Thyroid-Hormone Metabolism in the Kidney*

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Present knowledge of the metabolism of the thyroid hormones in the kidney is confined mainly to information obtained from experiments with ^{131}I-labelled thyroxine and tri-iodothyronine. Gross & Leblond (1951*a*) found that after the administration of [^{131}I]thyroxine to rats or mice, the kidney concentrated radioactivity from the blood. Subsequent chromatographic analysis of butanol extracts of kidney revealed the presence of at least four ^{131}I-labelled compounds: thyroxine, tri-iodothyronine, iodide and an unknown compound which was later shown to give rise to thyroxine on alkaline hydrolysis (Leblond & Cambron, 1952). The compound was also detected in bile and was thought to be a conjugate of thyroxine with glucuronic acid. Gross, Ford, Symchowicz & Horton (1957) found that [^{131}I]tri-iodothyronine when administered to rabbits gave rise to a metabolite in the kidney, plasma and liver which yielded tri-iodothyronine on hydrolysis with 0·2N-hydrochloric acid. The liver also contained a second conjugated compound which they identified as tri-iodothyronine glucuronide. Roche, Michel, Jouan & Wolf (1956) have shown that the kidney can deiodinate and deaminate [^{131}I]tri-iodothyronine, forming 3:3′-di-iodothyronine and tri-iodothyroacetic acid [4-(4-hydroxy-3-iodophenoxy)-3:5-di-iodophenylacetic acid].

Results obtained in experiments with synthetic thyroid hormones do not necessarily reflect the normal metabolism of endogenous thyroid hormone. A satisfactory qualitative and quantitative study of thyroid-hormone metabolism in the kidney has not yet been carried out. For this it is essential to label the endogenous thyroid hormones with ^{131}I; further, an analysis of the iodinated compounds present in the kidney must be carried out only after the administration of tracer doses of ^{131}I, in order to avoid radiation damage to the thyroid gland sufficient to cause the release of thyroglobulin into the circulation. The kidney is primarily an excretory organ and would be expected to partake in the removal of any abnormal iodinated compounds present in the circulation. Fletcher (1957) detected a number of compounds in the urine but not in the serum of patients treated with large doses of ^{131}I for thyroid carcinoma, and he suggested that they may be breakdown products of the thyroglobulin which was present in the serum of these patients. The maximum total ^{131}I in the kidneys after tracer doses of ^{131}I is such that it is impossible to detect and identify satisfactorily the iodinated compounds present in a butanol extract of the tissue. However, by absorption on Dowex-1 it is possible to separate and concentrate the iodinated compounds from a sufficient amount of tissue to permit their detection by chromatographic analysis (Galton & Pitt-Rivers, 1959).

The present work describes how this resin was used in a qualitative and quantitative study of thyroid-hormone metabolism in the kidneys of mice given very small doses of ^{131}I.

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METHODS AND MATERIALS

Preparation of tissue for analysis. Six male albino mice (20 g.) fed on diet 41 of Bruce & Parkes (1946) were used in each experiment. Each mouse received ^{131}I (10–20 μc) intraperitoneally and was killed 60–72 hr. later by exsanguination under ether anaesthesia. Urine was taken from the bladders with a syringe. The kidneys were removed, rapidly skinned and weighed, rinsed in ice-cold 0·9% NaCl soln. and homogenized in a pestle and mortar with thiouracil (2 mg.), 0·9% NaCl soln. (6 ml.) and a little acid-washed sand. Homogenization was carried out slowly with the mortar standing in an ice–salt freezing mixture. The homogenate was transferred to centrifuge tubes, a further 14 ml. of 0·9% NaCl soln. was added and the mixture was shaken and centrifuged, the temperature being kept below 5°. The volume of the extract in 0·9% NaCl solution was measured and a portion was estimated for radioactivity in a well-type scintillation counter.

Column analysis. The experiments were carried out at 4°. Dowex-1 was equilibrated with sodium acetate buffer at pH 5·6. Sodium metabisulphite (0·02 mg./ml.) was added to all the eluents to prevent any oxidation of iodide on the column. The kidney extract was passed through the column (3 cm., 1 cm. diameter) of resin four times. The column was then washed with buffer at pH 5·6 until the eluate contained no detectable amount of protein, followed by buffer at pH 3·6, acetic acid solutions at pH 3·0, 2·2 and 1·4, undiluted acetic acid and finally 3N-NaBr. At least 10 ml. of each solution was passed through the column. The eluates were collected into 10 ml. graduated centrifuge tubes and samples were estimated for radioactivity. The resin itself was counted in the scintillation counter in order to be sure that all the radioactivity had been discharged.

