SUMMARY

1. A study has been made of the substances excreted in the urine after the administration of butyl 4-hydroxy-3:5-diiodobenzoate (BHDB) to man, rabbits and rats.

2. The principal excretory products found in the urine of seven patients with thyrotoxicosis, two patients with myxoedema under treatment with thyroxine, and one normal volunteer were 4-hydroxy-3:5-diiodobenzoic acid and its methyl ether. These two acids accounted for 90% or more of the total urinary iodine. Less than 2% of the urinary iodine consisted of iodide.

3. The excretion of the methoxy acid appeared to be greater in patients with thyrotoxicosis than in other subjects studied. The implications of this observation in relation to processes of biological methylation are discussed.

4. In rabbits and rats treated with BHDB, only the hydroxy acid was isolated from the urine.

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The Biological Action of Substances Related to Thyroxine

8. THE EFFECTS OF BUTYL 4-HYDROXY-3:5-DIIODOBENZOATE ON THE DEIODINATION OF DIIODOTYROSINE AND THYROXINE IN RATS

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The demonstration of the presence of 3:5:3′-triiodo-L-thyronine in the thyroid gland and in circulating blood by Gross & Pitt-Rivers (1952*a*, 1953*a*) and Roche, Lissitzky & Michel (1952*a*, *b*), along with the parallel observations that it has several times the physiological activity of thyroxine (Gross & Pitt-Rivers, 1952*b*, 1953*b*; Gross, Pitt-Rivers & Trotter, 1952; Tomich & Woollett, 1953), has led to a reassessment of the nature of the thyroid hormone. Gross & Pitt-Rivers (1952*c*, 1953*b*) have suggested that triiodothyronine is the peripheral thyroid hormone. This substance may be produced by the condensation of diiodotyrosine with monoiodotyrosine in the gland, or alternatively, as a result of the deiodination of thyroxine, a process which may take place either in the gland or elsewhere (Gross & Pitt-Rivers, 1952*a*, 1953*b*; Michel, 1952; Roche, Lissitzky & Michel, 1953).

Experiments in which the thyroxine inhibitor, butyl 4-hydroxy-3:5-diiodobenzoate (BHDB), depressed the oxygen consumption of mice treated with thyroxine, but actually enhanced that produced by treatment with triiodothyronine (Sheahan, Wilkinson & Maclagan, 1951; Maclagan, Sprott & Wilkinson, 1952), support the latter hypothesis. It was suggested that by exercising a restraint on deiodination processes, BHDB would reduce the rate of formation of triiodothyronine thereby causing partial inhibition of its effects, whilst at the same time by retarding the breakdown of the triiodo compound it would extend its period of effective biological action. The experiments described in this paper were designed to test the first part of this hypothesis.

We decided first to use 3:5-diiodo-L-tyrosine in model experiments in rats, the results of which have

been briefly reported by Maclagan (1953). This involved the development of a method for the determination of iodide in urine in the presence of organically combined iodine compounds in order to avoid the difficulties experienced by Maclagan & Wilkinson (1954). Subsequently ^{131}I-labelled thyroxine was administered to rats and the effects produced by simultaneous treatment with BHDB were observed by measuring the urinary and faecal radioactivity. A brief account of this work has also appeared (Wilkinson & Maclagan, 1953).

MATERIALS AND METHODS

Animals. Twelve male rats each within 15 g. of the mean weight were used for each experiment. In different experiments the mean weights ranged from 100 to 230 g. Groups of three similarly treated animals were kept in metabolism cages arranged so that the urine and faeces could be collected separately. During the experiments the rats were fed on stock diet and tap water (both *ad lib.*).

Treatment of excreta. The urine, collected daily, and washings were combined and the volume measured.

When non-radioactive materials were used the mixture was acidified with 40% trichloroacetic acid (10% by volume) and clarified by shaking with Hyflo Super-cel (approx. 0·3 g.) and filtering through a Whatman no. 1 filter paper. A sample (2–10 ml.) of the filtrate was diluted to 25 ml. with water and the iodide content determined by electrometric titration (see below).

