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# METABOLIC PATHWAYS OF TETRAIODOTHYROACETIC ACID, TRIIODOTHYROACETIC ACID, TETRAIODOTHYROPROPIONIC ACID AND TRIIODOTHYROPROPIONIC ACID*†

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**Abstract**—Major metabolic pathways for L-thyroxine and L-3,5,3′–triiodothyronine involve removal of an iodine atom from the benzene ring with the side chain and thus inactivation of these hormones. Conjugates of the products 3,3′,5′–triiodothyronine and 3,3′–diiodothyronine are excreted in the bile of dogs with livers but accumulate in the blood and urine of dogs without livers. Analogs of the thyroid hormones labeled with $^{131}$I in the 3′ or 5′ position were studied in dogs with biliary fistulas and in hepatectomized dogs. In dogs with biliary fistulas tetraiodothyroacetic acid was metabolized much more slowly than was thyroxine; much less $^{131}$I was excreted in bile or urine. Large amounts of unchanged tetraiodothyroacetic acid with smaller amounts of 3,3′,5′–triiodothyroacetic acid were found in the blood. Tetraiodothyropropionic acid was partially deiodinated to 3,3′,5′–triiodothyropropionic acid which accumulated in the blood as the amount of unchanged tetraiodothyropropionic acid decreased. Both of these compounds of propionic acid were excreted in bile as glucosiduronides. 3,5,3′–Triiodothyroacetic acid and 3,5,3′–triiodothyropropionic acid were rapidly cleared from the blood and excreted in the bile chiefly as glucosiduronides, with small amounts of the ethereal sulfate conjugates of 3,3′–diiodothyroacetic acid and 3,3′–diiodothyropropionic acid. In dogs without livers, the sulfates of these 3,3′–diiodo–compounds were found in larger amounts in both blood and urine, but the major metabolites appeared to be sulfoconjugates of 3′–monoiodo-derivatives.

RECENT investigations have indicated that a major metabolic pathway for both L-thyroxine[1] and L-3,5,3′–triiodothronine[2, 3] in the dog involves removal of an iodine atom from position 5. By the loss of this iodine, thyroxine is converted to 3,3′,5′–triiodothyronine, and 3,5,3′–triiodothyronine is converted to 3,3′–diiodothyronine. Both of these products are inactive in calorigenic and antigoitrogenic assays.[4, 5] These products are found chiefly in conjugated form: the 3,3′,5′–triiodothyronine as the glucuronoside[1] and 3,3′–diiodothyronine as the sulfate[2, 3] in bile, or after hepatectomy in blood and urine of dogs.

The acetic acid and propionic acid analogs of the thyroid hormones have been synthesized and shown to have calorigenic activity.[6–9] Early work indicated that the acetic acid analogs acted more rapidly than did triiodothyronine,[10] but subsequent studies have shown them to be less active.[7–9] The acetic acid analogs may be formed metabolically from the thyroid hormones. Thus, tetraiodothyroacetic acid has been

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found when thyroxine was incubated with kidney slices[11] or homogenates.[12] Triiodothyroacetic acid is formed from 3,5,3′-triiodothyronine by an enzyme from the mitochondria of the rat kidney.[13] It also has been found in kidneys[14] and muscles[15] of rats after injection of 3,5,3′-triiodothyronine. The propionic acid analogs have been considered to be especially effective in producing metamorphosis in tadpoles.[16, 17] Frieden and Westmark,[18] however, found higher activity only when the analogs were administered in the immersion fluid and not when they were injected.

We have investigated the metabolic pathways of the acetic and propionic acid analogs of both thyroxine and 3,5,3′-triiodothyronine in dogs with biliary fistulas and in dogs without livers.

## EXPERIMENTAL

Dogs used in this study weighed from 4·6 to 14·7 kg. Tetraiodothyroacetic acid and tetraiodothyropropionic acid labeled with $^{131}I$ in the 3′ or 5′ positions, and 3,5,3′-triiodothyroacetic acid and 3,5,3′-triiodothyropropionic acid labeled in the 3′ position by Abbott Laboratories, Oak Ridge, Tennessee, were injected intravenously in single doses of 35 to 61 $\mu$c/kg and 0·6 to 3·8 $\mu$g/kg (tracer dosages). These injections were made 0·5 to 2 hr after total hepatectomy by the two-stage technic of Grindlay and Mann,[19] and usually either at similar intervals after cannulation of the common bile duct and cholecystectomy or 5 and 17 hr later. Injections of saline or, in a few cases, of a mixture of saline and dextran were made at the rate of 20 ml/hr into the hepatectomized dogs. Injections of saline were also given to dogs with the biliary fistules. Glucose was usually given to these dogs at the rate of 150 mg/kg/hr up to 250 mg as needed.

Continuous collections of bile and urine were made during a 6-hr and an 18-hr period except in hepatectomized dogs. Specimens of blood were collected at the end of each period. In the hepatectomized dogs the length of the second period depended on the condition of each animal. When this deteriorated the collections were terminated. The radioactivity in the bile, urine, and plasma was measured in a well-type NaI (thallium-activated) scintillation counter. The iodine compounds were extracted and separated by paper chromatography as previously described.[1] The conjugated $^{131}I$ compounds were eluted from one-dimensional strips with 0·02 N $NH_4OH$, dried, and incubated with bacterial-$\beta$-glucuronidase or Mylase P. Markers used on the chromatogram included iodide, 3,5,3′-triiodothyronine, 3,3′,5′-triiodothyronine, 3,3′-diiodothyronine, tetraiodothyroacetic acid, 3,5,3′-triiodothyroacetic acid, 3,3′,5′-triiodothyroacetic acid, 3,3′-diiodothyroacetic acid, tetraiodothyropropionic acid, 3,5,3′-triiodothyropropionic acid, 3,3′,5′-triiodothyropropionic acid, and 3,3′-diiodothyropropionic acid. $^{131}I$ compounds were in some cases first separated on the kieselguhr column and then on paper chromatograms prior to incubation with the enzymes.

## RESULTS

Two-dimensional chromatograms of the tetraiodothyroacetic acid and tetraiodothyropropionic acid, 3,5,3′-triiodothyroacetic acid and 3,5,3′-triiodothyropropionic acid shipments obtained from Abbott Laboratories, Oak Ridge, showed the presence of 10% or less of iodide. The radioactive spot just below tetraiodothyroacetic acid in the 3,3′,5′-triiodothyroacetic acid position, and a similar spot below tetraiodothyropropionic acid in the 3,3′,5′-triiodothyropropionic acid position accounted for 2–6% of the radioactivity, respectively. 2–9% of the radioactivity in 3,5,3′-triiodothyroacetic acid was found in the 3,3′-diiodothyroacetic acid position and 9% of $^{131}$I in the 3,5,3′-triiodothyropropionic acid shipments in the 3,3′-diiodothyropropionic acid position on chromatograms.

