Intracellular Distribution of Iodine-Containing Compounds in Rat Liver, Kidney and Heart

RICHARD W. HENINGER,[1] FRANK C. LARSON, AND EDWIN C. ALBRIGHT

Departments of Medicine and Physiology, University of Wisconsin Medical School, Madison, Wisconsin

ABSTRACT. The intracellular distribution and quantitation of endogenously labeled iodine-containing compounds in normal rat liver, kidney and heart was determined under steady state conditions. An isotope equilibrium method based on chronic feeding of ^{125}I-labeled diet of known specific activity was employed. Virtually all of the label appeared as T_4, T_3 and iodide. T_4 was more abundant than T_3 in all fractions of liver, and in all fractions except nuclei of kidney and heart. T_4 tended to concentrate in mitochondria, microsomes and soluble fraction and to be lowest in nuclei in all 3 tissues. This may be due to the available T_4 binding sites in these fractions. T_3 was more uniformly distributed among the 4 fractions. Quantities found per mg nitrogen were in the range of 0.1–0.7 mμg of T_4 and 0.02–0.35 mμg of T_3. Comparison of the subcellular distribution of endogenous hormone and exogenous hormone added *in vitro* to tissue homogenates indicated that the 2 methods of labeling gave different results. It would seem desirable to employ endogenously labeled hormone prepared under steady state conditions in biologic studies of thyroid hormone in cell free systems. (*Endocrinology* **78:** 61, 1966).

AN ISOTOPE equilibrium method, employing ^{125}I, for the quantitation of total iodine, iodide and iodine-containing compounds in tissues, plasma and excreta has been described (1). The technique permitted measurement of the exceedingly small quantities of iodine-containing compounds present in animals under physiological conditions. The present study extends these observations to intracellular iodine distribution in selected tissues.

Materials and Methods

Diets. The rats were fed a low iodine ration[2] containing 0.06 μg iodine[3] per g. To avoid producing iodine deficiency, sodium iodide (^{127}I) was added to raise the iodine content to 0.22 μg/g. Enough ration was obtained from the manufacturer to complete all of the experiments with one lot, avoiding variations in stable iodine content.

^{125}I (half-life 60 days) was obtained carrier-free from a commercial source.[4] Its purity was confirmed by chromatographic and decay analyses. Twenty millicuries of the radioisotope was mixed, as previously described (1), with the quantity of the ration required to feed the rats throughout the experiment.[5] The amount of iodine added to the diet as ^{125}I was negligible. Weighed aliquots were counted to establish the ^{125}I:^{127}I ratio and to check uniformity of mixing. The coefficient of variation of radioactivity in samples of labeled test diet was 6%.

Preparation of Tissue. Male albino rats (Holtzman) with an initial weight of 150 g were used in all experiments. To avoid a change in total iodine intake when feeding of labeled diet began, rats were maintained on the test diet without ^{125}I for 4 weeks. The rats were then fed the labeled diet for 30

Received June 30, 1965.

This study was supported by USPHS Grant AM 06605.

[1] Recipient of USPHS Graduate Training Grant 2A-5240 (C-1). *Present address:* Department of Physiology, University of Wisconsin Medical School, Madison 6, Wisconsin.

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

[3] Assayed by Albert L. Chaney Laboratories, Glendale, California.

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

[5] Although difficult to assess, it is unlikely that radiation damage to the thyroid has affected the distribution data in organs studied. Histologic appearance of thyroid follicles was normal and labeled thyroglobulin was not seen in the plasma.