Column analysis of the urine and serum was carried out in a similar manner.

Chromatographic analysis. A sample of urine was chromatographically analysed. The eluates containing radioactivity were pooled, evaporated to dryness under reduced pressure and the remaining acetic acid was removed in a stream of air. The residues were dissolved in methanol–aq. NH_3 soln. (sp.gr. 0·88) (3:1) and analysed by paper chromatography as described by Galton & Pitt-Rivers (1959). The solvent systems used were butan-1-ol–dioxan–aq. 2N-NH_3 soln. (4:1:5); butan-1-ol–2N-acetic acid (1:1); *tert.*-amyl alcohol–aq. 2N-NH_3 soln. (1:1). Carriers were used for identification and stained by the method of Gross & Leblond (1951*b*). The chromatograms were scanned with a strip counter or radioautographed on X-ray film.

Identification of the unknown metabolite

Preparation of compound. In order to increase the specific activity of the metabolite the mice used in these experiments were given 200 μc of ^{131}I. The kidneys from six mice were analysed on the column as previously described. The fraction eluted with 50% acetic acid (pH 1·4) was evaporated to dryness as previously described and the residue was dissolved in 0·1 ml. of methanol–aq. NH_3 soln. This solution was chromatographed on paper in butanol–dioxan–aq. NH_3 soln. The area containing the metabolite was located with an end-window Geiger–Müller tube, removed and cut into pieces.

Hydrolysis of metabolite. The paper containing the metabolite was placed in a counting tube and 0·3 ml. of the acid, alkali or buffer solution to be used was added. The paper was removed from the liquid after 1 min., squeezed well with forceps and lifted out of the tube. The ^{131}I in the tube was estimated in the scintillation counter before and after the paper had been removed to check that satisfactory elution had been achieved; it was invariably found that 90–95% of the total ^{131}I on the paper was eluted by this technique. Attempts to hydrolyse the compound were made with 2N-HCl at 100° for 18 hr., 5N-HCl at 100° for 1 hr. (SO_2 was bubbled through the 5N-HCl during hydrolysis to prevent oxidative destruction) and N-NaOH at 100° for 1 hr.

A crude preparation of β-glucuronidase was made by suspending scrapings of the mucosa of the large intestine of a rat in a few drops of 0·9% NaCl soln. Incubation of the metabolite was carried out in 0·1M-sodium phosphate buffer at pH 6·0, with 1 drop of this suspension, at 37° for 1, 4 or 18 hr.

Three arylsulphatases, free from β-glucuronidase, were incubated with the metabolite at 37° for 4 or 24 hr. These were a bacterial sulphatase from *Alcaligenes metalcaligenes*, in 0·1M-2-amino-2-hydroxymethylpropane-1:3-diol–HCl buffer at pH 9·0, a limpet sulphatase in 0·2M sodium acetate buffer at pH 5·5 and a rat-liver sulphatase in 0·1M-sodium phosphate buffer at pH 8·0. The ^{131}I-labelled compounds present in the incubation media or in the acid and alkali solutions were determined by chromatographic analysis.

Experiments with ^{35}S-labelled sulphate

The metabolite was prepared from the kidneys of six mice injected with ^{35}S-labelled sulphate in addition to 200 μc of ^{131}I; carrier-free $Na^{35}SO_4$ (250 μc in 0·5 ml. of 0·9% NaCl soln.) was injected twice subcutaneously into each mouse, 24 and 16 hr. before they were killed. The metabolite, purified by chromatography, was re-chromatographed in butanol–dioxan–aq. NH_3 soln. and the chromatogram was counted twice with a strip counter; the first time the end window was uncovered and the second time it was shielded with aluminium foil (11·4 mg./cm.^{2}). This shield will absorb all the ^{35}S radiation but allows about 60% of the ^{131}I radiation to pass through (J. R. Tata, personal communication). For comparative purposes this technique was carried out on a chromatogram containing only a single radioactive spot of ^{131}I. The proportion of radioactivity absorbed by the aluminium was determined by measuring the area under the peaks on the two records obtained from each chromatogram with a planimeter. The metabolite in this experiment was hydrolysed with the intestinal β-glucuronidase and the products of hydrolysis were analysed by chromatography to determine whether ^{35}S-labelled sulphate was released.