*Tetraiodothyroacetic acid*

This was metabolized by the dog much more slowly than was L-thyroxine. Only 4·2 and 5·7% of the $^{131}$I injected as tetraiodothyroacetic acid was excreted in urine and bile in 24 hr by dogs with biliary fistulas (Table 1), in contrast to 20·0 and 18·5% in dogs injected with thyroxine.[1] The low urinary output of $^{131}$I is indicative of decreased deiodination from the 3′ and 5′ positions, and thus of decreased formation of radioactive iodide. The low biliary output is indicative of decreased conjugation. Thirty per cent of the dose was circulating in the plasma in contrast to 7·5% after injection of thyroxine.[1] No accumulation of either radioactive iodide or conjugates was found in the plasma. Eighty-five per cent of the plasma $^{131}$I was in unaltered tetraiodothyroacetic acid. Deiodination from the 5 position, which is a mechanism

TABLE 1. DISTRIBUTION OF $^{131}$I IN DOGS IN THE 24 HOURS AFTER INJECTION OF ANALOGS OF THYROID HORMONES

| $^{131}$I compound injected | Body fluids | Per cent of dose of $^{131}$I in | |
|---|---|---|---|
| | | Dogs with biliary fistulas | Hepatectomized dogs |
| | Plasma | | |
| Tetraiodothyroacetic acid | | 30·3 ± 2·4 (5)* | 31·3 ± 4·6 (4)*† |
| Tetraiodothyropropionic acid | | 17·4 ± 2·1 (4) | 20·8 ± 3·7 (4) |
| 3,5,3′-Triiodothyroacetic acid | | 4·6 ± 1·3 (6) | 5·8 (3)‡ |
| 3,5,3′-Triiodothyropropionic acid | | 2·7 (3) | 12·9 ± 3·2 (5)‡ |
| | Urine | | |
| Tetraiodothyroacetic acid | | 4·2 ± 0·6 (4) | 5·4 ± 2·2 (4)† |
| Tetraiodothyropropionic acid | | 10·8 ± 3·5 (4) | 4·6 ± 1·3 (4) |
| 3,5,3′-Triiodothyroacetic acid | | 22·3 ± 3·3 (8) | 46·0 ± 10·0 (4)‡ |
| 3,5,3′-Triiodothyropropionic acid | | 23·4 ± 5·6 (5) | 21·4 ± 4·1 (5)‡ |
| | Bile | | |
| Tetraiodothyroacetic acid | | 5·7 ± 0·7 (4) | |
| Tetraiodothyropropionic acid | | 29·6 ± 6·4 (4) | |
| 3,5,3′-Triiodothyroacetic acid | | 42·1 ± 3·0 (8) | |
| 3,5,3′-Triiodothyropropionic acid | | 33·3 ± 7·8 (4) | |

* Means ± standard error; number in parentheses is number of dogs.

† Time after injection of tetraiodothyroacetic acid varied from 8–24 hr owing to the variable length of survival of the hepatectomized dogs.

‡ Time after injection of 3,5,3′-triiodothyroacetic acid and 3,5,3′-triiodothyropropionic acid varied from 12 to 24 hours.

for inactivation of thyroxine and its analogs, did occur to a limited degree, and 13% of the $^{131}$I in the plasma was found to be 3,3′,5′-triiodothyroacetic acid (Table 2). Chromatographic separation of $^{131}$I compounds in bile followed by enzymatic hydrolysis showed that both tetraiodothyroacetic acid and 3,3′,5′-triiodothyroacetic acid glucuronosides were excreted in bile (Fig. 1) in a ratio of approximately 2 : 1 (Table 3) with smaller amounts of the glucuronosides of 3,5,3′-triiodothyroacetic acid and 3,3′-diiodothyroacetic acid.

TABLE 2. $^{131}$I COMPOUNDS IN PLASMA

| Substance injected | After injection (hr) | $^{131}$I compounds | Per cent of plasma $^{131}$I* | |
|---|---|---|---|---|
| | | | Dogs with biliary fistula | Hepatectomized dogs |
| Tetraiodothyroacetic acid | 6 or 24 | Tetraiodothyroacetic acid | 85 ± 3 (4) | 90 ± 1 (7)† |
| | | 3,3′,5′-Triiodothyroacetic acid | 13 ± 2 (4) | 5 ± 1 (7) |
| | | Iodide | 1 ± 0·4 (4) | 3 ± 1 (7) |
| Tetraiodothyropropionic acid | 24 | Tetraiodothyropropionic acid | 31 ± 3 (4) | 85 ± 2 (5)‡ 55 ± 8 (4)§ |
| | | 3,3′,5′-Triiodothyropropionic acid | 50 ± 6 (4) | 6 ± 1 (5)‡ 30 ± 4 (4)§ |
| | | Iodide | 11 ± 4 (4) | 3 ± 1 (5)‡ 8 (3)§ |
| 3,5,3′-Triiodothyroacetic acid | 5–12 | 3,5,3′-Triiodothyroacetic acid | | 9 ± 4 (8) |
| | | Iodide | | 5 ± 2 |
| | | 3,5,3′-Triiodothyroacetic acid sulfate | | 28 ± 3 |
| | | 3,3′-Diiodothyroacetic acid sulfate | | 37 ± 4 |
| | | 3′-Monoiodothyroacetic acid sulfate | | 22 ± 5 |
| 3,5,3′-Triiodothyropropionic acid | 6–24 | 3,5,3′-Triiodothyropropionic acid | | 3 ± 1 (8) |
| | | Iodide | | 7 ± 3 |
| | | 3,5,3′-Triiodothyropropionic acid sulfate | | 29 ± 7 |
| | | 3,3′-Diiodothyropropionic acid sulfate | | 36 ± 4 |
| | | 3′-Monothyropropionic acid sulfate | | 24 ± 1 |

*Means ± standard error; number in parentheses is number of specimens.
† 8–24 hr after injection of tetraiodothyroacetic acid.
‡ 6 hr after injection of tetraiodothyropropionic acid.
§ 24 hr after injection of tetraiodothyropropionic acid.

