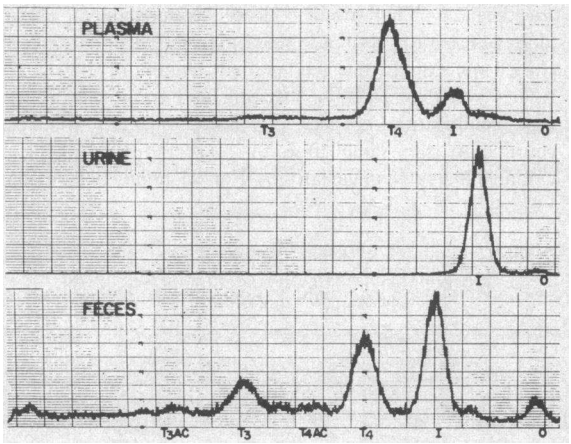

FIG. 2. RADIOCHROMATOGRAMS OF EXTRACTS OF PLASMA AND EXCRETA FROM RATS FED THE LOW-IODINE DIET, CONTAINING THE I^{125} LABEL, FOR 30 TO 40 DAYS. Solvent: tertiary-amyl alcohol saturated with 2 N ammonium hydroxide. O, origin; I, iodide; T_4, thyroxine; T_3, triiodothyronine; T_4AC, tetraiodothyroacetic acid; and T_3AC, triiodothyroacetic acid. Solvent front not shown.

The radioactivity was quantitated by planimetry of the area under the curve and by use of an automatic integrator attached to the scanner assembly. Radioautographs of the same strips were also prepared by use of Kodak nonscreen X-ray film to confirm the findings by scanning and to detect bands of low levels of radioactivity that might have been overlooked.

[5] Scanograter III, Atomic Accessories, Inc., Valley Stream, N. Y.

The labeled diet was continued until there was essentially no further increase in total radioactivity, after correction for physical decay, in any of the excised tissues. At this point, the rats were considered to be in a state of equilibrium with their diet in respect to relative I^{125} and I^{127} concentrations. The remaining rats were killed and their tissues processed as above. This provided a larger number of animals for statistical study.

Histological sections were prepared from thyroids of rats selected at random in order to evaluate any radiation damage to the thyroids. In all instances, the thyroid follicles appeared normal.[6]

[6] We are in debt to Dr. Joseph J. Lalich of the Department of Pathology for these studies.

RESULTS

The appearance of the radioactive label in the tissues, plasma, and excreta is shown in Figure 1. The ordinates reflect cumulative radioactivity. The radioactivity in all tissues increases rapidly for the first few days and then levels off. Since the labeled iodine will be continuously diluted by the stable iodine present in the body when feeding of the labeled diet begins, true equilibrium will be approached asymptotically. Very little increase in radioactivity in any of the tissues was observed

