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THYROXINE DEIODINATION DURING COLD EXPOSURE IN THE RAT

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SUMMARY

1. The urinary radio-iodide excretion following injection of [^{131}I]thyroxine and tri-iodothyronine is increased in acutely cold-exposed, thyroidectomized rats.

2. This cold response is demonstrable in animals which are fed or fasted or deprived of both food and water. Cold exposure does not increase the rate of urinary excretion of a tracer dose of radio-iodide. It is concluded that cold exposure accelerates the rate of deiodination of both thyroxine and tri-iodothyronine.

3. It is confirmed that both adrenaline and noradrenaline administered *in vivo* can enhance the rate of thyroxide deiodination.

4. The sympathetic blocking agents guanethidine and bethanidine reduce the extra deiodination of thyroxine induced by cold exposure. Bethanidine was also found to reduce the deiodination of tri-iodothyronine during cold.

5. There is a positive correlation between the deiodination of thyroxine and the urinary catecholamine excretion in the cold-exposed but not the warm-exposed rat.

6. It is suggested that the enhanced deiodination during cold exposure is mediated by the release of noradrenaline from the sympathetic nervous system.

INTRODUCTION

In a previous paper (Hillier, 1968) it was noted that the reduction in the biological half-life of thyroxine during cold exposure was only partly explained by acceleration of the biliary-faecal route of its excretion. The second major metabolic pathway for thyroxine is deiodination (Tata, 1964). The inorganic iodide liberated during this process is excreted into the urine and an estimate of the urinary radio-iodide excretion following injection of radioactive thyroxine can be used as an index of deiodination (Pitt-Rivers & Tata, 1959). In this paper an investigation of thyroxine deiodination during cold exposure is reported.

METHODS

Methods and materials used in this investigation have been described elsewhere (Hillier, 1968). Further procedures however need description.

Urine collection was made from animals kept in metal metabolism cages into polythene funnels; course metal grids at the base of the funnels were used to trap faeces. In early experiments urine was collected into about 10 ml. of a potassium iodide solution (10 g/100 ml.) in order to prevent any possible adsorption of iodide on to the glass. This procedure proved unnecessary however and was later abandoned. In order to make the animals empty their bladders at the end of a collection period a few drops of ether were placed on their backs. Special care was taken to ensure all urine was washed off from the sides of the collection funnels and the faeces. This was done by making the volume of collected urine up to about 90 ml. and using this to wash over the funnels and faeces several times. The volume was then made up to exactly 100 ml. and 2 ml. aliquots of this taken for radioactive assay.

Special precautions were taken when urine was to be measured for catecholamines. The floor of the metabolism cage was made of thin glass rods, a polythene faeces trap was used and the urine was collected into 20 ml. of 0·05 N hydrochloric acid. Under these conditions the catecholamines were stable over the collection period (Leduc, 1961).

The method of catecholamine extraction and estimation was that used by Euler & Lishajko (1961). Leduc (1961) found that about 90% of the catecholamine in the urine of acutely cold exposed rats is noradrenaline. In this present study the catecholamine in urine was estimated as though it were all noradrenaline (separation of adrenaline and noradrenaline is much more difficult). This introduces a slight error in the results because the fluorescence produced by the lutine from adrenaline is about twice as intense as that from noradrenaline with the filter sets used. This means in practice that the values for total catecholamine obtained by this method are about 5–10% higher that they should be (since about 5–10% of the urine catecholamine is adrenaline), the error is however a consistent one and does not influence the interpretation of the results. A Direct Reading Fluorimeter, Model 27 A (Electronic Instruments Ltd.) was used with Ilford filters Nos. 501 (primary) and 109 (secondary).

The radioactive tri-iodothyronine, thyroxine and inorganic iodide were obtained from the U.K. Atomic Energy Authority, The Radiochemical Centre at Amersham. The method of storage of tri-iodothyronine used was the same as that for thyroxine described elsewhere (Hillier, 1968).