The average level of $^{131}$I in the plasma of hepatectomized dogs from 8 to 24 hr after the injection of tetraiodothyroacetic acid and the average output of $^{131}$I in the urine were similar to those in the dogs with biliary fistulas (Table 1). Most of the $^{131}$I in the plasma was in unchanged tetraiodothyroacetic acid, but small amounts of free

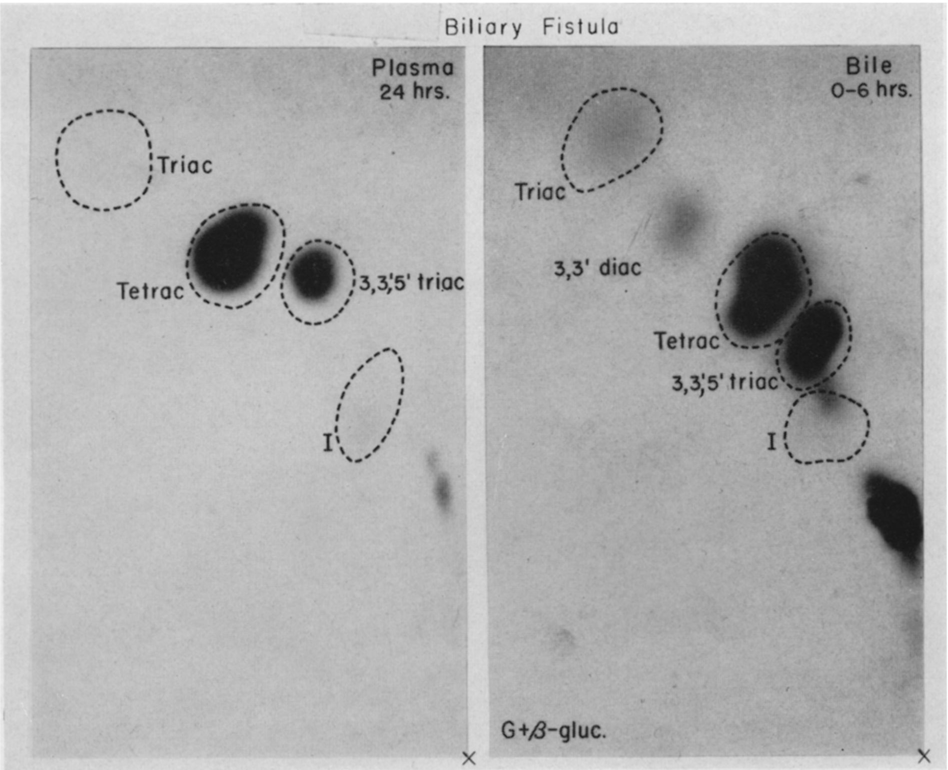

FIG. 1. Tetraiodothyroacetic acid administered to a dog with a biliary fistula.

*Left.* A typical radioautograph of a two-dimensional paper chromatogram of $^{131}$I compounds in plasma 24 hr after administration of tetraiodothyroacetic acid to a dog with a biliary fistula. An aliquot of an extract of plasma was applied to the filter paper at spot X with nonradioactive markers of 3,5,3′-triiodothyroacetic acid (triac), tetraiodothyroacetic acid (tetrac), 3,3′,5′-triiodothyroacetic acid (3,3′,5′ triac), and iodide. The chromatograms were developed in butanol-dioxane-ammonia in the vertical direction and in *tert*amyl-alcohol-ammonia in the horizontal direction. The positions of the markers are indicated by dotted lines. Eighty-one per cent of the radioactivity was found in the tetraiodothyroacetic acid spot and 16 per cent in the 3,3′,5′-triiodothyroacetic acid spot.

*Right.* A similar chromatogram of the hydrolytic products liberated from the glucuronoconjugates in bile by β-glucuronidase. Thirty per cent of the radioactivity was found in the tetraiodothyroacetic acid (tetrac) spot, 26% in the 3,3′,5′-triiodothyroacetic acid (3,3′,5′ triac) spot, 2% in 3,5,3′-triiodothyroacetic acid (3,5,3′ triac), and 2% in 3,3′-diiodothyroacetic acid (3,3′ diac). Radioactivity was also found below iodide in unidentified unhydrolyzed conjugates. The original 10 × 12-inch chromatograms have been trimmed to a 6 × 8·5-inch area from spot X which contained all of the radioactive spots.

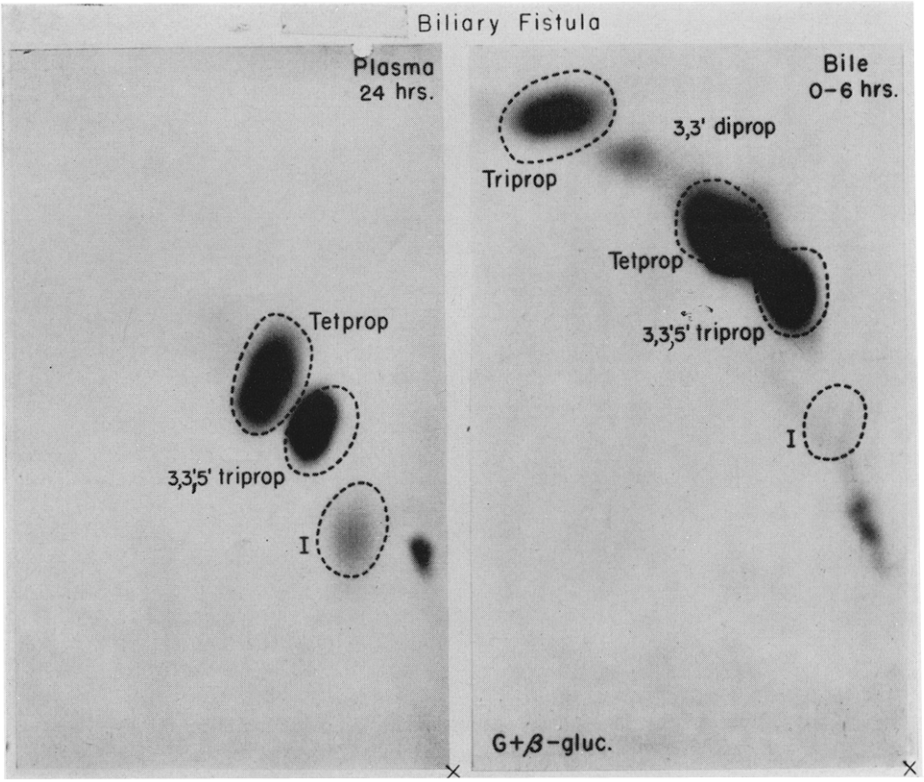

FIG. 2. Paper chromatograms after administration of tetraiodothyropropionic acid to a dog with a biliary fistula.