RESULTS

The 0·9% NaCl soln. extract (19·4 ml.) from six kidneys (2·77 g.) contained at least 98% of the total ^{131}I. It was evident from the column analysis of the kidney extract (Table 1) that thyroglobulin, mono- or di-iodotyrosine, which would have been discharged from the resin by acetate buffer at pH 3·6 and acetic acid solutions at pH 3·0 and 2·2, were not present. Two iodinated compounds, one of which was identified as thyroxine, were detected by chromatographic analysis of the fraction eluted with 50% acetic acid (pH 1·4) (Figs. 1 and 2). Most of the ^{131}I in this fraction (80–95%) was in the form of a metabolite which could not be identified with any synthetic thyroxine analogue available. On some of the radioautographs a trace of radioactivity corresponding to tri-iodothyronine was detected. The amount of organically bound ^{131}I present varied between 26 and 70% of the total ^{131}I in the kidney solution. The highest value was always obtained in the mice killed between 60 and 72 hr. after the injection of ^{131}I.

The efficiency of the resin in absorbing the radioactivity from the saline solution was considerably less than that observed when only thyroxine and iodide were present (Galton & Pitt-Rivers, 1959). In order to determine the nature of the unabsorbed ^{131}I-labelled compounds, six mice were each given 200 μc of ^{131}I. They were killed 72 hr. later and the kidneys were analysed on the column. The protein solution was passed through the column four times and the radioactivity which remained in the solution was investigated by butanol extraction followed by chromatographic analysis. It was found that practically all the ^{131}I was in the form of the unidentified metabolite described above (Fig. 2); only a trace amount of radioactivity corresponding to thyroxine was detected and this was of doubtful significance. In this experiment

Table 1. *Column analysis of extract of kidney in sodium chloride solution*

Fraction	pH or solvent	Volume	Total counts/min.	Percentage of total ^{131}I
Kidney extract	7·4	19·4	28 984	100
1*	5·6	10·2	1 999	15·5
2	5·6	8·3	2 424	
3	5·6	10·3	—	—
4	3·6	10·0	—	—
5	3·0	10·0	—	—
6	2·2	10·0	—	—
7	2·2	6·0	—	—
8	1·4	10·3	8 240	54
9	1·4	12·0	5 796	
10	1·4	7·4	1 325	
11	1·4	7·4	296	
12	3N-NaBr	30·0	8 820	30·5
Total eluted material			28 900	

* Fraction containing most of the original protein, etc.

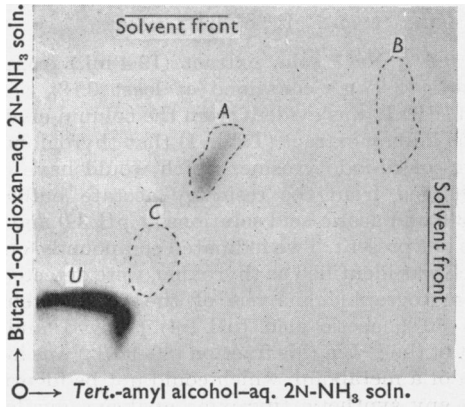

Fig. 1. Radioautograph of two-dimensional chromatogram prepared from kidney fraction eluted at pH 1·4. Solvent systems: butan-1-ol–dioxan–aq. 2N-NH_3 soln. (4:1:5) and *tert.*-amyl alcohol–aq. 2N-NH_3 soln. (1:1). *A*, Thyroxine; *B*, tri-iodothyronine; *C*, iodide; *U*, unknown metabolite.

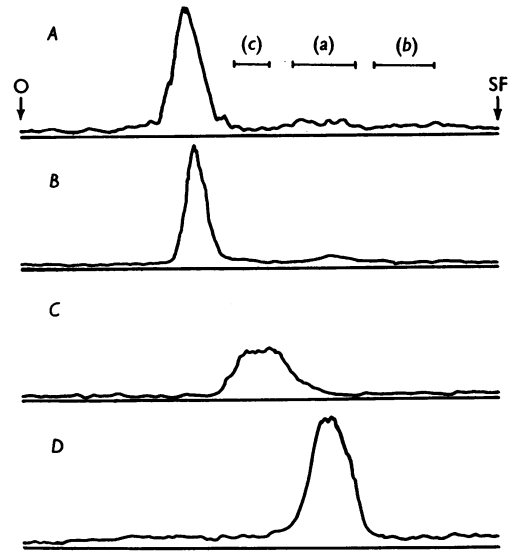

Fig. 2. Scans of radioactive chromatograms prepared from *A*, butan-1-ol extract of kidney solution from column; *B*, kidney fraction eluted from column at pH 1·4; *C*, urine; *D*, serum fraction eluted at pH 1·4. Solvent system: butan-1-ol–dioxan–aq. 2N-NH_3 soln. (*a*) Thyroxine; (*b*) tri-iodothyronine; (*c*) iodide. O, Origin; SF, solvent front. Maximum scale represents about 0·005 μc of ^{131}I.