In the experiments using radioactive substances the urine was suitably diluted if necessary and counted in a liquid counter (Veall, 1948) connected to a standard scaler. The faecal pellets, also collected daily, were pounded in a mortar with N-NaOH (20 ml.), and, after dilution with water (80 ml.), the mixture was comminuted in an Atomix homogenizer. The volume of the resulting suspension was adjusted so that it contained 10%, w/v, of the faeces. This suspension was then suitable for use in the liquid counter.

Solutions of ^{131}I-labelled thyroxine. Considerable difficulty was experienced in obtaining a pure sample of thyroxine labelled in the 3′:5′ positions with ^{131}I, for the product prepared by the method of Clayton, Free, Page, Somers & Woollett (1950) contained only about 10% of the theoretical radioactivity and was found to be seriously contaminated with triiodothyronine and iodide.

A satisfactory supply of radiothyroxine (3 mg.) in 0·2 N-Na_2CO_3 (15 ml.) (135 μc/ml.), received from the Radiochemical Centre, Amersham, was used in the experiments reported. After storage for 3 weeks, however, even this material underwent deterioration, and paper chromatograms showed the presence of considerable amounts of iodide (Fig. 2). It is therefore important to use the solution soon after preparation.

Chromatographic methods. Unidimensional chromatograms were run in a butanol:dioxan:ammonia mixture (Gross, Leblond, Franklin & Quastel, 1950) by vertical ascent for 18 hr. on Whatman no. 1 chromatographic paper.

The non-radioactive chromatograms were developed with the ceric sulphate-arsenious acid reagent as described elsewhere (Bowden & Maclagan, 1954). Autoradiographs of the chromatograms containing radioactive materials were prepared using Ilford Industrial *B* X-ray film with an exposure varying from 4 hr. to 4 days according to the intensity of the spots.

Electrometric titration of iodide. The titration unit consisted of a 50 ml. beaker containing a Ag:Ag_2S electrode (Hiltner, 1933; Sand, 1941) connected to the negative terminal of a Marconi pH meter used as a potentiometer. The positive terminal was connected to a calomel electrode which was linked by means of a KNO_3:agar bridge with the contents of the beaker. A cross-over switch incorporated in the electrode leads enabled the potential to be reversed during titration. As it was found that variations in the speed at which the contents of the beaker was stirred affected the rate of equilibration, a geared Citenco motor was used to drive the glass paddle stirrer.

The Ag:Ag_2S electrode consisted of a single coil of S.W.G. 16 Ag wire about 3 cm. in diameter on which a thin but even layer of Ag_2S was electrodeposited. This electrode could be used for about 20 titrations in water, but required replacement after about 12–15 such operations in urine. Electrode failure was usually characterized by 'flattening' of the curve.

An iodide solution (usually 25 ml.) acidified with 40% trichloroacetic acid (1 ml.) was used for each titration with 0·001 N-, 0·002 N- or 0·0001 N-$AgNO_3$ according to the amount of iodide present. When larger volumes were used a 100 ml. beaker was employed in such a manner that the electrode was immersed to approximately the same depth on each occasion. The initial potential of an iodide solution was about −180 mv in water and about −130 mv in urine. The potential at the end point was usually in the range 0 to +40 mv in water and −10 to +10 mv in urine. Titrations were continued until the potentials reached +200 mv in water or +80 mv in urine.

Iodine-free distilled water was used for the preparation of all solutions and for the dilution of samples throughout this work.

EXPERIMENTAL AND RESULTS

Determination of urinary iodide in the presence of organically combined iodine

The accuracy of electrometric titration with a silver:silver sulphide electrode for the determination of iodide was tested by performing a number of recovery experiments in water and in urine. Solutions containing known amounts of iodide and organically combined iodine were prepared by methods similar to the following:

Diiodotyrosine (125 mg.) was dissolved in 0·1 N sodium hydroxide (6 ml.) and the volume diluted to 250 ml. with water. 2–12 ml. of this solution were added to water or urine (25–50 ml.) containing added iodide (20–500 μg.); the mixture was acidified with 40% trichloroacetic acid (1–2 ml.), and titrated with silver nitrate as described in the previous section.