*Left.* Twenty-four hr after the injection, 36% of the $^{131}I$ in plasma was found in the tetraiodothyropropionic acid (tetprop) spot and 48% in the 3,3′,5′-triiodothyropropionic acid (3,3′,5′ triprop) spot. *Right.* After hydrolysis of the glucuronoconjugates present in bile after the injection of tetraiodothyropropionic acid, 12% of the radioactivity was found in the 3,5,3′-triiodothyropropionic acid (triprop) spot, 4% in the 3,3′-diiodothyropropionic acid (3,3′ diprop) spot, 35% in the tetraiodothyropropionic acid (tetprop) spot, and 43% in the 3,3′,5′-triiodothyropropionic acid (3,3′,5′ triprop) spot.

3,3′,5′-triiodothyroacetic acid also were present (Table 2). Variable amounts of unchanged tetraiodothyroacetic acid and iodide were excreted in the urine (Table 4), but the total amount of $^{131}$I excreted in the urine was small (Table 1).

### *Tetraiodothyropropionic acid*

Tetraiodothyropropionic acid was metabolized by the dog much more rapidly than was tetraiodothyroacetic acid and, in some ways, more rapidly than was thyroxine.

TABLE 3. $^{131}$I CONJUGATES IN BILE*

| Substance injected | Total glucuronosides (G region), per cent biliary $^{131}$I | Hydrolytic products after β-glucuronidase | Per cent of total glucuronosides† |
|---|---|---|---|
| Tetraiodothyroacetic acid | 74 ± 6 (5) | 3,5,3′-Triiodothyroacetic acid | 5 (3) |
| | | Tetraiodothyroacetic acid | 42 ± 5 (4) |
| | | 3,3′,5′-Triiodothyroacetic acid | 19 ± 3 (4) |
| | | 3,3′-Diiodothyroacetic acid | 2 (3) |
| Tetraiodothyropropionic acid | 42 ± 3 (8) | 3,5,3′-Triiodothyropropionic acid | 11 ± 0·4 (6) |
| | | Tetraiodothyropropionic acid | 34 ± 1 (6) |
| | | 3,3′,5′-Triiodothyropropionic acid | 44 ± 3 (6) |
| | | 3,3′-Diiodothyropropionic acid | 5 ± 0·3 (6) |
| 3,5,3′-Triiodothyroacetic acid | 74 ± 3 (10) | 3,5,3′-Triiodothyroacetic acid | 86 ± 7 (5) |
| | | 3,3′-Diiodothyroacetic acid | 3 ± 1 (5) |
| 3,5,3′-Triiodothyropropionic acid | 77 ± 3 (7) | 3,5,3′-Triiodothyropropionic acid | 81 ± 5 (5) |
| | | 3,3′-Diiodothyropropionic acid | 16 ± 4 (5) |

* Most specimens of bile were collected during the first 6 hr after injection, a few during the next 18 hr, and a few during the entire 24-hr period.

† Means ± standard error; number in parentheses is number of specimens.

The average level of $^{131}$I in the plasma 24 hr after the injection of tetraiodothyropropionic acid into dogs with biliary fistulas was 17·4% of the dose. This was intermediate between the levels of 30·3% after injection of tetraiodothyroacetic acid (Table 1) and 7% after thyroxine. Chromatographic separation of the $^{131}$I compounds in plasma showed that partial deiodination from position 5 with formation of 3,3′5′-triiodothyropropionic acid proceeded rapidly (Table 2; Fig. 2). The average concentration of free 3,3′,5′-triiodothyropropionic acid in plasma was 50% of the plasma $^{131}$I, compared with 31% for unchanged tetraiodothyropropionic acid. Conjugation with glucuronic acid proceeded more rapidly with tetraiodothyropropionic acid and its metabolites than with tetraiodothyroacetic acid or thyroxine. The average excretion of $^{131}$I in the bile (Table 1) was one-third greater after injection of tetraiodothyropropionic acid than after injection of thyroxine. Both tetraiodothyropropionic acid and 3,3′,5′-triiodothyropropionic acid glucuronosides were excreted in large amounts in the bile with smaller amounts of 3,5,3′-triiodothyropropionic acid and 3,3′-diiodothyropropionic acid glucuronosides (Fig. 2; Table 3).

TABLE 4. $^{131}$I COMPOUNDS IN URINE

| Substance injected | After injection (hr) | | $^{131}$I compounds | Per cent of urinary $^{131}$I* | |
|---|---|---|---|---|---|
| | First period | Second period | | Dogs with biliary fistulas† | Hepatectomized dogs |
| Tetraiodothyroacetic acid | 0–6 | 6–24 | Tetraiodothyroacetic acid | | 5–59 (6)† |
| | | | Iodide | | 23–68 (4) |
| Tetraiodothyropropionic acid | 0–6 | 6–24 | Tetraiodothyropropionic acid | 1–10 (5) | 4–75 (5) |
| | | | Iodide | 62–94 (5) | 7–44 (4) |
| 3,5,3′-Triiodothyroacetic acid | 0–3 | 6–24 | 3′-Monoiodothyroacetic acid sulfate | | 68 ± 3 (10)† |
| | 0–6 | | 3,3′-Diiodothroacetic acid sulfate | | 14 ± 3 (10) |
| | | | Iodide | 54–100 (6) | 10 ± 2 (10) |
| 3,5,3′-Triiodothyropropionic acid | 0–1 | | 3′-Monoiodothyropropionic acid sulfate | 28–43 (3) | 75 ± 4 (12) |
| | 0–6 | 6–24 | Iodide | 37–45 (3)<br>76–98 (6) | 15 ± 3 (12) |

* Range of values or means ± standard error; number of specimens in parentheses.

† In the second period urine was collected from 6 to 24 hr in the dogs with biliary fistulas but for a shorter time in some of the hepatectomized dogs, as shown in Table 1.