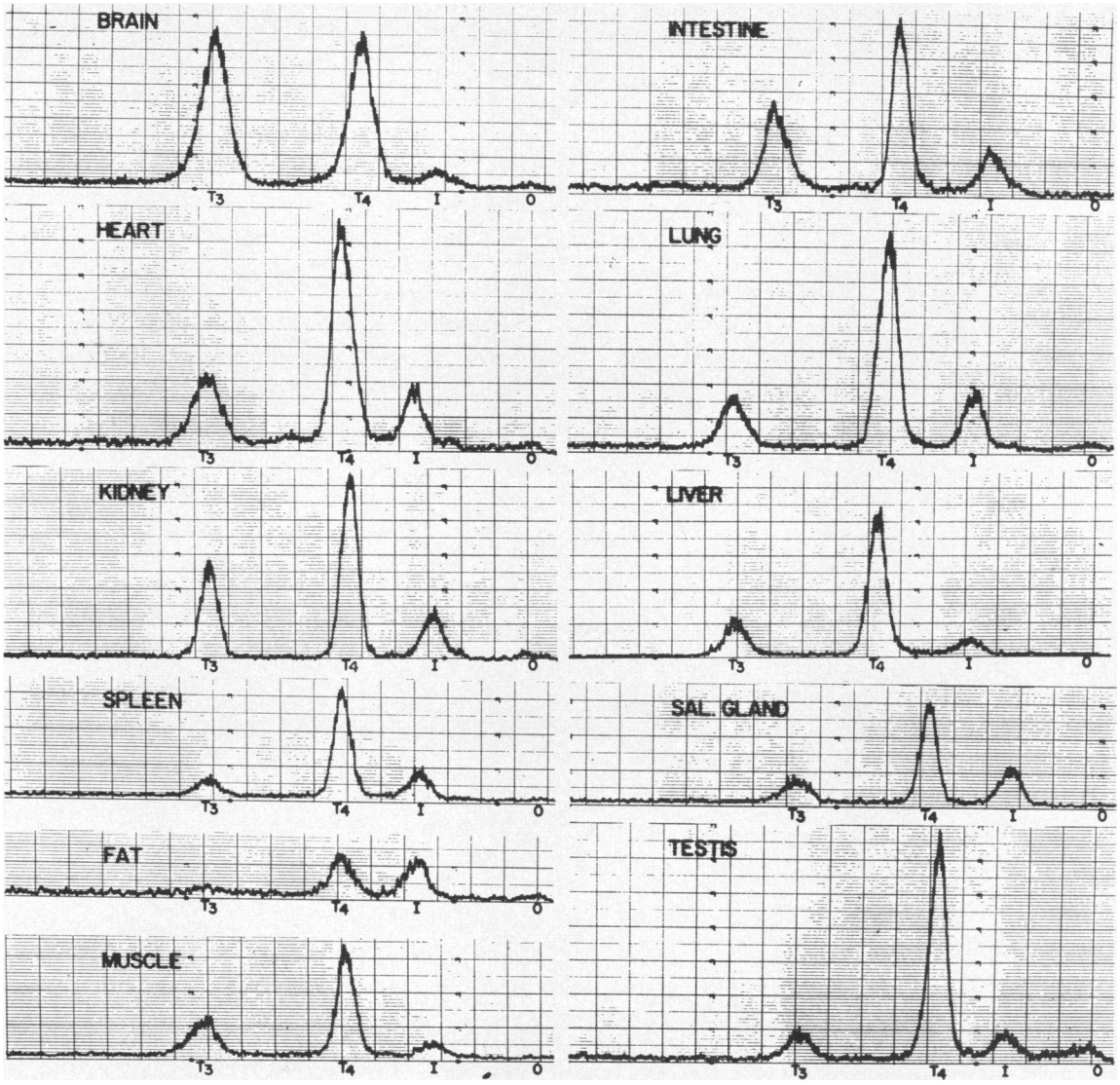

FIG. 3. RADIOCHROMATOGRAMS OF EXTRACTS OF SELECTED TISSUES FROM RATS FED THE LOW-IODINE DIET, CONTAINING I^{125} LABEL, FOR 30 TO 40 DAYS. Solvent: teriary-amyl alcohol saturated with 2 N ammonium hydroxide. O, origin; I, iodide; T_4, thyroxine; and T_3, triiodothyronine. Solvent front not shown.