The results obtained with aqueous iodide solutions are given in Table 1, which shows that satisfactory recoveries can be obtained with iodide concentrations as low as 10 μg. in 50 ml. water, despite the presence of organically combined iodine. In

Table 1. *Effect of organically combined iodine (diiodotyrosine) on the recovery of added iodide from water*

Organic iodine (I) added (μg.)	Iodide (I^-) added (μg.)	Iodide found: In absence of added iodide (μg.)	Iodide found: In presence of added iodide (μg.)	Recovery of added iodide (μg.)	Recovery of added iodide (%)
0	13·4	0	12·3, 12·6	12·3, 12·6	92, 94
0	26·8	0	25·7, 25·8	25·7, 25·8	96, 96
0	131	0	127, 128	127, 128	97, 98
0	448	0	447	447	100
975	13·4	0·4	14·3, 14·3	13·9, 13·9	104, 104
4880	26·8	3·0	28·8, 29·8	25·8, 26·8	96, 100
1912*	131	12*	138, 134	126, 122	96, 93
650	448	1	447	447	100

* A solution of diiodotyrosine several days old was used in this series of determinations (see Table 3).

Table 2. *The effect of organically combined iodine (diiodotyrosine) on the recovery of added iodide from urine*

Organic iodine (I) added (μg.)	Iodide (I^-) added (μg.)	Iodide found: In absence of added iodide (μg.)	Iodide found: In presence of added iodide (μg.)	Recovery of added iodide (μg.)	Recovery of added iodide (%)
0	22	2	23, 21, 18	21, 19, 16	95, 86, 73
0	43	2	39, 41	37, 39	86, 91
0	101	2	112, 110	110, 108	109, 107
0	223	2	237	235	105
0	490	2	480	478	98
1 480	25	4	29	25	100
2 960	49	4	49, 49	45, 45	92, 92
5 920	98	5	98	93	95
11 840	196	21	223, 221	202, 200	103, 102
29 600	490	29	526, 526	497, 497	101

Table 3. *The effect of storage on the iodide content of solutions of diiodotyrosine* (20 ± 2°)

Diiodotyrosine (dissolved in 25 ml. 0·06N-NaOH) (μg.)	Period of storage (days)	Iodide found (μg.)	Iodide found (% of total I)
32 600	0	12·0	0·063
34 100	1	16·6	0·083
34 100	3	24·8	0·124
32 600	4	30·0	0·157

urine the recovery of added iodide was also satisfactory (Table 2), but the presence of large quantities of organic iodine reduced the sharpness of the end point thereby causing a slight diminution in the sensitivity of the method. In spite of this, 25 μg. added to 50 ml. urine were recovered in the presence of 2·5 mg. diiodotyrosine. As this concentration is equivalent to a daily output of iodide of the order of 0·5–1·0 mg., it is probable that the method would be useful for the study of iodide excretion in patients receiving iodine-containing drugs. Similar results were obtained when the organic iodine was added in the form of 4-hydroxy-3:5-diiodobenzoic acid and its methyl ether, compounds found in human urine after BHDB therapy (Maclagan & Wilkinson, 1951, 1954). The sulphide electrode proved to be much more stable in urine than the iodide electrode previously used and was generally much more satisfactory.

It was necessary to prepare the diiodotyrosine solutions immediately before use in these experiments, for it was found that, contrary to expectations, this substance undergoes significant hydrolysis on standing in weak alkaline solution. The iodide produced during storage was demonstrated by titration (Table 3) and amounted to 0·16% after 4 days.

The effect of BHDB on the deiodination of diiodotyrosine in rats

BHDB (500 mg.) was dissolved in 0·2N sodium carbonate (12 ml.), the pH was adjusted to 7·8 by the dropwise addition of N hydrochloric acid and the resulting suspension made up to 20 ml. with distilled water. The diiodotyrosine solution (25 mg./ml.) was prepared similarly. These materials were injected subcutaneously into rats, each weighing 100 ± 15 g., as follows:

Three rats each received two injections (1 ml./rat) of BHDB suspension on the first day of the experiment and one injection daily on each of the next 4 days.