An unidentified radioactive spot was found near the solvent front in both butanol–dioxane–ammonia and *tert*amyl–alcohol–ammonia in chromatograms of bile collected either during the the first 6 hr or the next 18 hr after the injection of tetraiodothyropropionic acid. Approximately one-fourth of the biliary $^{131}$I was found in this spot. Radioactivity was not found in this region in chromatograms of plasma which contained large amounts of tetraiodothyropropionic acid or in chromatograms of urine. In a few chromatograms of commercial tetraiodothyropropionic acid, 3% of the radioactivity was found at the solvent front. Stasilli *et al.*[4] have reported decomposition of both tetraiodothyroacetic acid and tetraiodothyropropionic acid in alkaline solutions.

Partial deiodination from the 5 position of tetraiodothyropropionic acid with formation of 3,3′,5′-triiodothyropropionic acid occurred as readily in the hepatectomized dog as in the dog with a biliary fistula. Six hr after injection of tetraiodothyropropionic acid, 85% of the $^{131}$I present in plasma was in free tetraiodothyropropionic acid and 6% in free 3,3′,5′-triiodothyropropionic acid. By hour 24, after injection of tetraiodothyropropionic acid, these percentages were 55 for tetraiodothyropropionic acid and 30 for 3,3′,5′–triiodothyropropionic acid. By hour 44, 15% of the plasma $^{131}$I in one hepatectomized dog was in unchanged tetraiodothyropropionic acid, and 66% was in 3,3′,5′–triiodothyropropionic acid. Partial deiodination from the 3′ or 5′ position of tetraiodothyropropionic acid with formation of radioactive iodide was greatly diminished in the hepatectomized dog. Variable amounts of free tetraiodothyropropionic acid and iodide were excreted in the urine of the hepatectomized dog (Table 4), but the total amount of $^{131}$I excreted in the urine was small (Table 1).

### 3,5,3′–*Triiodothyroacetic acid*

This substance, like 3,5,3′–triiodothyronine and 3,5,3′–triiodothyropropionic acid, was cleared more rapidly from blood and metabolized faster than the corresponding tetra-iodo compounds. $^{131}$I from injected 3,5,3′–triiodothyroacetic acid was excreted much more rapidly in urine and bile than tetraiodothyroacetic acid was (Table 1). 3,5,3′–Triiodothyroacetic acid glucuronoside was excreted in large amounts in the bile with small amounts of 3,3′–diiodothyroacetic acid glucuronoside (Fig. 3; Table 3). Ethereal sulfate conjugates of 3,5,3′–triiodothyroacetic acid, 3,3′–diiodothyroacetic acid and, apparently, 3′–monoiodothyroacetic acid also were excreted in the bile. The first two substances were identified by use of appropriate markers on the chromatograms. Evidence for the presence of 3′–monoiodothyroacetic acid, for which no marker was available, is circumstantial and is based simply on the observation that this hydrolytic product of a sulfoconjugate has $R_f$ values just below those of 3,3′–diiodothyroacetic acid in both butanol–dioxane–ammonia and *tert* amyl–alcohol–ammonia as shown previously, such a lowering of $R_f$ values in these solvents occurs in the conversion of thyroxine to 3,3′,5′–triiodothyronine, of 3,5,3′-triiodothyronine to 3,3′–diiodothyronine, and of this to 3′–monoiodothyronine; as well as in the conversion of tetraiodothyroacetic acid to 3,3′,5′–triiodothyroacetic acid, of tetraiodothyropropionic acid to 3,3′,5′–triiodothyropropionic acid, and of 3,5,3′–triiodothyroacetic acid to 3,3′–diiodothyroacetic acid. It is very likely that the corresponding loss of one atom of iodine in the conversion of 3,3′–diiodothyroacetic acid to 3′–monoiodothyroacetic acid would result in a similar decrease of $R_f$ values. Loss of an iodine atom next to the phenolic group has the opposite effect and thus results in an increase in the $R_f$ values as in the conversion of thyroxine to 3,5,3′–triiodothyronine and of 3,5,3′–triiodothyronine to 3,3′–diiodothyronine.

The same stepwise deiodination of 3,5,3′–triiodothyroacetic acid that occurs in dogs with livers was also found in hepatectomized dogs, but conjugation of this substance or its metabolites with glucuronic acid was not observed after removal of the liver. Most of the $^{131}$I which was circulating in the blood plasma from 12 to 24 hr after the injection of this substance was found to be in sulfates of 3,5,3′–triiodothyroacetic acid, 3,3′–diiodothryoacetic acid, or 3′–monoiodothyroacetic acid. The major metabolite excreted in the urine by the hepatectomized dog appeared to be 3′–monoiodothyroacetic acid. Small amounts of 3,3′–diiodothyroacetic acid were also excreted.

### 3,5,3′–*Triiodothyropropionic acid*

When 3,5,3′–triiodothyropropionic acid was injected into dogs with biliary fistulas, much of the $^{131}$I was excreted rapidly in the bile. Most of this was found conjugated with glucuronic acid, but small amounts of 3,3′-diiodothyropropionic acid glucuronide also were found. 3,5,3′-Triiodothyropropionic acid sulfate, 3,3′-diiodothyropropionic acid sulfate, and two unidentified sulfates also were found (Fig. 4). One of these last was also found in urine and appeared to be 3′-monoiodothyropropionic acid. Most of the $^{131}I$ in the urine was iodide. When 3,5,3′-triiodothyropropionic acid was injected into a hepatectomized dog, sulfoconjugation and deiodination of the ring with the alanine side chain were the chief mechanisms by which this substance was metabolized. Three different ethereal sulfate conjugates, 3,5,3′-triiodothyropropionic acid sulfate, 3,3′-diiodothyropropionic acid sulfate, and an unidentified sulfate were found in the plasma (Table 2), but only the last one was found in the urine (Fig. 4; Table 4). When this was hydrolysed by Mylase P, the radioactivity travelled on the chromatogram to a spot just below 3,3′-diiodothyropropionic acid, which suggests that this was 3′-monoiodothyropropionic acid.

## COMMENT

Information on the metabolism of tetraiodothyroacetic acid, tetraiodothyropropionic acid, 3,5,3′-triiodothyroacetic acid, and 3,5,3′-triiodothyropropionic acid is of value for assessing the possible importance of these substances in the metabolic pathways of the thyroid hormones and, indeed, for a better understanding of the sequence of events in these pathways. Thus, although tetraiodothyroacetic acid has been found previously as a metabolite of thyroxine in kidney slices[11] or homogenates,[12] and in the bile of dogs with livers,[1] the present studies show that it is metabolized so slowly that it could not be involved in a major metabolic pathway of thyroxine. If tetraiodothyroacetic acid were an important intermediate in the metabolism of thyroxine, it would accumulate, because it is so slowly metabolized. The absence of such accumulation after the administration of thyroxine indicates that tetraiodothyroacetic acid is not an important intermediate in the dog. These studies also indicate that when thyroxine is converted to tetraiodothyroacetic acid, deiodination from the 3′ or 5′ position is greatly decreased. Conjugation with glucuronic acid is greatly decreased (particularly in the extrahepatic tissues), but partial deiodination from the 3 or 5 position of tetraiodothyroacetic acid occurs readily in dogs with or without livers.