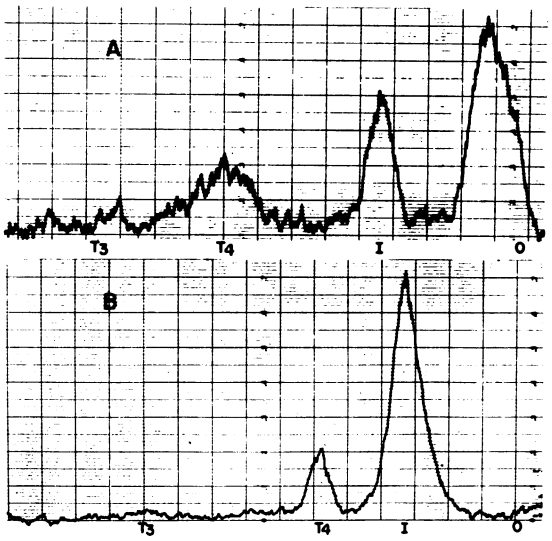

FIG. 4. RADIOCHROMATOGRAMS OF EXTRACTS OF RAT KIDNEYS. Rats were fed A) high-iodine diet consisting of Rockland rat ration, or B) low-iodine diet supplemented with potassium iodide, chromatographed in tertiary-amyl alcohol: ammonium hydroxide solvent system. Note the difference in material remaining at the origin. O, origin; T_4, thyroxine; T_3, triiodothyronine; I, iodide. Solvent front not shown.

between days 23 and 30; therefore we assumed that equilibrium had been approximated after day 30.

Radiochromatograms of extracts of plasma, feces, urine, and selected tissues from rats fed the low-iodine labeled diet for 30 to 40 days are shown in Figures 2 and 3. Peaks appeared that could be identified as iodide, thyroxine, and triiodothyronine. In the chromatograms of tissues (Figure 3), iodide, thyroxine, and triiodothyronine were always present. Some labeled material as yet unidentified remained at the origin in both solvent systems. The amount of this material, relatively small in the low-iodine group, often exceeded 50% of the total activity (Figure 4A) in the high-iodine group fed Rockland rat ration. When the iodine content of the low-iodine diet was raised by adding potassium iodide to equal that of the Rockland rat ration, this peak was not observed (Figure 4B), and thus may be an artifact produced by the Rockland rat ration.

In the chromatograms of feces (Figure 2), in addition to iodide, thyroxine, triiodothyronine, and origin material, two other peaks having the mobility of triiodo- and tetraiodothyroacetic acid appeared. Chromatograms of urine revealed chiefly iodide and small amounts of origin material, as shown in Figure 2. Trace amounts of triiodothyronine and several unidentified bands appeared on radioautographs of chromatograms of urine. Chromatograms of plasma (Figure 2) always revealed thyroxine and iodide, and usually small peaks of triiodothyronine and origin material. In addition, a small peak (3 to 5% of total activity) with a chromatographic mobility similar to diiodotyrosine often appeared.

The quantitative distribution of iodine among various components in rat tissues, plasma, feces, and urine is given in Tables II (high-iodine group) and III (low-iodine group). The major

TABLE II

*Iodine content of plasma, selected tissues, and excreta from rats fed high-iodine diet**

	Total iodine	Origin	Iodide	Thyroxine
Brain	4.64 ± 0.57	3.03 ± 0.86	1.16 ± 0.52	0.80 ± 0.31
Heart	16.28 ± 2.6	9.89 ± 1.61	6.10 ± 0.23	1.73 ± 0.87
Small intestine	25.83 ± 6.16	13.9 ± 3.1	12.2 ± 2.9	2.34 ± 1.02
Kidney	43.03 ± 7.0	21.2 ± 3.5	19.9 ± 3.2	6.85 ± 1.96
Liver	35.52 ± 6.32	14.6 ± 3.4	11.5 ± 3.1	11.2 ± 4.4
Lung	31.79 ± 3.7	11.8 ± 1.89	15.1 ± 1.00	4.48 ± 1.93
Muscle	9.88 ± 1.47	8.42 ± 0.83	1.81 ± 1.59	0.85 ± 0.43
Salivary gland	25.92 ± 3.4	15.7 ± 2.8	7.49 ± 1.20	1.56 ± 1.03
Spleen	23.72 ± 2.68	10.5 ± 1.1	10.6 ± 1.4	2.32 ± 1.06
Testis	20.99 ± 2.54	13.08 ± 1.12	6.81 ± 1.36	1.38 ± 0.68
Urine	21,180 ± 6,240		21,180 ± 6,240	
Feces†	7,860 ± 3,360	2,050 ± 890	3,880 ± 1,380	
Plasma	64.11 ± 8.48	17.6	42.6	5.03

* Calculated from SA of the diet and expressed as millimicrogram per gram tissue, millimicrogram per 24-hour urine or feces, and millimicrogram per milliliter plasma. No. of animals = 20.