Table 4. *The effect of BHDB on the urinary iodide in rats injected with diiodotyrosine*

(Diiodotyrosine was given in doses of 25 mg./rat/day from day 1, BHDB in doses of 50 mg./rat on day 0 followed by 25 mg./rat/day from day 1, as described in the text. The effect of BHDB is expressed as the ratio of the urinary iodide of groups receiving diiodotyrosine + BHDB/groups receiving diiodotyrosine alone, × 100.)

	Urinary iodide (mg./group of three rats)			
200 g. rats treated with	After 24 hr.	After 48 hr.	After 72 hr.	After 96 hr.
Diiodotyrosine	3·38	13·38	17·14	21·87
BHDB	0·37	0·63	0·98	1·32
Diiodotyrosine + BHDB	2·62	4·83	8·63	13·47
Untreated controls	0·014	0·032	0·054	0·063
Effect of BHDB	77	36	50	62
100 g. rats treated with				
Diiodotyrosine	12·12	15·00	19·36	22·40
BHDB	0·30	1·70	2·12	2·54
Diiodotyrosine + BHDB	4·78	7·88	10·01	11·96
Untreated controls	0·004	0·024	0·046	0·057
Effect of BHDB	39	53	52	53

A second group of three rats was given the diiodotyrosine solution (1 ml./rat) each day for 4 days commencing on the second day of the experiment.

The third group of three rats received similar injections of both BHDB and diiodotyrosine, at the same times as the two preceding groups.

A fourth group of three similar rats served as untreated controls.

Twelve other rats, each weighing 200 ± 20 g., were divided into four further groups which were treated in exactly the same manner as the 100 g. rats listed above.

The urine, collected daily from each group, was treated as described in the preceding section. The iodide contents of the various samples are listed in Table 4. It is clear that the urinary output of iodides in rats treated with diiodotyrosine is markedly reduced by the simultaneous administration of BHDB. In the experiments summarized, the iodide output when both substances are given is reduced to about 50% of that produced by diiodotyrosine alone. If allowance were made for the iodide produced by breakdown of the BHDB, the difference would be even more marked. It would appear that 2–3 mg. of iodide is excreted in the urine after a total dose of 150 mg. BHDB (equivalent to 85 mg. iodine). Thus it seems that in the rat the degree of iodide liberation from BHDB is slightly higher than the maximum of 2% indicated in man by our earlier experiments (Maclagan & Wilkinson, 1954).

Similar experiments were performed in which the dose of BHDB was reduced to 10 mg. and 5 mg./rat per day. In the former the iodide output following the administration of diiodotyrosine was reduced by approx. 19% when BHDB was given at the same time, but the results of the 5 mg. experiment were not significant.

The effect of BHDB on the urinary iodide of rats treated with thyroxine labelled with ^{131}I

As the amount of iodide liberated during the metabolism of a small dose of thyroxine would be minute in comparison with that released by the breakdown of a much larger dose of BHDB, the methods used in the diiodotyrosine experiments could not be applied to the study of iodide excretion after thyroxine treatment. We therefore used ^{131}I-labelled thyroxine, the iodine of which would be distinguished from that of the BHDB by its radioactivity.

The general procedure may be illustrated by the following typical experiment. 1 ml. of a suspension of BHDB (50 mg./ml.) in 1% aqueous carboxymethylcellulose* was given orally to each of six rats, each weighing 230 ± 20 g., on the first day of the experiment. Another group of six similar rats received 1% aqueous carboxymethylcellulose (1 ml./rat). The first group was given 0·5 ml. of the BHDB suspension per rat and the second group 0·5 ml. of the medium per rat on each of the next 4 days. On the second day of the experiment, each rat was injected subcutaneously with 0·5 ml. thyroxine solution (100 μg., 67 μc). The rats were then placed in metabolism cages in four groups of three, two groups having received thyroxine alone and two, thyroxine + BHDB. The urine and faeces were collected daily and treated as described in the previous section. The radioactivity of each sample was measured.

A second experiment in which a dose of 4 μg. thyroxine/rat was given was also performed with the same number of animals.

When ^{131}I-labelled thyroxine is administered to animals it is probable that the urinary radioactivity

* We are indebted to Dr R. Michel for suggesting the use of this suspending medium.