If thyroxine could be converted by the dog to tetraiodothyropropionic acid, then conjugation with glucuronic acid would be enhanced in dogs with livers but would not occur in extrahepatic tissues. Deiodination from the 3′ or 5′ position would be greatly inhibited, but deiodination from the 3 or 5 position would proceed readily.

3,5,3′-Triiodothyronine, which is formed metabolically from thyroxine by loss of an iodine atom in the 5′ position, produces its calorigenic effect more rapidly than does thyroxine and is itself more rapidly metabolized. Since the second iodine atom in the prime position is much more rapidly removed than is the first one, radioactive iodide is formed and excreted more rapidly in the urine after injection of triiodothyronine than of thyroxine. Deiodination occurs from the 3 or 5 position of triiodothyronine more rapidly than from thyroxine, and 3,3′-diiodothyronine and even 3′-monoiodothyronine accumulate in the hepatectomized dog. Conjugation with glucuronic acid occurs much more rapidly with 3,5,3′-triiodothyronine than with thyroxine in dogs with livers. After removal of the liver, little if any 3,5,3′-triiodothyronine is conjugated with glucuronic acid, but thyroxine and its metabolite,

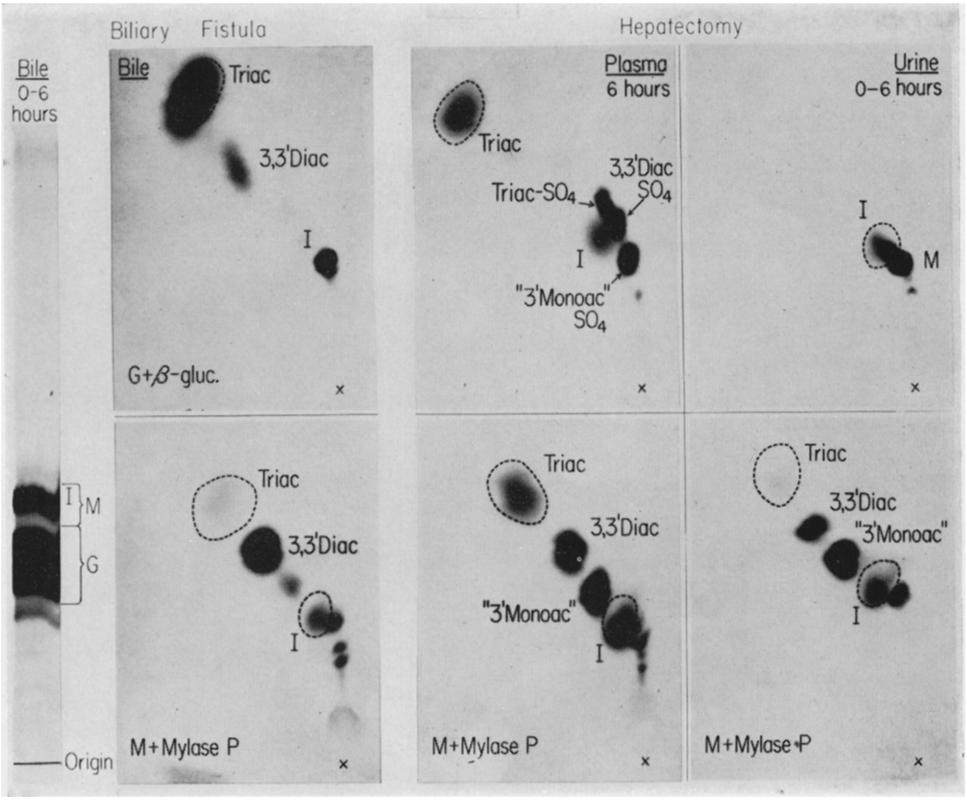

FIG. 3. 3,5,3′- Triiodothyroacetic acid injected.

*Two left panels.* Radioautograph of a typical one-dimensional chromatogram of bile from a dog with a bile fistula after injection. The chromatogram was developed in butanol-dioxane-ammonia. The brackets indicate the G and M regions, which were cut out for elution of the $^{131}$I compounds. Thirty-one per cent of the $^{131}$I injected as 3,5,3′-triiodothyroacetic acid had been excreted in the bile by this dog, 66% of the biliary $^{131}$I was found in the G region and 26% in the M region. When the G region was hydrolysed with β-glucuronidase, most of the radioactivity was found in the 3,5,3′-triiodothyroacetic acid (triac) spot with a smaller amount in the 3,3′-diiodothyroacetic acid (3,3′ diac) spot. When the M region was hydrolysed with Mylase P, most of the radioactivity was found in the 3,3′-diiodothyroacetic acid spot.

*Two right panels.* Chromatograms of hepatectomized dogs. The chromatogram of plasma 6 hr after injection showed the presence of free 3,5,3′-triiodothyroacetic acid (triac) and three sulfoconjugates. The chromatogram directly below this showed that when the sulfonconjugates (M region) were hydrolysed with Mylase P, the hydrolytic products were 3,5,3′-triiodothyroacetic acid, 3,3′-diiodothyroacetic acid (3,3′ diac), and 3′-monoiodothyroacetic acid (3′ monoac).

The chromatogram of urine excreted after the injection of 3,5,3′-triiodothyroacetic acid showed the presence of two conjugates in the M region. The chromatogram directly below this showed that hydrolysis with Mylase P liberated some 3,3′-diiodothyroacetic acid but chiefly 3′-monoiodothyroacetic acid sulfate.