The solutions of radio-active hormone were contaminated with iodide (determined chromatographically). Iodide usually accounted for between 5 and 10% of the total activity. On injection into rats this inorganic radio-iodide is very rapidly excreted especially if the animals are fed, so that about 80% is lost into the urine within 24 hr (see results). If the original thyroxine solution therefore had a 5% iodide contamination and it was used to determine the thyroxine deiodination over a 30 hr period 5% of the dose of radio-activity found in the urine would not have been derived from deiodinated thyroxine. For this reason the iodide contamination of samples of hormone were determined and a correction made; in the example given above if the total urine radio-activity accounted for 29% of the dose, then 5% would be removed to give the true deiodination value of 24%. This correction was made in all cases.

The following drugs were used. Guanethidine sulphate (C.I.B.A.) administered in aqueous solution (15 mg/ml.); bethanidine sulphate (Burroughs Wellcome) also in aqueous solution (40 mg/ml.); 'Adrenalin in Oil' (Parke Davis & Co.) containing 2·0 mg. adrenaline/ml. peanut oil; noradrenaline (Light and Co.), a suspension of this in peanut oil was made containing 200 μg./0·05 ml.

Results are given as a mean $\pm$ one standard deviation and the significance of differences

between means was estimated by Student's method. In one experiment (illustrated in Fig. 6) a correlation coefficient (r) was estimated by a method described by Fisher (1932). The significance of the observed correlation coefficient was also estimated by a method described there.

RESULTS

Thyroxine deiodination during cold exposure

Male rats were used which had been thyroidectomized for 24 hr. Radioactive thyroxine was given intraperitoneally at a dose of 1·5 μg/100 g and the urine collected for the next 30 hr. At the end of that period a blood sample was taken. Over the collection period the animals were fasted. The experimental animals were placed in the cold room immediately after the injection.

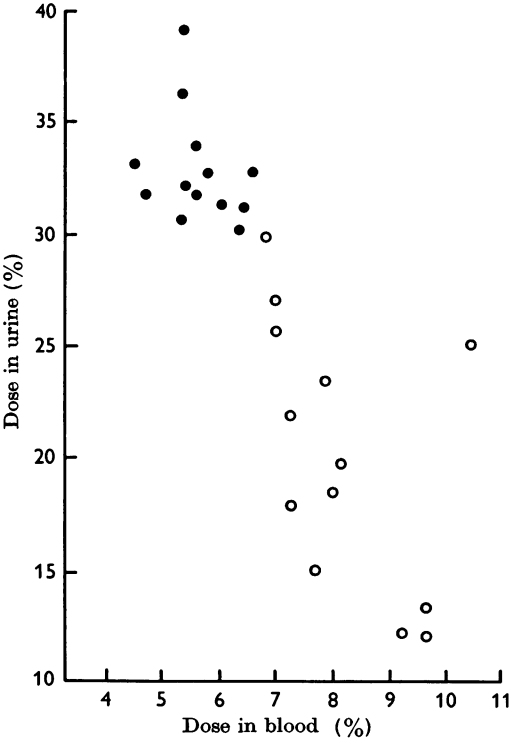

Fig. 1. The urinary excretion of radio-iodide following injection of radioactive thyroxine over the first 30 hr period, together with the percentage of the dose of thyroxine remaining in the blood at 30 hr. Warm-exposed rats ○; cold-exposed rats ●.

The results are shown in Fig. 1. At 30 hr the experimental group had 1·74 % less of the dose of thyroxine remaining in its blood ($P < 0·001$) and had 12·6 % more of the dose in its urine ($P < 0·001$). Chromatographic

analysis of the urine radioactivity showed that more than 97 % of it was inorganic iodide.

Tri-iodothyronine deiodination during cold-exposure

A similar experiment was performed using radioactive tri-iodothyronine. Male rats were used which had been thyroidectomized for 2 days. Radioactive tri-iodothyronine was given intraperitoneally at a dose of 0·014 μg/100 g.

