6-phosphate, arginine phosphoric acid, phosphoglycerate and free orthophosphate.

3. Evidence was obtained that all the P atoms of the ATP and ADP fractions were equally labelled.

4. The histological distribution of the ^{32}P was studied by an autoradiographic technique. A relatively high concentration of ^{32}P in the gut of the thorax was found but appeared to be of the same chemical composition as that of the muscle.

5. The effects of motor activity and cyclopropane anaesthesia upon the distribution of labelled intermediates appeared to be consistent with current views in muscle biochemistry.

6. The phosphorylated compounds of resting housefly and mammalian muscle have been compared. Relatively low phosphagen and high phosphoglycerate levels in the insect were noted.

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The Biological Action of Substances Related to Thyroxine

10. THE DE-IODINATION OF THYROXINE IN THYROIDECTOMIZED RATS*

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In previous communications (Wilkinson & MacLagan, 1953, 1954; Wilkinson, Sprott, Bowden & MacLagan, 1954) it has been shown that, in intact rats, the output of urinary iodide after a single injection of ^{131}I -labelled thyroxine or triiodothyronine is considerably reduced by the simultaneous administration of butyl 4-hydroxy-3:5-diiodobenzoate (BHDB). In contrast with these

results, Roche, Deltour & Michel (1953) reported that, in thyroidectomized rats, BHDB brought about an increase in the urinary iodide excretion after a single dose of radioactive thyroxine. Our work has therefore been extended to thyroidectomized animals in an endeavour to explain these conflicting results.

A brief report of some of the results has been published elsewhere (Wilkinson & Feetham, 1954).

* Part 9, Wilkinson & MacLagan (1954).

MATERIALS AND METHODS

Materials. Thyroxine labelled in the 3':5' positions with ^{131}I , purchased from the Radiochemical Centre, Amersham, was prepared for injection as described by MacLagan & Wilkinson (1954a). Immediately before use, the purity of the solutions was checked by paper chromatography, the thyroxine being located on the chromatograms by the method of Bowden & MacLagan (1954) or by autoradiography.

BHDB was administered in suspension (50 mg./ml.) in 1% (w/v) aqueous carboxymethylcellulose.

Animals. Male rats, weighing 160–180 g., were thyroidectomized as described by Gross & Pitt-Rivers (1953). After recovery from the operation the rats were checked for completeness of removal of the gland by comparing their weights with those of control animals and also by measuring individually their rates of O_2 consumption by the method of MacLagan & Sheahan (1950). Eleven days after operation each rat consumed 0.81–1.13 ml. O_2 /g./hr., which compares with control values in the range 1.4–1.6 ml. O_2 /g./hr. All rats which gained more than 10 g. in weight during the 15 days before the beginning of the excretion experiments were rejected.

Some confirmation of the completeness of the thyroidectomies was obtained from the fact that, of the first batch of twenty-four animals used in a preliminary experiment, all but seven died during the 4th week after operation. The deaths occurred during a spell of wintry weather when the temperature of the animal room fell by about 5° . The survivors, obviously affected by the cold, were maintained on thyroxine (approx. $2\mu\text{g./rat/day}$, as recommended by Barker *et al.* 1950), after which they recovered. Subsequently, the excretion experiments were carried out during the 3rd week after operation.

When the excretion measurements had been completed, twelve of the animals were maintained on thyroxine for 3 weeks, after which no radioactivity could be detected in the excreta. They were then placed on a low-iodine diet (oatmeal and tap water) for 4 days. Each rat was then given a single subcutaneous injection of carrier-free Na^{131}I ($5\mu\text{C.}$) and, 4 hr. later, was killed with chloroform. The trachea in the thyroid region was dissected, weighed, and dissolved in 0.4N-NaOH (10 ml.). The radioactivity of the resulting solutions was measured in a liquid counter. The tracheal uptake was less than 0.4% of the injected dose except in three cases when it was about 1%, whilst similar measurements with control animals gave thyroid uptakes of over 10%. In view of the fact that these measurements were made 6 weeks after thyroidectomy it is reasonable to assume that some measure of thyroid regeneration had occurred in the rats showing 1% iodine uptake.