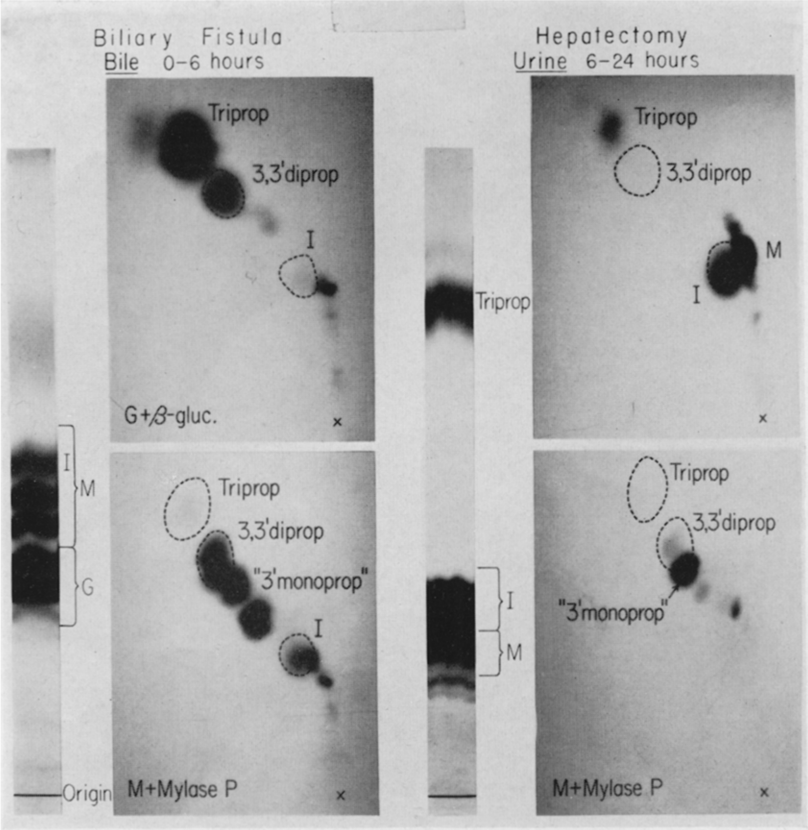

FIG. 4. 3,5,3′-Triiodothyropropionic acid injected.

*Left panels.* A one-dimensional chromatogram of bile from a dog after the injection. The dog excreted 35% of the injected $^{131}$I in the bile. The G and M region which were eluted are indicated by brackets. Shown also is a two-dimensional chromatogram of the hydrolytic products of the G region liberated by β-glucuronidase. They were 3,5,3′-triiodothyropropionic acid (triprop) and 3,3′-diiodothyropropionic acid (3,3′diprop), and they accounted for 73 and 18% of the radioactivity, respectively. When the M region was hydrolysed by Mylase P, 1% of the radioactivity was found in the 3,5,3′-triiodothyropropionic acid spot, 24% in the 3,3′-diiodothyropropionic acid, 26% in the 3′-monoiodothyropropionic acid (3′ monoprop), and 35% in an unidentified spot below this.

*Right panels.* A one-dimensional and a two-dimensional chromatogram (upper) of urine excreted by a hepatectomized dog are shown also. The dog excreted 21% of the dose of $^{131}$I in the urine; 62% of this was found in the M region, 30% in the iodide spot, and 3% in the 3,5,3′-triiodothyropropionic acid (triprop) spot. When the M region was eluted and hydrolysed with Mylase P, 90% of the $^{131}$I was found in the 3′-monoiodothyropropionic acid (3′ monoprop) spot and 4% in the 3,3′-diiodothyropropionic acid (3,3′ diprop) spot.

3,3′,5′-triiodothyronine, are readily conjugated with it. 3,5,3′-Triiodothyronine and its partially deiodinated derivatives are readily conjugated with sulfuric acid even in the hepatectomized dogs, but thyroxine is conjugated only to a limited extent.

3,5,3′-Triiodothyroacetic acid and 3,5,3′-triiodothyropropionic acid, like 3,5,3′-triiodothyronine, are metabolized much faster than are the corresponding tetra-iodo compounds. Conjugation with glucuronic acid proceeds more rapidly with the first two tri-iodo compounds. Correspondingly, smaller amounts are conjugated with sulfate. If the liver is removed, conjugation with glucuronic acid practically ceases, and sulfate conjugation predominates. Deiodination occurs readily at the 3′ position, and large amounts of radioactive iodide are excreted in the urine. After removal of the liver this type of deiodination as usual decreases, and deiodination at the 3 and 5 positions predominates. In fact, it appears that both of these iodine atoms can be removed, and thus the 3′ compounds accumulate.

Hatfield and co-workers[20] have shown that tetraiodothyroacetic acid and tetraiodothyropropionic acid disappear from the blood more slowly than does thyroxine after their injection in the rat. Deiodination of tetraiodoacetic acid occurred more slowly than thyroxine;[21] less $^{131}$I was excreted in the bile of man,[22] and less in urine and feces of the rat.[23] The only product of deiodination of tetraiodothyroacetic acid, tetraiodothyropropionic acid, 3,5,3′-triiodothyroacetic acid, or 3,5,3′-triiodothyropropionic acid found in homogenates or slices of spleen, kidney, or liver by Yamazaki and Slingerland[24] was iodide. The half-life of tetraiodothyroacetic acid in man was 3·7 days in contrast to 18 hr for 3,5,3′-triiodothyroacetic acid.[22]

3,5,3′-Triiodothyroacetic acid has been studied previously more extensively than tetraiodothyroacetic acid. Larson and Albright[25] showed that 3,5,3′-triiodothyroacetic acid was taken up rapidly from the blood by the liver. Large amounts of 3,5,3′-triiodothyroacetic acid glucuronoside and smaller amounts of the sulfoconjugate have been found in bile.[25–29] Less iodide was excreted in the urine after the administration of 3,5,3′-triiodothyroacetic acid than after administration of triiodothyronine.[25–28] Identification of 3,5,3′-triiodothyroacetic acid sulfate in bile and plasma was accomplished by Roche and co-workers[29] and Closon.[30]

Variations have been found in the binding of the thyroid hormones and their analogs to serum proteins, but it is difficult to correlate these findings with the rate of metabolism of these substances. The analogs are bound to sites other than thyroxine, since they do not displace thyroxine. Less tetraiodothyroacetic acid than thyroxine exists in the free state in serum.[31] 3,5,3′-Triiodothyroacetic acid has a low affinity for the thyroxine-binding protein of human serum.[32]

The present studies demonstrate the importance of deiodination from the 3 and 5 positions of the acetic and propionic acid derivatives of the thyroid hormones in the dog. This type of deiodination, which also occurs with thyroxine and 3,5,3′-triiodothyronine, appears to be a fundamental mechanism. It differs from the better known type of deiodination involving removal of labeled iodine from the prime positions with subsequent excretion of radioactive iodide and would lead to the excretion of nonradioactive iodide which would escape detection. The radioactive, partly deiodinated products formed by deiodination from the 3 and 5 positions normally appear in the bile, but they are readily formed in the extrahepatic tissues and thus accumulate in the blood of the hepatectomized dog. Partly deiodinated products of 3,5,3′-triiodothyroacetic acid and 3,5,3′-triiodothyropropionic acid are excreted in the urine by these dogs. It has been shown by Stasilli *et al.*[4] that both positions 3 and 5 must be occupied by iodine atoms if any of these compounds is to have calorigenic or antigoitrogenic activity. Thus, deiodination from the 3 and 5 positions can be considered to be a common mechanism for inactivation of these compounds.