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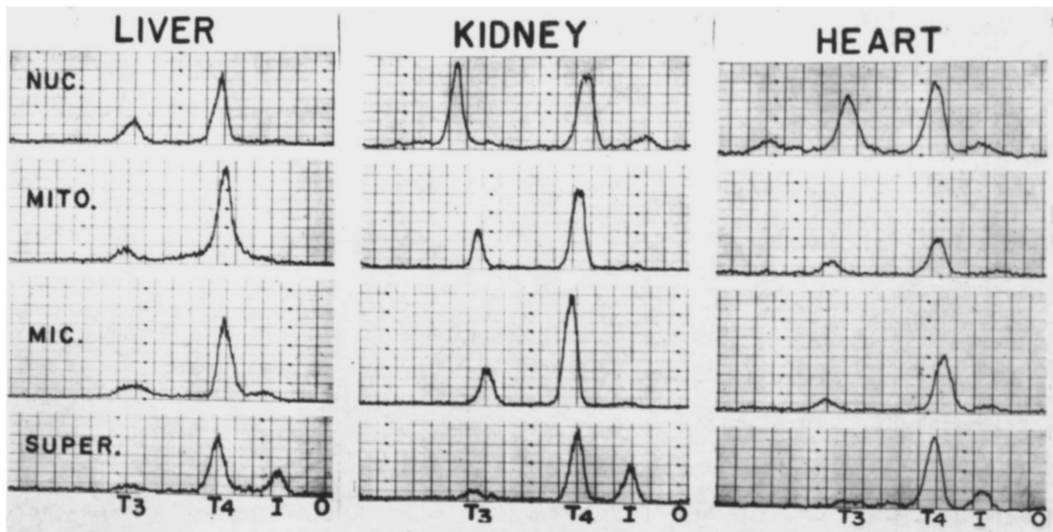

FIG. 1. Radiochromatograms of extracts of nuclei, mitochondria, microsomes and soluble fraction of liver, kidney and heart from rats fed diet labeled with ^{125}I for 30 days. Solvent: tertiary-amyl alcohol saturated with 2N ammonium hydroxide. O, origin; I, iodide; T_4, thyroxine; and T_3, triiodothyronine. Solvent front not shown.

days before sacrifice. This period was sufficient to establish equilibrium between the specific activity of the diet and that of all the iodine pools in the body (1). The rats were killed by exsanguination under ether anesthesia. The liver, kidney and heart were excised, blotted free of blood, rinsed with water and chilled on ice.

Homogenization and Fractionation. The whole organs were homogenized in 0.25M sucrose. Four fractions were prepared by successive centrifugation: 1) a nuclear fraction separated at 700 $\times g$ for 15 min, 2) a mitochondrial fraction separated at 8500 $\times g$ for 15 min, 3) a microsomal fraction separated at 105,000 $\times g$ for 60 min, and 4) a soluble fraction, the supernatant of the last centrifugation. The mitochondrial and microsomal fractions were not washed. The nuclear pellet was washed to remove unbroken cells and debris, by resuspending twice in 0.25M sucrose containing 0.5% Triton X100 and 0.001M magnesium chloride and centrifuging again at 700 $\times g$ for 5 min each time. The efficacy of this procedure was confirmed by microscopic examination of the pellet, which revealed nothing but unbroken nuclei. Further washing was avoided as it was unnecessary and resulted in loss of a significant fraction of radioactivity.

The particulate fractions were suspended in water. One ml aliquot of each fraction was counted in a well-type scintillation counter to determine the total radioactivity. Duplicate 0.1 ml aliquots were analyzed for total nitrogen content by the micro-Kjeldahl method. The remainder of the fraction was extracted for chromatography. An aliquot of the diet was counted at the same time to correct for physical decay and instrument variation.

Extraction and Separation of Products. The fractions were extracted with 10 volumes of *n*-butanol. Approximately 3–5 mg of thiouracil was added as a precaution against instability of iodinated organic compounds. The butanol extracts were concentrated *in vacuo* and subjected to descending chromatography for 48 hr with a *t*-amyl alcohol system (2), which permits excellent separation of thyroxine, triiodothyronine, 3,5-diiodothyronine and iodide, and of T_4 and T_3 from their corresponding acetic and propionic acid analogues.[6] Colorimetrically identifiable quan-

[6] With this solvent system, the following RF values were observed: iodide 0.16, thyroxine 0.21, tetraiodothyroacetic acid 0.27, tetraiodothyropropionic acid 0.29, triiodothyronine 0.40, diiodothyronine 0.46, triiodothyropropionic acid 0.50, and triiodothyroacetic acid 0.56. Chromatography with 3 other solvent systems (butanol-acetic acid-water, collidine-water, butanol-ethanol-ammonia) confirmed the results obtained with *t*-amyl alcohol, which was therefore used routinely.