Excretion experiments. Groups of three thyroidectomized rats were given BHDB (50 mg./rat) orally on the first day of the experiment and 25 mg./rat on each succeeding day. Labelled thyroxine ($2.5\text{--}20\mu\text{g./rat}$) was injected subcutaneously on the second day. Further groups received thyroxine only.

Each group was kept in a metabolism cage from which the urine and faeces were collected daily. The excreta were treated as already described (Wilkinson *et al.* 1954) and the radioactivity was measured.

RESULTS

The effect of BHDB on the output of urinary radioactivity after the injection of a single $5\mu\text{g./rat}$ dose of ^{131}I -labelled thyroxine in thyroidectomized rats is shown in Fig. 1. It will be observed that it has produced a substantial reduction similar to that found in intact rats (Wilkinson & MacLagan, 1953; Wilkinson *et al.* 1954). Comparable results were obtained when $20\mu\text{g./rat}$ doses of thyroxine were given.

Since the timing of the administration in these and the experiments with intact rats differed from that employed by Roche *et al.* (1953), an experiment was performed using their conditions. BHDB (25 mg./rat) was given orally on each of the 3 days immediately preceding the thyroxine injection. Of the two groups of three rats treated with BHDB, one showed an appreciable reduction in the excretion of urinary radioactivity, whilst the output of the other was indistinguishable from that of the control groups. The rats in this group, however, were constipated during the first day of the collection.

Further experiments were therefore performed in which BHDB was given on the day preceding the thyroxine injection and on the next 2 days only. The results obtained appear in Fig. 2. During the first 2 days after the administration of thyroxine the urinary radioactivity of the treated groups was much reduced as compared with that of the controls. Subsequently, however, the output increased until after 5 days it exceeded that of the controls. The change in the rate of excretion coincided with the onset of severe constipation in both BHDB-treated

Fig. 1. The effect of BHDB on the urinary radioactivity of thyroidectomized rats treated with a single subcutaneous injection of ^{131}I -labelled thyroxine ($5\mu\text{g./rat}$). O, the combined output of a group of three rats treated with thyroxine alone; ●, the combined output of three rats treated with thyroxine + BHDB (see text for dosage).

groups. During the 48 hr. (3–5 days) each group of three rats produced only about 0.5 g. faecal pellets, instead of the normal 10–20 g./day.

The results of the faecal measurements in this and earlier experiments are summarized in Table 1. It will be observed that in each case where a low faecal output is recorded, severe constipation appears to

be the immediate cause. This seems to be more liable to occur when BHDB is administered together with minimal thyroxine dosage.

DISCUSSION

Since it has been established that the urinary radioactivity of rats treated with thyroxine labelled in the 3':5' positions with ¹³¹I is almost entirely due to iodide (Wilkinson *et al.* 1954), it follows from the results shown in Fig. 1 that BHDB reduces the output of urinary iodide. Consequently, it may be concluded that it interferes with the de-iodination of thyroxine in thyroidectomized animals in much the same way as in intact rats.

A possible explanation may be suggested to account for the surprising results of Roche *et al.* (1953) who, using one group of three rats, found exactly the opposite effect. Their rats were treated with BHDB on each of the 3 days preceding the thyroxine injection only. It is therefore probable that the concentration of the drug in the tissues after the thyroxine was given would be too low to exert any inhibitory effect, for it has been shown that, in the rabbit, BHDB is very rapidly excreted (Maclagan & Wilkinson, 1954*b*). In our experiments however, BHDB was given daily before and after the administration of thyroxine so as to ensure an adequate concentration in the tissues throughout the experiment.

This difference in timing of the BHDB treatment might well account for the absence of a reduction in the urinary iodide output in the experiments of Roche *et al.*, but it could hardly explain the increase they reported. We therefore decided to vary the

Fig. 2. The effect of stopping treatment with BHDB on the urinary radioactivity of thyroidectomized rats given a single subcutaneous injection of ¹³¹I-labelled thyroxine (2.5 µg./rat). ○, the combined output of a group of three rats treated with thyroxine alone; ●, the combined output of a group of three rats treated with thyroxine + BHDB (see text for dosage).