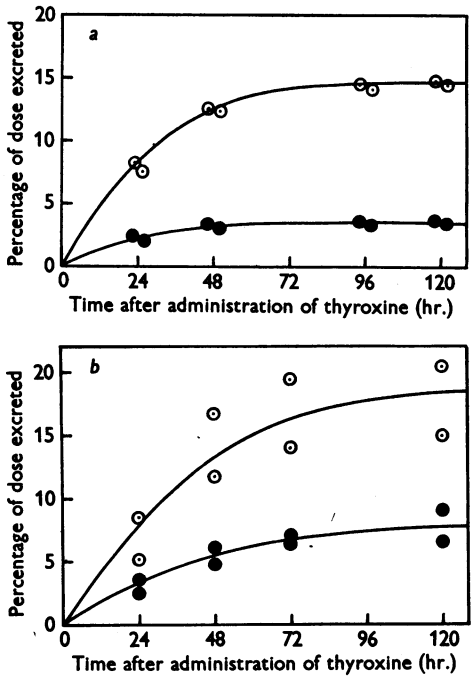

Fig. 1*a*, 1*b*. The effect of BHDB upon the urinary radioactivity of rats treated with a single subcutaneous injection of ^{131}I-labelled thyroxine. ⊙, the combined output of a group of three rats treated with (*a*) 100 μg. thyroxine/rat, and (*b*) 4 μg. thyroxine/rat; ●, the combined output of a group of the rats treated with daily oral doses of 25 mg. BHDB/rat and in addition single doses of (*a*) 100 μg. thyroxine/rat, and (*b*) 4 μg. thyroxine/rat.

is mainly due to inorganic iodide, while thyronine derivatives are the principal sources of the faecal radioactivity (Gross & Leblond, 1951*a*, *b*; Roche, Michel & Tata, 1952). Consequently, the urinary radioactivity should serve as a measure of the extent of deiodination occurring in rats treated with radiothyroxine alone or together with BHDB (see below). The results (Fig. 1) show that BHDB produces a marked reduction in the urinary radioactivity of the groups receiving both substances, irrespective of whether the dose of thyroxine is large (100 μg.) or only slightly above physiological levels (4 μg.).

The sample of radiothyroxine solution used in the 4 μg. experiment had undergone some decomposition during the 3 weeks which elapsed between the two experiments (Fig. 2). In order to minimize exchange between ^{131}I and ^{127}I, Clayton *et al.* (1950), after confirming the experience of Miller, Anderson, Madison & Salley (1944) and Frieden, Lipsett & Winzler (1948), have recommended that solutions of labelled thyroxine be kept at an alkaline pH. We have observed that, under such conditions, the iodide content is likely to increase presumably because of hydrolysis, so that about 6 % of the radioactivity was in the form of iodide at the time of the second experiment. This has tended, of course, to obscure the effects of BHDB in this experiment, though they are still quite obvious.

The studies of the faecal radioactivity disclosed no significant difference between the output of the two groups of animals. The combined urinary and faecal excretion accounted for about 80–90 % of the dose during the 5 days of the experiment in which 100 μg. were given, and 50–70 % when 4 μg. were injected. In the first 24 hr. of the 100 and 4 μg. experiments, 40–50 % and 10–20 %, respectively, of the dose were excreted in the faeces. These figures are essentially similar to the results obtained in bile by Taurog, Briggs & Chaikoff (1951) using ^{131}I-labelled thyroxine, and by Klitgaard, Lipner, Barker & Winnick (1953) using [1-^{14}C]thyroxine.

