The Identification of the Acetic Acid Analogues of Thyroxine and Tri-iodothyronine in Mammalian Tissues*

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The acetic acid analogues of thyroxine and tri-iodothyronine, namely tetraiodothyroacetic acid [4-(4'-hydroxy-3':5'-di-iodophenoxy)-3:5-di-iodophenylacetic acid] and tri-iodothyroacetic acid [4-(4'-hydroxy-3'-iodophenoxy)-3:5-di-iodophenylacetic acid] have been synthesized (Harington & Pitt-Rivers, 1952; Pitt-Rivers, 1953) and shown to possess considerable biological activity when assayed by the goitre-prevention test in rats (Pitt-Rivers, 1953). Pitt-Rivers suggested that tetra- and tri-iodothyroacetic acids might arise during the peripheral metabolism of the thyroid hormones by oxidative deamination and decarboxylation of the alanine side chain of the parent amino acid. In support of this hypothesis Roche, Michel & Tata (1954) found that rats injected with large doses of tetra- or tri-iodothyroacetic acid excrete the corresponding pyruvic acid analogue in their urine and bile. Traces of tri-iodothyroacetic acid have been detected in the kidney and muscle of rats given small doses tri-iodothyronine (Roche, Michel, Jouan & Wolf, 1956), and tetraiodothyroacetic acid was found in the serum of dogs injected with [^{131}I]-thyroxine (Flock, Bollman, Grindlay & McKenzie, 1957). Tomita, Lardy, Larson & Albright (1957) detected tetra- or tri-iodothyroacetic acid in rat-kidney homogenates or cell-free extracts after incubation with thyroxine or tri-iodothyronine. Tata has obtained evidence that brain and muscle slices and homogenates can convert thyroxine and tri-iodothyronine into their respective acetic acid analogues (Tata, 1957; Tata, Rall & Rawson, 1957). It therefore appears that certain tissues can convert thyroxine and tri-iodothyronine into the corresponding acetic acid analogues, but it does not necessarily follow that this is a normal metabolic process. The experiments described below were carried out to determine whether the thyroacetic acids are formed from endogenous thyroid hormones after the administration of radioactive iodide.

METHOD AND MATERIALS

Three male albino mice (20 g.) were used for each experiment; the animals were fed for 3 weeks before the experiment on an iodine-poor diet consisting of wholemeal flour (88% extracted) 1000 parts, gluten 660 parts, I.C.F. 200 parts and yeast 140 parts (I.C.F., $CaHPO_4$ 40 parts, $FeSO_4,7H_2O$ 2 parts and flour 200 parts). Deionized water was administered *ad lib.* Animals were given a single intraperitoneal injection of ^{131}I (1 mc) and groups were killed by exsanguination under ether anaesthesia 12–48 hr. later.

Preparation of tissue extracts. The liver and kidneys were removed, freed from surrounding tissues, rinsed rapidly in ice-cold 0·9% NaCl soln. and frozen in a beaker in dry ice. After partial thawing, tissues were cut into small pieces, homogenized in metal-free glass and Teflon homogenizers immersed in a freezing mixture and transferred to ice-cold centrifuge tubes with a little 0·9% NaCl soln. The pH of the homogenates was reduced to 2 with 5N-HCl and they were extracted five times with equal volumes of butan-1-ol. The pooled extracts were evaporated to dryness under reduced pressure below 40°; the residues were transferred to centrifuge tubes with four 0·5 ml. portions of methanol–aq. NH_3 soln. (sp.gr. 0·88) (3:1). After centrifuging, the supernatant liquids, which contained nearly all of the radioactivity, were concentrated in a stream of air and recentrifuged. The final supernatant solution was used for chromatographic analysis.

Chromatographic analysis. Ascending chromatography on Whatman no. 1 paper was used throughout. Solvent systems were butan-1-ol–dioxan–aq. 2N-NH_3 soln. (4:1:5) and *tert.*-amyl alcohol–aq. 2N-NH_3 soln. (1:1). The dioxan was purified by Vogel's (1946) method. Thiouracil (0·01 mg./ml.) was added to the solvent systems to prevent any oxidation of iodide during chromatography. The butanol extracts contained, in addition to the iodinated compounds, fat and lipid materials from the homogenized tissues, which made chromatographic analysis difficult. The following technique was therefore adopted. Preliminary one-dimensional chromatograms of the tissue extracts were developed in both butanol–dioxan–aq. NH_3 soln. and *tert.*-amyl alcohol–aq. NH_3 soln. Separate spots, 0·5 cm. in diameter, were applied across the paper at the origin. Carrier tetra- and tri-iodothyroacetic acid were developed with a sample of the extract at one side of the paper. The spots were separated to allow the solvent to pass more evenly up the paper. After development the carriers were stained by the method of Gross & Leblond (1951) and the ^{131}I-labelled compounds on the corresponding carrier-free parts of the chromatograms were eluted into methanol–aq. NH_3 soln. (sp.gr. 0·88) (3:1). The eluates were concentrated under reduced pressure below 40° and analysed by two-dimensional chromatography. Since the fatty material had a small R_F value in the two solvent systems used it was not present in these final eluates. The radioactive spots on the chromatograms were located by radioautography on Kodak No-screen X-ray film.