the concentration of radioactivity in the fraction eluted with NaBr was high enough to permit chromatographic analysis; the ^{131}I was entirely in the form of inorganic iodide.

Column analysis of serum was also carried out (Table 2). No radioactivity was detected in the fraction eluted with buffer at pH 3·6. This indicates that thyroglobulin was not present in the serum. Chromatographic analysis of the radioactive material eluted with 50 % acetic acid revealed that only thyroxine was present; there was no evidence that the unidentified metabolite present in kidney occurred in serum (Fig. 2).

All detectable radioactive material in the urine was absorbed by the resin and could be eluted only with NaBr. Chromatographic analysis of a sample of urine confirmed that all the radioactivity was in the form of inorganic iodide; clearly the metabolite detected in the kidney was not secreted into the urine.

The ratio organic ^{131}I/inorganic ^{131}I was 2·3 in the kidney and 1·8 in the serum. These values were calculated from column analyses of serum and tissues from the same group of mice (Tables 1 and 2). In another experiment the total ^{131}I concentration in the kidneys and serum was about equal. The radioactivity in the urine was about 100 times

Table 2. *Column analysis of serum*

Fraction	pH or solvent	Volume	Total counts/min.	Percentage of total ^{131}I
Serum	7·4	2·47	23 275	100
1*	5·6	10·0	1 190	5
2	3·6	10·0	—	—
3	3·0	10·0	—	—
4	2·2	10·0	—	—
5	1·4	9·6	8 275	
6	1·4	7·7	3 873	59
7	1·4	7·3	1 533	
8	3N-NaBr	30·0	8 310	36
	Total eluted material		23 181	

* Fraction containing most of the original protein, etc.

as high as in either serum or kidney and consisted entirely of iodide; since small amounts of urine are always present in kidney extracts this would give a falsely high value for kidney inorganic ^{131}I. However, in spite of this higher proportion of organic iodine, it was evident that the concentration of free thyroxine in the kidney was small compared with that in the serum.

Identification of unknown metabolite

2N-Hydrochloric acid had little or no hydrolytic effect on the metabolite even at 100° for 18 hr. However, with 5N-HCl the metabolite was hydrolysed completely in 1 hr. When the hydrolysate was analysed by chromatography, only thyroxine was detected. The metabolite was also hydrolysed with N-NaOH but some deiodination of the liberated thyroxine also occurred. Hydrolysis of the metabolite was achieved within 1 hr. by the crude intestinal-enzyme preparation, but the three sulphatases had no effect on the compound; no thyroxine was detected in the media even after incubation for 24 hr.

From the control chromatogram it was estimated that the aluminium shield covering the end window of the strip counter absorbed 40 % of the ^{131}I radiation. When a chromatogram containing the metabolite prepared from mice which had received ^{131}I and $^{35}SO_4^{2-}$ was scanned with and without this filter, only 40 % of the total radiation was absorbed, indicating that the metabolite contained no significant amount of sulphur. This conclusion was confirmed in the hydrolysis experiment. When the hydrolysate was chromatographically analysed, only one radioactive spot which corresponded to carrier thyroxine was detected; there was no evidence of any free radioactive sulphate.

DISCUSSION

The experiments described above indicate that at least 98 % of the total iodine present in the kidney is distributed among three substances: iodide, thyroxine and a third compound not previously identified in this organ. Tri-iodothyronine was occasionally found, but only in very small amounts. Although 60–70 % of the total iodine in the kidney is organically bound only 5–10 % of this is in the form of free thyroxine; at least half of the kidney iodine is in the form of the unknown metabolite. These estimations are subject to error owing to the presence of urine in the kidney tubules; it is possible that the ratio of organic to inorganic iodine in the kidney cells is higher than was estimated here.