† The figures represent only the fraction of iodine (30 to 60%) that was extracted by the method employed.

TABLE III

*Iodine content of selected extrathyroidal tissues, excreta, and plasma from rats fed low-iodine diet**

	Total iodine	Origin	Iodide	Thyroxine	Triiodothyronine	T_3:T_4†
Brain	2.55 ± 0.83	0.20 ± 0.12	0.32 ± 0.13	1.18 ± 0.13	0.97 ± 0.18	0.82
Fat	9.35 ± 0.62	0.52 ± 0.95	3.53 ± 1.83	4.49 ± 1.94	0.81 ± 0.16	0.18
Heart	7.29 ± 0.88	0.51 ± 0.37	1.41 ± 0.42	3.56 ± 1.25	1.51 ± 0.35	0.43
Small intestine	12.44 ± 2.4	0.60 ± 0.43	2.46 ± 0.74	5.47 ± 1.85	3.37 ± 1.85	0.62
Kidney	18.96 ± 1.75	1.17 ± 0.38	3.04 ± 1.29	9.26 ± 2.08	5.08 ± 1.83	0.55
Liver	16.82 ± 1.19	0.43 ± 0.30	3.31 ± 1.41	11.05 ± 1.63	1.97 ± 0.29	0.18
Lung	11.08 ± 1.1	0.37 ± 0.31	2.44 ± 1.28	6.47 ± 0.99	1.75 ± 0.91	0.39
Muscle	2.44 ± 0.25	0.21 ± 0.18	0.39 ± 0.16	1.17 ± 0.26	0.55 ± 0.25	0.47
Salivary gland	6.75 ± 0.89	0.46 ± 0.37	1.20 ± 0.56	3.50 ± 1.09	1.32 ± 0.28	0.38
Spleen	8.4 ± 0.71	0.95 ± 0.40	2.70 ± 0.90	3.17 ± 0.05	1.39 ± 0.74	0.44
Testis	4.86 ± 0.98	0.40 ± 0.21	1.25 ± 0.49	2.73 ± 0.36	0.36 ± 0.34	0.13
Urine	1,350 ± 570	Trace	1,350 ± 570		Trace	
Feces‡	1,310 ± 380	72.0 ± 24.0	393 ± 239	487 ± 154	266 ± 93	0.55
Plasma	33.13 ± 3.5	0.72 ± 1.3	9.0 ± 4.3	21.9 ± 4.4	0.67 ± 0.74	0.03

* Calculated from SA of the diet and expressed as mμg per g tissue, mμg per 24 hours urine or feces, and mμg per ml plasma. No. of animals = 9.

† Triiodothyronine to thyroxine ratio.

‡ The figures represent only the fraction of iodine (30 to 60%) extracted by the method employed.

difference between these two groups is in the amount of iodine in the origin material and as iodide. Both origin iodine and iodide were much higher in all tissues examined in the high-iodine group. The large amount of radioactivity as iodide on the chromatograms in the high-iodine group raised the background level to the point that accurate measurement of the radioactivity present as thyroxine and triiodothyronine was not feasible. For this reason, the low-iodine group was selected for quantitation of thyroxine and triiodothyronine.

Among tissues examined (Table III), thyroxine iodine was in highest concentration in liver and kidney, and in lowest concentration in skeletal muscle and brain. Triiodothyronine iodine was most abundant in kidney and small intestine and least abundant in muscle and testis. The relative concentration of triiodothyronine to thyroxine expressed as the T_3: T_4 ratio was the lowest in testis (0.13), fat (0.18), and liver (0.18) and highest in brain (0.82). This ratio in most tissues varied from approximately 0.4 to 0.6.