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Carriers. Thyroxine, tri-iodothyronine, tetra- and tri-iodothyroacetic acid, the corresponding iodothyropropionic acids and iodide were developed with the eluates on many chromatograms for identification purposes.

Control experiments. In order to check that no artifacts were produced from thyroid hormones present in the tissues, during extraction and analysis, experiments were carried out in which thyroxine or tri-iodothyronine biosynthetically labelled with ^{131}I was added to normal liver and kidney before homogenization. The tissues were subsequently extracted and analysed as described above.

RESULTS

Liver. Two-dimensional chromatographic analysis of the radioactive eluates obtained from the preliminary chromatograms, followed by radioautography, revealed that a number of iodinated compounds were present (Fig. 1), some of which were identified with one or other of the carriers; tri-iodothyronine, iodide and thyroxine were always present (spots *A*, *B*, *D*), and a compound (spot *C*) which had the same R_F values as tri-iodothyroacetic acid in the two solvent systems was observed on chromatograms of extracts prepared from mice killed later than 18 hr. after ^{131}I administration. The maximum total liver radioactivity was reached between 18 and 24 hr.; compound *C* was not detected at 12 hr. when relatively little organic ^{131}I was present in the liver. Compound *C* was detected on chromatograms developed with and without carrier. It represented only 0·1 % of the total liver radioactivity; this value was obtained from preliminary chromatograms by locating the relevant area with carrier tri-iodothyroacetic acid; the value was only approximate since no correction could be made for any decomposition of the compound which might have occurred during extraction, or for additional radioactivity from other compounds incompletely separated from it during chromatography. No tetraiodothyroacetic acid was detected in the livers of any mice even after exposure of the chromatograms for 3 weeks.

Kidney. Tri-iodothyroacetic acid was never found in the kidney; as the total radioactivity was considerably less than that in liver from the same animal, the technique may not have been sensitive enough to detect traces of this compound. However, analysis of the ^{131}I eluted from the tetra-iodothyroacetic acid area of the preliminary chromatograms revealed that this acid and tri-iodothyronine, only partially separated, were present (Fig. 2).

Serum. Serum from each group of mice was analysed chromatographically to determine whether any thyroglobulin was present in the blood, thereby indicating radiation damage to the thyroid. Thyroglobulin was only detected 22 hr. after injection of ^{131}I. The amount then increased rapidly and at 48 hr. after ^{131}I administration represented at least 60 % of total serum ^{131}I.

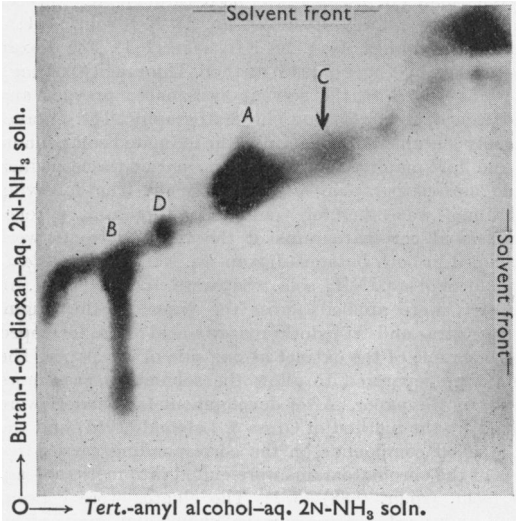

Fig. 1. Radioautograph of chromatogram of liver extract containing tri-iodothyroacetic acid. Solvent systems: butan-1-ol–dioxan–aq. 2N-NH_3 soln. and *tert.*-amyl alcohol–aq. 2N-NH_3 soln. *A*, Tri-iodothyronine; *B*, iodide; *C*, tri-iodothyroacetic acid; *D*, thyroxine.