Table 1. The effect of changes in the timing of the oral administration of BHDB on the faecal radioactivity of thyroidectomized rats treated with ¹³¹I-labelled thyroxine

Each row of figures refers to the combined excretion of a group of three rats. Dosage of BHDB: A, 25 mg./rat on each of 3 days preceding that on which the thyroxine was injected; B, 50 mg./rat on day before thyroxine injection, and 25 mg./rat on each of next 2 days; C, 50 mg./rat on day before thyroxine injection and 25 mg./rat daily throughout the experiment. ‘+’ indicates that rats were severely constipated.

Dosage of		Percentage of dose excreted				
		In faeces after (hr.)				
		24	48	72	96	120
Thyroxine (µg./rat)	BHDB					
2.5	None	27.4	43.8	51.9	—	57.9
		17.2	30.3	40.2	—	54.9
2.5	A	1.4 +	20.2 +	—	27.3 +	28.8 +
		18.7	28.1	—	36.8 +	38.3 +
2.5	B	19.5	26.7	32.8 +	—	33.4 +
		22.4	29.7	32.9 +	—	39.8 +
5	None	18.0	39.8	50.7	—	55.3
		24.1	39.2	50.2	—	56.1
5	C	31.0	51.4	57.6	—	61.5
		26.5	47.4	55.7	—	59.0
20	None	29.2	46.1	—	52.2	52.7
20	C	36.4	48.2	—	54.7	55.7

timing of the BHDB treatment and first repeated their experiment in duplicate. One group showed no decrease of urinary iodide output, whilst the other showed appreciable diminution. The effect of conditions intermediate between those used in our experiments and those of the French workers was investigated in another experiment in which the BHDB treatment was stopped 24 hr. after the thyroxine injection. During the first 2 days the inhibitory effect of BHDB was evident (Fig. 2), but later the iodide output increased in a manner similar to that reported by Roche *et al.*

The change in the rate of iodide excretion in this experiment coincided with the onset of severe constipation in both groups of BHDB-treated rats. Instead of the normal 10–20 g./day of faeces these rats excreted less than 0.5 g./day. The constipation appeared to be due to loss of appetite rather than to a direct effect of the withdrawal of BHDB. A study of the faecal radioactivities (Table 1) suggests that small doses of thyroxine (2.5 µg./rat) were insufficient to overcome the systemic effects of 3 days' treatment with BHDB, whilst larger doses of thyroxine were capable of preventing loss of appetite and the resulting constipation.

In a relative degree of intestinal stasis, thyroxine and its metabolic products excreted in the bile would undergo a much greater degree of re-absorption than in a normal gut. These re-absorbed substances would then be subjected to further de-iodination and more iodide would be available for excretion by the kidney. Thus the net result of severe constipation would be the diversion of radioactivity to the urine. Such an effect might account for the results of Roche *et al.* (1953) for in a footnote they report that in their experiments the faecal radioactivity was minimal.

We therefore suggest that BHDB reduces the rate of de-iodination of thyroxine in thyroidectomized as well as in intact rats, but the use of urinary iodide output as a measure of this process is only possible

when the administration of the BHDB is correctly timed and when complications due to constipation do not occur.

SUMMARY

1. The output of urinary iodide by thyroidectomized rats after the injection of [3':5'-¹³¹I]thyroxine was substantially reduced by the simultaneous administration of butyl 4-hydroxy-3:5-diiodobenzoate.

2. A probable explanation of some conflicting results reported in the literature is given.

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The Hydration of the Cornea

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In the dead eye the cornea swells, at the same time losing its transparency; similar changes are observed when the isolated cornea is kept in physiological saline. These, and other, observations suggest that the cornea, *in vivo*, has a constant tendency to absorb water from its surrounding fluids, a tendency that is opposed by dehydrating factors which cease to operate on the death of the

animal. Essentially, the cornea consists of a number of collagenous laminae—the stroma—separated from the tears by the epithelium, and from the aqueous humour by the endothelium (Fig. 1); it is only at the limbus, through the conjunctival capillaries, that the cornea comes into direct relationship with the blood. Under the electron microscope the structural units of the laminae are