Thyroxine iodine concentration in plasma (Table III) greatly exceeded that found in tissues, being twice that of liver and eighteen times that of skeletal muscle. Triiodothyronine iodine concentration, on the other hand, was less in plasma than in any tissue with the possible exception of skeletal muscle and testis. The total iodine in plasma according to this method was 33.1 mμg per ml. The protein-bound iodine determined by a standard method [7] was 31.0 mμg per ml (3.1 μg per 100 ml).

The pattern of excretion of labeled substances reflected the dietary intake of I^{125}. Fifteen times as much radioactivity, almost exclusively iodide, appeared in the urine of the high-iodine group as in that of the low-iodine group. Correspondingly, six times as much radioactivity appeared in the feces. According to the data obtained from the low-iodine group, fecal radioactivity was distributed between thyroxine (37%), triiodothyronine (20%), iodide (30%), origin material (5%), and remainder, including triiodo- and tetraiodothyroacetic acid (8%).

DISCUSSION

For the measurement of iodine in tissues, Van Middlesworth reported a method involving feeding I^{131}-labeled diet until equilibrium was established between the SA of the diet and the SA of all the iodine pools of the animals. At equilibrium, the I^{131} in any pool or in any compound was assumed to be proportional to its total iodine content. This procedure was applied by Van Middlesworth (7) and by Pitt-Rivers and Rall (8) to the measurement of various iodine pools. The use of I^{131} as a label for this purpose is limited by its relatively short half life (8 days). Van Middlesworth, noting that it required several half

[7] Bioscience Laboratories, Los Angeles, Cal.

lives to reach equilibrium, proposed a correction for this limitation by labeling the diet each day with increasing amounts of I^{131} in increments calculated to correct for physical decay (7). Although this may accomplish an approximate correction for the total body iodine, one may question whether it would establish uniform labeling of the various organic iodine-containing compounds, each with different and long biologic half lives. This disadvantage is, to some extent, overcome by the use of I^{125}.

Radioiodine-125 was introduced as a tracer isotope by Myers and Vanderleeden (9). It decays by orbital electron capture, emitting for the most part soft (27 Kev) X rays, with a convenient half life of 60 days. Since I^{125} does not emit a charged particle, as does I^{131}, radiation exposure is less per millicurie. Detection is possible either by use of scintillation detectors or by gas-flow counters. With the latter, counting efficiency is greatly increased by using argon-isobutane instead of the more standard helium mixtures.

Our method depends upon the establishment of equilibrium between the SA of the diet and all of the iodine pools in the body. We recognize that the tracer will be continuously "diluted" by the stable iodine present in the body at the beginning of the feeding; therefore, the SA in the body will approach the SA of the diet asymptotically, and true equilibrium will not be achieved. Absolute equilibrium is not essential, however, for useful estimations. Any value in excess of 90% equilibrium for all iodine pools should be adequate for our purpose.

Van Middlesworth and Intoccia (10) concluded that equilibrium was reached between 5 and 50 days, depending on the iodine content of the diet. Pitt-Rivers and Rall (8) stated that 97% equilibrium was obtained after 11.7 days. Morel and Simon (11) found that "somewhere over 80%" was reached after 21 days. In our study, since very little increase in total radioactivity of any tissues occurred between days 23 and 30, we assumed that equilibrium had been nearly achieved after day 30. Therefore, rats fed the labeled diet for at least 30 days were used for the quantitative part of this study.