In order to exclude the possibility that BHDB produced these effects by altering the permeability of the kidney to iodide, similar experiments were performed in which radioactive iodide was used in place of the radiothyroxine. 150 g. (± 15 g.) rats were given subcutaneous injections of Na^{131}I (50 μg., 0·5 μc/rat). No significant difference was

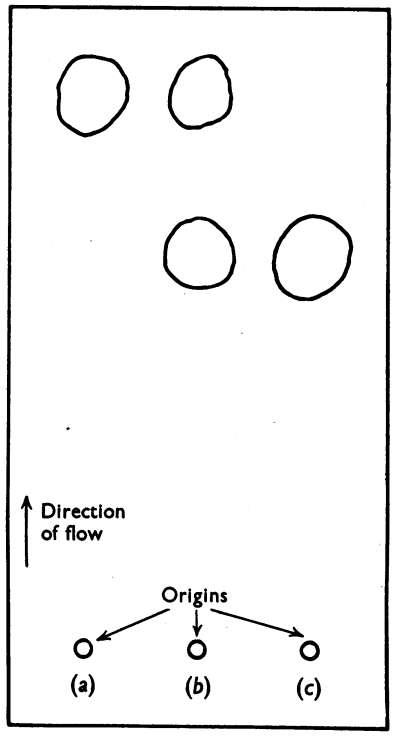

Fig. 2. Autoradiographs of paper chromatograms of a solution of ^{131}I-labelled thyroxine in 0·02 N-Na_2CO_3 run (*a*) 1 day, and (*b*) 3 weeks, after preparation; (*c*) iodide marker.

observed between the urinary or faecal radioactivities of those groups of rats receiving iodide alone and those having iodide plus BHDB in this or in a similar experiment in which potassium iodide (75 μg.) containing $Na^{131}I$ (carrier-free, 5 μc/rat) was given.

Table 5. *The effect of the precipitation of added protein on the radioactivity of the urine of rats treated with* ^{131}I*-labelled thyroxine*

Materials used	Radioactivity (counts/min.) Before protein treatment	After protein precipitation	Recovery of radioactivity (%)
Urine of rats treated with			
^{131}I-Thyroxine	4569	4449	98
	4064	3865	95
^{131}I-Thyroxine + BHDB	1463	1433	98
	1661	1540	93
$Na^{131}I$ in			
Water	2188	2133	98
	5486	5380	98
Urine	7796	7631	98
	3978	3972	100
^{131}I-Thyroxine in			
Water	1134	74	6
	1756	150	8
Urine	1891	138	7
	1068	70	6

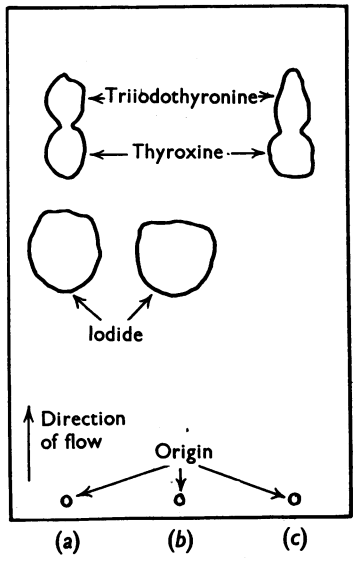

Fig. 3. Paper chromatograms of a solution of thyroxine, triiodothyronine and iodide in urine: (*a*) original solution; (*b*) solution after precipitation of added protein; (*c*) butanol extract of protein precipitate.

Evidence in support of the view that the urinary radioactivity consisted mainly of iodide was obtained by the addition of normal human serum or plasma (1 ml.) to the urine (10 ml.). The mixture was shaken and, after 5 min., treated with 40% trichloroacetic acid (1 ml.) and again mixed thoroughly. After 5 min. it was centrifuged at 3000 rev./min. The supernatant liquid (6 ml.) was diluted to 10 ml. with water and counted in a liquid counter. The count obtained was compared with that given by 5 ml. of the original urine similarly diluted to 10 ml. The radioactivity of the urines of both groups of rats was not significantly reduced by this treatment. Solutions of radioiodide behaved similarly but labelled thyroxine was almost completely removed by co-precipitation with protein (Table 5). When faecal suspensions were submitted to this procedure, less than 6% of the radioactivity was retained by the supernatant fluid after removal of the precipitate. It appears, therefore, that the principal radioactive components of the faeces are thyronine derivatives.