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TABLE 1. Iodine compounds of subcellular fractions of liver, kidney and heart*

Organ-fraction	Total Iodine	Iodide	Thyroxine	Triiodothyronine	T_3/T_4
Liver					
Nuclei	$0.48 \pm .09$	$0.01 \pm .01$	$0.32 \pm .10$	$0.13 \pm .04$	0.41
Mitochondria	$0.84 \pm .18$	$0.03 \pm .02$	$0.55 \pm .13$	$0.11 \pm .03$	0.20
Microsomes	$0.94 \pm .26$	$0.04 \pm .03$	$0.70 \pm .22$	$0.12 \pm .05$	0.17
Soluble	$0.58 \pm .06$	$0.03 \pm .03$	$0.42 \pm .10$	$0.12 \pm .03$	0.29
Kidney					
Nuclei	$0.30 \pm .07$	$0.01 \pm .01$	$0.11 \pm .05$	$0.13 \pm .04$	1.18
Mitochondria	$0.52 \pm .10$	$0.02 \pm .02$	$0.36 \pm .10$	$0.13 \pm .03$	0.36
Microsomes	$0.54 \pm .11$	$0.02 \pm .02$	$0.46 \pm .18$	$0.11 \pm .06$	0.24
Soluble	$1.20 \pm .15$	$0.03 \pm .03$	$0.75 \pm .19$	$0.35 \pm .11$	0.47
Heart					
Nuclei	$0.05 \pm .01$	$0.01 \pm .02$	$0.02 \pm .01$	$0.02 \pm .01$	1.00
Mitochondria	$0.27 \pm .08$	$0.02 \pm .01$	$0.17 \pm .07$	$0.06 \pm .02$	0.35
Microsomes	$0.45 \pm .14$	$0.04 \pm .04$	$0.24 \pm .08$	$0.08 \pm .03$	0.33
Soluble	$0.45 \pm .09$	$0.04 \pm .02$	$0.28 \pm .08$	$0.06 \pm .03$	0.21

* Expressed as mμg/mg nitrogen, except column T_3/T_4, which is a ratio. Values are the mean ±standard deviation. No. animals = 13.

tities of thyroxine, triiodothyronine and iodide were added to the origin just before chromatography. The position of the thyronine compounds and iodide was determined with 4-amino-antipyrine reagent and palladium chloride reagent, respectively (1). Radioactivity on the strips was localized as described previously (1), except that a 4-fold increase in sensitivity was achieved by substitution of an ultrathin crystal scintillation detector[7] for the gas-flow counter in the scanner assembly. Radioactivity was quantitated by planimetry of the area under the curve inscribed by the automatic recording rate-meter. Typical radiochromatograms are shown in Fig. 1.

Distribution of Total Iodine Following in Vitro *Addition of Labeled Compounds.* Six animals were fed the diet without ^{125}I. The tissues were prepared in the usual manner and homogenized. At this point both ^{131}I-labeled thyroxine[8] and triiodothyronine[8] were added to each homogenate in the quantity known (1) to be present in the liver, kidney and heart.[9] After mixing, the homogenates were fractionated and the fractions were treated as described above under *Homogenization and Fractionation* to determine total radioactivity and nitrogen content. These fractions were not chromatographed, since in this experiment we were interested in comparing distribution with 2 methods of labeling. For this purpose the comparison of total iodine was judged to be sufficient.

[7] Atomic Accessories, Inc., Valley Stream, N. Y.

[8] Obtained from Abbott Laboratories, Oak Ridge, Tennessee.

[9] The quantities of labeled compound added per g of homogenate were as follows: liver, 11 mμg T_4 and 2 mμg T_3; kidney, 9 mμg T_4 and 5 mμg T_3; heart, 3.5 mμg T_4 and 1.5 mμg T_3.

Results

As shown in Fig. 1, essentially all of the label appeared as thyroxine, triiodothyronine and iodide. The trace quantity of unidentified labeled substance seen in heart nuclear fraction is thought to be insignificant as it did not appear routinely.