Early efforts to measure the iodine content of tissues have been reviewed by Elmer (12). That any of the methods employed were sufficiently sensitive to give reliable results is doubtful. The introduction of the catalytic method by Sandell and Kolthoff (13) in 1937 provided a method sensitive enough to permit accurate assay of serum protein-bound iodine content. This method, adapted by Barker (14), was applied to tissues by Carr and Riggs (15) and by Klitgaard, Dirks, Garlick, and Barker (16), with highly divergent results, possibly owing to variation in the total iodine content of experimental diets. These as-

TABLE IV

Iodine content of tissues as determined by various methods

Author: Exp. animal: Analysis:	Sturm* Man Total iodine	Baumann (19) Bovine Total iodine	Klitgaard (16) Rat PBI†	Carr (15) Rat PBI	McClendon (20) Dog PBI	Present work Rat $T_4 + T_3$
	μg/100 g	*μg/100 g*	*μg/100 g*	*μg/100 g*	*μg/100 g*	*μg/100 g*
Tissue						
Brain	12					0.22
Diaphragm			18.0			
Heart	100		21.5	1.1	6.1	0.51
Intestine	35			1.7		0.88
Kidney	20	0.5–4.0	32.6	2.8	8.0	1.43
Liver	110		25.9	5.8	8.4	1.30
Lung	42			2.8		0.82
Muscle	30		9.5	0.6	5.8	0.17
Ovary		2.0				
Pancreas	44			1.1		
Salivary gland	550					0.48
Spleen	400	0.5–1.0		1.8		0.46
Testis	1.65	0.6		1.0		0.31
Plasma			5.4	1.5	4.8	2.26

* Quoted from Elmer (12).
† PBI = protein-bound iodine.

says for protein-bound iodine, as well as earlier assays for total iodine, are summarized in Table IV. Protein-bound iodine values are higher than those obtained in the present study, in which the iodine content of specific thyronines, thyroxine, and triiodothyronine has been determined. This difference can be accounted for, in part at least, if the origin material noted in the chromatograms is protein bound.

Carr and Riggs (15) found the concentration of protein-bound iodine in liver and kidney to be significantly higher than in plasma and attributed this to thyroxine accumulation. In none of the tissues examined in our study did the concentration of thyroxine exceed that of plasma. Their results may be due to the presence of origin material in tissues and its relative absence in plasma.

Triiodothyronine was consistently present in all tissues, and with the possible exception of skeletal muscle, in higher concentration than in plasma. This suggests either that the triiodothyronine is formed intracellularly, or that there is a trapping mechanism responsible for this concentration gradient. The very high concentration of triiodothyronine in kidney, together with previous evidence that this tissue has the most active thyroxine monodeiodinase activity (17), lends support to the first hypothesis. On the other hand, triiodothyronine injected intravenously does accumulate in certain organs, reaching levels 10 hours after injection that are higher than in plasma (18). Moreover, the organs in which the highest levels were attained were the same as were observed in our study (i.e., kidney, small intestine, and liver). Studies are underway to determine the order of appearance and disappearance of the labeled compounds at the time of starting and stopping the labeled diet, respectively. This may yield information bearing on the question of the origin of triiodothyronine.

SUMMARY

An isotope-equilibrium method using an I^{125}-labeled diet of known total iodine content is described for the quantitative estimation of total iodine and iodine-containing compounds in body tissues, plasma, and excreta of the rat.

Radiochromatograms of plasma, feces, and tissues revealed iodide, thyroxine, and triiodothyronine. The feces, in addition, contained small amounts of triiodo- and tetraiodothyroacetic acid. Urine contained virtually only iodide. Although most of the radioactivity was associated with these known compounds, several unknown peaks of varying mobility were observed.

The calculated concentration of thyroxine iodine in tissues ranged from 1 mμg per g in muscle and brain to 11 mμg per g in liver. Plasma contained 22 mμg per ml. The concentration of triiodothyronine iodine in tissues ranged from 0.4 mμg per g in testis and muscle to 5 mμg per g in kidney. Plasma contained 0.7 mμg per ml. Concentration of total iodine, iodide, and certain unknown compounds varied with total iodine content of the diet.

ACKNOWLEDGMENT

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