Further confirmation was obtained by submitting a solution of thyroxine (1 mg.), triiodothyronine (1 mg.) and potassium iodide (1 mg.) in normal urine (25 ml.) to the protein-precipitation treatment. The solution (20 ml.) was treated with human plasma (2 ml.) and 40% trichloroacetic acid (2 ml.) as described above. The supernatant liquid and the remainder of the original solution were reserved for paper chromatography. The precipitate was resuspended in 2% trichloroacetic acid (20 ml.) and centrifuged again, the supernatant solution being discarded. The washing procedure was repeated and the precipitate dissolved in 0·4N sodium hydroxide (5 ml.). The solution was acidified to pH 3 with hydrochloric acid and extracted with *n*-butanol (6, 4, 2 ml.). The butanol extract was then chromatographed. The papers were developed by the ceric sulphate: arsenious acid method of Bowden & Maclagan (1954), and the positions of the spots compared with markers. As anticipated, the supernatant solution gave one spot only, corresponding to that occupied by iodide in the marker (Fig. 3*b*). The butanol extract gave two spots, the positions of which indicated the presence of thyroxine and triiodothyronine (Fig. 3*c*). It is therefore clear that the precipitation of added protein with trichloroacetic acid is an efficient method for the separation of iodide from thyronine derivatives.

DISCUSSION

The suggestion by Gross & Pitt-Rivers (1952*c*, 1953*b*) that triiodothyronine is the active form of thyroid hormone has focused attention on its biosynthesis. Michel (1952) and Roche *et al.* (1953) have suggested that triiodothyronine may arise in

the thyroid by the condensation of one molecule of monoiodotyrosine with one molecule of diiodotyrosine, by a method analogous to that proposed by Harington (Harington & Barger, 1927; Harington, 1944; Pitt-Rivers, 1948) for the biosynthesis of thyroxine. The alternative possibility is that triiodothyronine may arise as a result of the deiodination of thyroxine. Indeed, the experiments of Gross & Leblond (1951*a*, *b*) in which triiodothyronine was found in the plasma and excreta of both intact and thyroidectomized rats treated with radioactive thyroxine have been interpreted by Gross & Pitt-Rivers (1952*b*) as a probable indication of this process. If triiodothyronine is the active form of the hormone then extra-thyroidal sites must be at least as important in deiodination as the thyroid, since thyroidectomized animals respond to thyroxine and to anti-thyroxine compounds (Barker, Dirks, Garlick & Klitgaard, 1951). The observation of Maclagan *et al.* (1952) that BHDB inhibited the effect of thyroxine in intact mice, but enhanced that of triiodothyronine, is also consistent with the view that triiodothyronine arises as the result of deiodination of thyroxine. These results suggest that BHDB acts by inhibiting deiodination processes, thus preventing the formation of triiodothyronine from thyroxine and, at the same time, reducing its rate of decomposition (Maclagan & Wilkinson, 1952).

The fact that BHDB depresses the output of urinary iodide after the administration of diiodotyrosine to intact rats (Table 4) and also depresses the output of radio-iodide in intact rats treated with ^{131}I-labelled thyroxine (Fig. 1) strongly supports this theory. The suggestion advanced by Sheahan *et al.* (1951) that inhibition of thyroxine occurs as a result of competition between thyroxine and the inhibitor for a site on an enzyme surface may thus be extended, as we are now able to postulate that the enzyme concerned is that responsible for the conversion of thyroxine to triiodothyronine. It is interesting to note that, despite the inability of Roche *et al.* (1952*b*) to demonstrate deiodination of thyroxine by the enzyme found in various tissue preparations (Roche, Michel, Michel & Lissitzky, 1951), Gross & Pitt-Rivers (1953*b*) regard this as the probable mode of synthesis of triiodothyronine.

It may be pointed out that, with the possible exception of the lactating cow (Bartlett, Burt, Folley & Rowland, 1953), animals pre-treated with BHDB or similar substances represent the only biological systems known at the present time to respond differently to thyroxine and triiodothyronine.

SUMMARY

1. An electrometric method for the determination of small amounts of iodide in urine in the presence of relatively large quantities of organically combined iodine has been studied. Using a silver: silver sulphide electrode the method can be applied to the determination of about 20 μg. of iodide in the presence of 1·5 mg. organic iodine in 50 ml. urine.

2. A method for the separation of thyronine derivatives from solutions containing iodide has been developed. This procedure was used to confirm that the urinary radioactivity after treatment with thyroxine labelled in the 3′:5′ positions with ^{131}I was due to iodide.