The quantitative distribution of iodine as thyroxine, triiodothyronine and iodide in the various fractions of liver, kidney and heart, expressed as mμg/mg of nitrogen, is shown in Table 1. Thyroxine was more abundant than triiodothyronine in all fractions of liver and in all fractions except the nuclei of kidney and heart. Thyroxine, moreover, tended to concentrate in mitochondria, microsomes and soluble fraction and to be lowest in the nuclei in all three tissues. Triiodothyronine seemed to differ somewhat as it was more uniformly distributed throughout all four fractions. This dis-

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TABLE 2. Thyroxine and triiodothyronine in subcellular fractions of liver, kidney and heart*

Fraction	Liver		Kidney		Heart	
	T_4	T_3	T_4	T_3	T_4	T_3
Nuclei	11.8	4.6	3.2	4.0	4.0	3.9
Mitochondria	15.2	2.6	11.5	4.2	8.7	2.9
Microsomes	18.8	3.4	8.3	1.8	12.6	4.3
Soluble	23.3	6.7	42.0	19.7	34.9	6.9

* Expressed as a percentage of the sum of the total iodine in the 4 tissue fractions, present as thyroxine and triiodothyronine in each of the fractions. Total iodine in each fraction was calculated as follows: mμg total iodine per mg nitrogen × total nitrogen in fraction.

parity of thyroxine and triiodothyronine with respect to the nucleus is emphasized by comparing the ratio of triiodothyronine to thyroxine concentrations in the various fractions. It will be seen that this ratio was highest in the nuclear fraction for each tissue examined. The soluble fraction of kidney showed the highest concentration of both thyroxine and triiodothyronine among the various fractions of the tissues studied. Iodide was uniformly distributed among the fractions. Comparison between the three tissues with respect to total iodine content (Table 1) revealed that, for any given fraction, heart contained the smallest quantity and liver the largest. Kidney contained somewhat less than liver.

The distribution of thyroxine and triiodothyronine in the subcellular fractions expressed as a percentage of the sum of the total iodine in the four tissue fractions is shown in Table 2. The concentrations of thyroxine and triiodothyronine used in the calculations are those given in Table 1. On a percentage basis, there was more thyroxine in the soluble fraction than in any particulate fraction regardless of the tissue examined. Among the particulate fractions the nuclei contained the least thyroxine in all three tissues. Triiodothyronine appeared to be fairly uniformly distributed among the fractions, with the single exception of a greater amount in soluble fraction of kidney.

To investigate possible differences in distribution of endogenous and exogenous hormone among subcellular fractions, a comparison was made with the distribution of total iodine following addition of labeled thyroxine and triiodothyronine to homogenized tissues. Table 3 shows the results of this study. The data are expressed as a percentage of the sum of total iodine of the four fractions. In each tissue the nuclear fraction contained a significantly higher percentage of iodine after endogenous labeling than after *in vitro* addition of labeled hormones. The same was true with mitochondria of liver. The difference with kidney mitochondria was small and not statistically significant. Microsomes and soluble fraction of liver and soluble fraction of kidney contained a significantly lower percentage of iodine with endogenous labeling than with *in vitro* addition. The difference with kidney microsomes was not significant. Heart mitochondria were significantly lower and soluble significantly higher with endogenous labeling. No significant difference was seen with heart microsomes.

Discussion

The isotope equilibrium method for the determination of iodine-containing

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TABLE 3. Distribution of total iodine in tissue fractions following endogenous labeling (E) or addition *in vitro* (V)*

Fraction		Liver %	p value	Kidney %	p value	Heart %	p value
Nuclei	E	18.0 ±8.0		8.7 ±3.3		10.0 ±1.7	
	V	7.6 ±4.8	<.01	1.7 ±0.9	<.001	5.3 ±1.2	<.001
Mitochondria	E	22.8 ±4.9		16.8 ±2.7		14.4 ±3.5	
	V	11.2 ±4.6	<.001	14.7 ±1.9	NS	28.7 ±1.7	<.001
Microsomes	E	24.9 ±3.7		10.9 ±1.6		22.4 ±6.3	
	V	36.0 ±7.9	<.001	12.7 ±2.5	NS	19.7 ±5.0	NS
Soluble	E	34.2 ±3.7		63.6 ±2.1		53.1 ±6.6	
	V	44.1 ±4.6	<.001	70.8 ±2.6	<.001	46.3 ±6.2	<.001

* Expressed as a percentage of the sum of total iodine of the 4 fractions. Total iodine in each fraction was calculated as follows: mμg total iodine per mg nitrogen × total nitrogen in fraction. Values for endogenous labeling were calculated from data in Table 1. All values are the means, with standard deviation. No. of animals: E = 13, V = 6.