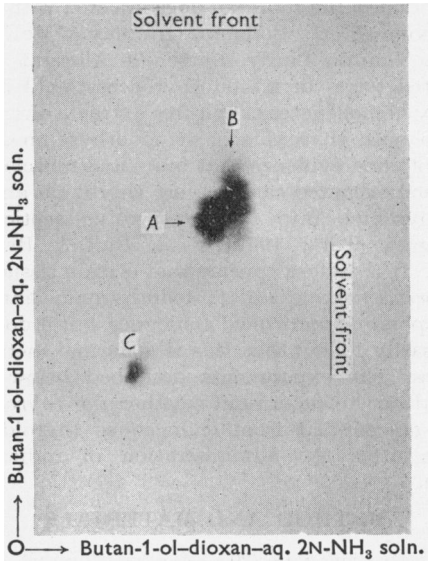

Fig. 2. Radioautograph of chromatogram of kidney extract containing tetraiodothyroacetic acid. Solvent system: butan-1-ol–dioxan–aq. 2N-NH_3 soln. *A*, Tetraiodothyroacetic acid; *B*, tri-iodothyronine; *C*, iodide.

Control experiments. No tetra- or tri-iodothyroacetic acid was detected in the kidney or liver to which ^{131}I-labelled thyroxine or tri-iodothyronine had been added before extraction.

DISCUSSION

Certain derivatives of thyroxine and tri-iodothyronine have been found to be inseparable by any analytical procedure so far examined. These include the corresponding thyroacetic acid and thyropropionic acid analogues. It may therefore be premature to identify tri-iodothyroacetic acid as a thyroid-hormone metabolite in biological material. However, certain facts support the hypothesis that the unknown metabolite found in the liver of mice after injection of ^{131}I is indeed this acid. When extracts were developed in the presence of carrier tri-iodothyroacetic acid a more diffuse spot of the ^{131}I-labelled compound was formed than that observed on carrier-free chromatograms, and the shape of the radioactive spot revealed by radioautography and that of carrier visualized by staining were very similar. These phenomena were not seen when carrier tri-iodothyropropionic acid was employed; it was sometimes found that although the radioactive spot and carrier spot overlapped, they could not be superimposed. Further, tri-iodothyroacetic acid might be produced from tri-iodothyronine in the tissues via the well-known biochemical pathway of oxidative deamination, whereas the propionic acid analogue cannot; reductive deamination of amino acids is not known to take place in biological tissues.

Since tetra- and tri-iodothyroacetic acid were not found to be artifacts formed during extraction or chromatography it was concluded that they represent metabolites of the thyroid hormones. Although the dose of ^{131}I used in this work was large and traumatic changes in the thyroid gland inevitably occurred, the thyroacetic acids were detected in the tissues before any thyroglobulin appeared in the blood.

Certain observations of Thibault and her colleagues support the suggestion that tetra- and tri-iodothyroacetic acid may be active thyroid-hormone metabolites. Gross, Pitt-Rivers & Thibault (1953) found that tri-iodothyronine, like thyroxine, when injected into rats stimulates the oxygen consumption only after a latent period. Thibault (Thibault & Pitt-Rivers, 1955; Thibault, 1956) observed that the corresponding thyroacetic acids produced an immediate increase in oxygen consumption both *in vivo* and in rat kidney slices *in vitro*. The fact that these derivatives have now been identified in certain tissues might lend support to this view. However, the immediate effects of tetra- and tri-iodothyroacetic acid have not been confirmed (Barker & Lewis, 1956; Dickens & Salmony, 1956; Wiswell & Asper, 1958), and it therefore cannot be said at present that the hypothesis has been justified by experimental evidence.

Although tetra- and tri-iodothyroacetic acid are formed in the tissues, this does not necessarily imply that they are biologically important; degradation of the thyroid hormones by deamination and decarboxylation in the tissues may represent only a minor process in thyroid-hormone metabolism.

Probably the most important metabolic pathway for the thyroid hormones is deiodination, and the large amount of work on this subject has been summarized by Tata *et al.* (1957), but it is not certain whether this results in the formation of partially iodinated compounds. Many organs and extravascular tissues of vertebrates possess some deiodinating activity; whether the deiodination of the thyroid hormones is directly related to their physiological activity has yet to be discovered.

SUMMARY

1. The acetic acid analogues of thyroxine and tri-iodothyronine have been identified in the kidneys and liver of mice given large doses of ^{131}I.

2. These compounds constitute approximately 0·1 % of the total radioactivity in the tissues.

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