3. Solutions of radio-thyroxine in 0·02N sodium carbonate undergo considerable decomposition on storage. After 3 weeks about 6% of the radioactivity was due to iodide.

4. The urinary iodide after the injection of diiodotyrosine or thyroxine was markedly reduced by the simultaneous administration of butyl 4-hydroxy-3:5-diiodobenzoate in intact rats.

5. These results therefore support the hypothesis that butyl 4-hydroxy-3:5-diiodobenzoate exerts its anti-thyroxine effect by interference with the conversion of thyroxine into triiodothyronine.

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Studies in Carotenogenesis

10. SPIRILLOXANTHIN SYNTHESIS BY WASHED CELLS OF *RHODOSPIRILLUM RUBRUM*

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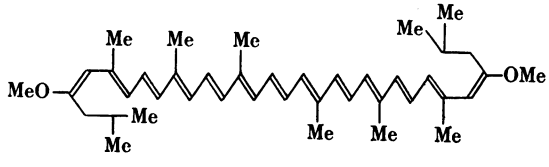
It has been known for some time that the photosynthetic bacterium *Rhodospirillum rubrum* synthesizes, in addition to bacteriochlorophyll, a single carotenoid spirilloxanthin (Polgar, van Neil & Zechmeister, 1944). This pigment appears to be identical with rhodoviolascin isolated by Karrer & Solmssen (1938) from a mixed culture of *Rhodovibrio*, and to have the following structure



Recently, Goodwin & Osman (1952, 1953*a*) have investigated the general conditions governing spirilloxanthin synthesis in growing *Rsp. rubrum*. The present paper reports an investigation into the synthesis of this pigment in washed cells. Part of this work has already been reported briefly (Goodwin & Osman, 1953*b*).

EXPERIMENTAL

Cultures. The strain of *Rsp. rubrum* used was the same as that examined previously (Goodwin & Osman, 1953*a*). 'Normal' cultures were grown on our standard medium, which is very similar to that used by Gest & Kamen (1949), containing per litre: $MgSO_4, 7H_2O$ (0·20 g.), $CaCl_2$ (0·0375 g.), biotin (5 μg.), L-glutamic acid (4·0 g.), DL-malic acid (3·5 g.), sodium citrate, $2H_2O$ (0·8 g.), yeast extract (Difco) (0·25 g.), KH_2PO_4 (0·18 g.). The pH was adjusted to 7·0. 'Diphenylamine' cultures were grown on the same medium containing diphenylamine at a level of 1/140 000. The cultures were grown at 30° in a glass incubator (Garton, Goodwin & Lijinsky, 1951) illuminated with two banks of 6 × 75 w tungsten incandescent lamps. In order to get a more exact temperature control, some of the transfer experiments were carried out at 30° in a similarly illuminated, thermostatically controlled water bath.

Except in the case of those cultured in the presence of a $NaHCO_3/CO_2$ buffer, anaerobic cultures were grown in glass-stoppered Pyrex bottles (200 ml.) completely filled with medium. When buffering with $NaHCO_3/CO_2$ was required, the cultures (100 ml.) were placed in 250 ml. Erlenmeyer flasks in a large desiccator which was flushed with an argon: CO_2 (95:5, v/v) mixture. A valve was inserted in the bung of the desiccator in order to allow for the expansion of the gas mixture when the desiccator was placed in the incubator. Aerobic cultures were grown in 250 ml. Pyrex conical flasks containing 100 ml. of medium.

Washed suspensions were obtained by centrifuging 3- to 4-day-old growing cultures, washing the bacterial mass with sterile distilled water and then re-centrifuging. After the washing had been twice repeated, these cells were ready for resuspension in new media under conditions similar to those described for growing cultures. The density of the suspensions was adjusted to between 20 and 50 mg. dry wt. of cells/100 ml.

Analytical procedures. Dry weights were determined turbidimetrically by measuring the $E_{1\,cm.}$ of the bacterial suspension at 1 μ. and reading off the weights on a standard curve (Goodwin & Osman, 1953*a*).