compounds in tissues (3) is based on the feeding of ^{125}I-labeled diet of known total iodine content until equilibrium is established between the specific activity of the diet and all of the iodine pools in the animal. At equilibrium, the quantity of the labeled compound in the tissue may be calculated from its radioactivity and the specific activity of the diet. This analytic procedure offers several advantages: 1) A very high level of sensitivity, exceeding that obtainable by chemical analysis. Because of this, it is now feasible to examine subcellular compartments for their specific iodine content. 2) Utilization of endogenously labeled hormone. Exogenous thyroxine or triiodothyronine may not be distributed or metabolized in exactly the same way as endogenous hormone released from the thyroid. 3) Quantitation of labeled compounds under steady state conditions. Although pulse-type experiments yield information of value, they do not resemble physiologic events. 4) Uniform labeling in 3′,5′, 3,5 positions, which permits detection of reaction products bearing iodine only in the A ring.

It is difficult to compare results of this study with previous reports because of major differences in experimental methods. Carr and Riggs (4) determined protein-bound iodine in rat liver nuclei, mitochondria and supernatant (consisting of cytoplasm and extracellular fluid). Microsomal fraction was not examined, nor was dietary iodine intake specified. They reported that protein-bound iodine occurred in approximately equal concentrations in the various fractions. Tabachnick, Bonnycastle and Salter (5) measured radioactivity of rat liver cell fractions at 24, 48 and 72 hours after administration of ^{131}I to intact animals and labeled thyroxine to thyroidectomized animals. They found no specific localization of radioactivity and very little difference between results obtained in the two studies. Lee and Williams (6) studied distribution of radioactivity in rat liver fractions at 5, 15, 30 and 60 minutes after intravenous injection of labeled thyroxine. Their findings indicated no specific localization of radioactivity within particular fractions. Tata (7) found in acute experiments that labeled hormone was not concentrated exclusively in any of the subcellular fractions of rat liver and skeletal muscle although the largest part was recovered

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from the soluble fraction. Our data indicate that under steady state conditions there is no exclusive localization of thyroid hormone to a particular subcellular fraction. To this extent the present findings are comparable to the reports cited above.

The qualitative distribution of the radioactive label indicates that hormonal iodine is present in subcellular fractions only as thyroxine and triiodothyronine. The use of multiple solvent systems failed to reveal the presence of other iodinated compounds. An exceedingly small proportion of the label was present as iodine.

Quantitatively, the generally greater abundance of thyroxine compared with triiodothyronine is in keeping with results on whole tissues reported earlier (1). The tendency of thyroxine to concentrate in mitochondria, microsomes and soluble fraction is probably related to the number of available thyroxine binding sites in these fractions, as suggested by Tata (7) for liver and skeletal muscle. Whether this localization has relevance to mitochondrial oxidative metabolism or microsomal protein synthesis must remain conjectural. The relatively more uniform distribution of triiodothyronine among the four fractions may reflect weaker binding affinity for this compound.

It is conceivable that the fractionation procedure itself may affect iodine distribution. If the results following endogenous labeling were due to artifactual redistribution at the time of fractionation, one might expect there to be less difference between the distributions following endogenous and exogenous labeling. Because significant differences were observed, it is believed unlikely that the endogenous distribution data are due to artifact.

Comparison of the subcellular distribution of endogenous hormone under steady state conditions and exogenous hormone added *in vitro* indicates that the two methods of labeling give different results. With liver and kidney, more of the exogenous hormone is found in the soluble and microsomal fractions and less in the mitochondria and nuclei compared to endogenously labeled hormone. This may be due to competition by unlabeled endogenous hormone for available binding sites on mitochondria and nuclei, or to interference with penetration of exogenous hormone into mitochondria and nuclei by the strong thyroxine binding property of the soluble fraction. In contrast to liver and kidney, heart mitochondria have a relatively higher affinity for exogenous hormone, suggesting that differences in distribution of endogenous and exogenous hormone are not consistent for all tissues. In view of the demonstrated differences in subcellular distribution of endogenously labeled and exogenous hormone, it would seem preferable to employ the former, prepared under steady state conditions, in biologic studies of thyroid hormone in cell-free systems.

Acknowledgment

The assistance of Miss Sue Ames, medical technologist, is gratefully acknowledged.

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