## CONCLUSIONS

(1) Tetraiodothyroacetic acid is so slowly metabolized by the dog that it cannot be considered to be an intermediate in a major metabolic pathway of thyroxine.

(2) Tetraiodothyropropionic acid is conjugated with glucuronic acid even more rapidly than is thyroxine.

(3) Extrahepatic tissues do not conjugate significant amounts of tetraiodothyroacetic or tetraiodothyropropionic acid with glucuronic acid. These tissues have limited ability to remove iodine from the phenolic ring of tetraiodothyroacetic acid or tetraiodothyropropionic acid but readily remove iodine atoms from the non-phenolic ring, chiefly with the formation of 3,3′,5′-triiodothyroacetic acid and 3,3′,5′-triiodothyropropionic acid.

(4) 3,5,3′-Triiodothyroacetic acid and 3,5,3′-triiodothyropropionic acid, like 3,5,3′-triiodothyronine, are much more rapidly metabolized than are the corresponding tetra-iodo compounds.

(5) Both 3,5,3′-triiodothyroacetic acid and 3,5,3′-triiodothyropropionic acid are excreted in large amounts in bile as glucuronosides and in small amounts as ethereal sulfates. Extrahepatic tissues conjugate these substances and their partly deiodinated derivatives with sulfate and not glucuronic acid.

(6) Partially deiodinated derivatives, 3,3′-diiodothyroacetic acid and 3,3′-diiodothyropropionic acid, are excreted in the bile as sulfates. In hepatectomized dogs, the chief metabolites of 3,5,3′-triiodothyroacetic acid and 3,5,3′-triiodothyropropionic acid appear to be 3′-monoiodothyroacetic acid and 3′-monoiodothyropropionic acid.

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## REFERENCES

1. E. V. FLOCK, J. L. BOLLMAN, J. H. GRINDLAY and G. H. STOBIE, *Endocrinology* **69**, 626 (1961).
2. E. V. FLOCK, J. L. BOLLMAN and J. H. GRINDLAY, *Proc. Mayo Clin.* **35**, 75 (1960).
3. E. V. FLOCK, J. L. BOLLMAN and J. H. GRINDLAY, *Endocrinology* **67**, 419 (1960).
4. N. R. STASILLI, R. L. KROC and R. I. MELTZER, *Endocrinology* **64**, 62 (1959).
5. W. L. MONEY, S. KUMAOKA, R. W. RAWSON and R. L. KROC, *Ann. N.Y. Acad. Sci.* **86**, 512 (1960).
6. R. PITT-RIVERS, *Lancet* **2**, 234 (1953).
7. S. B. BARKER and W. J. LEWIS, *Proc. Soc. exp. Biol., N.Y.* **91**, 650 (1956).
8. S. B. BARKER, *Endocrinology* **59**, 548 (1956).
9. J. G. WISWELL and S. P. ASPER, JR., *Bull. Johns Hopkins Hosp.* **102**, 115 (1958).
10. O. THIBAULT and R. PITT-RIVERS, *Lancet*, **1**, 285 (1955).
11. S. B. BARKER, Discussion. *Ciba Found. Coll. Endocr.* **10**, 165 (1957).
12. K. TOMITA, H. A. LARDY, F. C. LARSON and E. C. ALBRIGHT, *J. biol. Chem.* **224**, 387 (1957).
13. E. C. ALBRIGHT, F. C. LARSON, K. TOMITA and H. A. LARDY, *Endocrinology* **59**, 252 (1956).

14. J. Roche, R. Michel, P. Jouan and W. Wolf, *C.R. Acad. Sci., Paris* **241**, 1880 (1955).
15. J. Roche, R. Michel and P. Jouan, *Bull. Soc. chim. biol.* **38**, 941 (1956).
16. T. C. Bruice, R. J. Winzler and N. Kharasch, *J. biol. Chem.* **210**, 1 (1954).
17. K. Tomita and H. A. Lardy, *J. biol. Chem.* **219**, 595 (1956).
18. E. Frieden and G. W. Westmark, *Science* **133**, 1487 (1961).
19. J. H. Grindlay and F. C. Mann, *Surgery* **31**, 900 (1952).
20. W. B. Hatfield, F. F. Davidoff, J. E. Ross and D. F. Tapley, *Endocrinology* **66**, 676 (1960).
21. N. F. Maclagan and D. Reid, *Ciba Found. Coll. Endocr.* **10**, 190 (1957).
22. W. L. Green and S. H. Ingbar, *Advances in Thyroid Research* p. 406. Trans. Fourth Int. Goitre Conference, London, July, 1960. New York, Pergamon Press (1961).
23. J. H. Wilkinson, *Biochem. J.* **73**, 334 (1959).
24. E. Yamazaki and D. W. Slingerland, *Endocrinology* **64**, 126 (1959).
25. F. C. Larson and E. C. Albright, *Endocrinology* **63**, 183 (1958).
26. J. Roche, R. Michel, N. Etling and P. Jouan, *C.R. Soc. Biol., Paris* **150**, 1320 (1956).
27. J. Roche and R. Michel, *C.R. Acad. Sci., Paris* **245**, 748 (1957).
28. J. Roche, R. Michel, P. Jouan and W. Wolf, *C.R. Soc. Biol., Paris* **150**, 461 (1956).
29. J. Roche, R. Michel, J. Closon and O. Michel, *Bull. Soc. chim. biol.* **40**, 2125 (1958).
30. J. Closon, *Recherches sur la Sulfoconjugaison des Hormones Thyroidiennes.* Theses de l'Université de Paris, Serie A, No. 856. Imprimérie Derououx, Liège (1959).
31. L. K. Christensen, *Endocrinology* **67**, 407 (1960).
32. J. Robbins and J. E. Rall, *J. clin. Invest.* **34**, 1331 (1955).