It was concluded that these results reflect the normal metabolism of iodine in the kidney because, first, the dose of ^{131}I used was small and radiation damage to the thyroid was not likely. The results obtained from the analysis of serum supported this view; no thyroglobulin was detected. Secondly, it was observed that the ratio of organic to inorganic ^{131}I in the kidney did not reach a maximum until between 50 and 65 hr. after administration of ^{131}I; after this interval the ratio remained approximately constant. This was taken as an indication that equilibration between the ^{131}I and ^{127}I in the kidney had been reached. Thirdly, the technique permits a quantitative analysis of at least 98 % of the total ^{131}I present in the kidney; in the kidney tissue the amount of radioactive material which was insoluble in sodium chloride solution was small and the resin showed no detectable radioactivity after the iodide had been eluted with sodium bromide solution.

The possibility that the metabolite was an artifact produced during the analytical procedure was eliminated as it was not found in serum or urine which were subjected to the same procedure. Further, the metabolite was detected in the kidneys of mice treated with larger doses of ^{131}I which were not analysed on the column but were extracted with butanol.

On hydrolysis the metabolite yielded thyroxine. It was rapidly hydrolysed when incubated with scrapings of the mucosa of rat large intestine which contains both β-glucuronidase and sulphatases

among other enzymes. It was not possible to obtain β-glucuronidase in a pure state free from sulphatase activity, but the sulphatases used were freshly prepared and stated to be free from β-glucuronidase (K. S. Dodgson, personal communication). None of the three sulphatases was capable of hydrolysing the metabolite. These enzymes are known to hydrolyse tyrosine-*O*-sulphate and some of its derivatives but since there was no direct evidence that thyroxine-*O*-sulphate would be hydrolysed by the sulphatases, these experiments do not provide unequivocal proof that the metabolite is not the ethereal sulphate of thyroxine. However, the experiments carried out on the metabolite prepared from mice treated with both $^{35}SO_4^{2-}$ and ^{131}I substantiated the view that the metabolite did not contain sulphur.

It is now generally accepted that the thyroid hormones undergo conjugation with glucuronic acid in the liver and are excreted as such a conjugate in the bile. It is not unreasonable to suppose that the kidney contains a similar mechanism for conjugating thyroid hormones. It was therefore concluded from the results obtained in the present work that the kidney metabolite is a conjugate of thyroxine with glucuronic acid. This compound is probably identical with that detected by Gross & Leblond (1951*b*) in the kidneys of mice treated with [^{131}I]thyroxine; their metabolite was stable to N-hydrochloric acid but yielded thyroxine on alkaline hydrolysis. The absence of the metabolite from the serum and urine indicates that both its formation and metabolic breakdown must take place in the kidney tissue itself.

The concentration of organic iodine though not that of unconjugated thyroxine is approximately the same in the kidney and serum. This contrasts with the finding of Tata (1958) that muscle and brain contain comparatively low concentrations of organic iodine. Further, there is no evidence that conjugation of the thyroid hormones takes place in these tissues. Tata (1958) has suggested that the transport of thyroxine from the serum into the cells is dependent on the different thyroxine-binding properties of serum, extracellular and intracellular proteins. This results in a high serum/tissue thyroxine concentration ratio. In the kidney, however, the glucuronide-forming mechanism may compete with the proteins for the intracellular thyroxine with the result that some thyroxine is dissociated from the proteins and conjugated, thus permitting more thyroxine to pass into the cell than would otherwise be possible. There is no reason to suppose that the kidney is dependent for normal function on a concentration of thyroxine many times that in muscle and brain. It is more likely that this conjugating system is part of a mechanism whereby the kidney assists in the regulation of blood thyroxine concentration; the glucuronide undergoes metabolic breakdown without having exerted any biological effect, and the physiological action of the thyroid hormone is brought about by the trace amounts of free thyroxine in the kidney. The way in which thyroxine glucuronide is metabolized is unknown; the assumption that it is physiologically inert, which forms the basis of these suggestions, has yet to be justified.

SUMMARY

1. The anion-exchange resin Dowex-1 was used to separate the iodinated compounds from the kidneys of mice treated with small doses of ^{131}I.

2. 60–70 % of the ^{131}I in the kidney after 70 hr. was organically bound.

3. Chromatographic analyses of the organic iodine compounds after elution indicated that only small amounts of thyroxine were present.

4. Most of the organic ^{131}I occurred in a metabolite of thyroxine which was shown to be a conjugate. This metabolite was not present in the serum or urine.

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