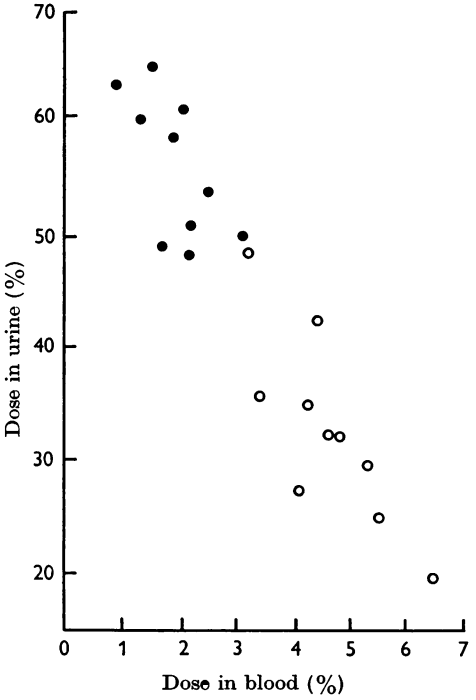

Fig. 2. The urinary excretion of radio-iodide following injection of radioactive tri-iodothyronine over the first 30 hr, together with the percentage of the dose of tri-iodothyronine remaining in the blood at 30 hr. Warm-exposed rats ○ ; cold-exposed rats ●.

The results are shown in Fig. 2. At 30 hr the experimental group had 2·67 % less of the dose remaining in its blood ($P < 0·001$) and had 22·7 % more of the dose in its urine ($P < 0·001$).

It was possible that cold exposure was not affecting deiodination but the rate at which inorganic iodide was being cleared by the kidney. This suggestion was unlikely for several reasons.

(i) In control experiments the rate of excretion of a tracer dose of radio-iodide was examined both in intact rats and rats treated with 5 mg

of potassium perchlorate (to inhibit any thyroid uptake of iodide). No evidence of any acceleration in the rate of iodide excretion during cold exposure was obtained (Table 1).

(ii) If the animals were fed 15·0 g Remington diet the rate at which a tracer dose of radioiodide was excreted was increased (Table 1). Following injection of radioactive thyroxine, feeding the Remington Diet caused a rise in the deiodination rate which was similar in size to that caused by cold exposure. Table 1 gives therefore some indication of the sort of difference in renal excretion of iodide which must be induced by cold if its effect was to be due to an action on the kidney and not on deiodination. (The extra deiodination induced by Remington feeding is probably due to its salt content since it can be reproduced by feeding glucose containing equivalent amounts of sodium chloride but not by feeding glucose alone.)

(iii) When both control and cold-exposed animals were fed 15·0 g Remington Diet, because they have a more rapid iodide excretion, both groups showed this extra apparent deiodination when compared to fasted animals. They still, however, showed a normal cold response.

(iv) Halmi, King, Widner, Hass & Stuelke (1958) have shown that chloride excretion accelerates the excretion of radio-iodide and it has been reported that in rats fed *ad libitum* acute cold exposure can induce a brief negative chloride balance (Baker, 1960). Examination of the urinary chloride excretion in fasted and fed rats was therefore undertaken. The results (Table 2) failed to show any difference between warm and cold exposed animals.

(v) A normal cold response was shown by animals deprived of both food and water.

The effect of sympathetic blocking agents on the extra deiodination induced by cold

The effects of two potent inhibitors of noradrenaline release were investigated, guanethidine and bethanidine (Boura & Green, 1965).

(i) *Guanthidine.* Forty-six male rats were used which had been thyroidectomized for 24 hr. Radioactive thyroxine was given intraperitoneally at a dose of 1·0 μg/100 g and urine collected for the next 24 hr when blood samples were taken. The experimental animals were exposed to a cold environment after the radioactive injection and all of the animals were each given 15·0 g Remington Diet. Half of the animals received guanethidine sulphate at a dose of 1·5 mg/100 g; two injections were given at this dose level 50 and 26 hr before death (Boura & Green, 1965).

The results are shown in Fig. 3. At 24 hr the untreated groups were showing a normal cold response; the cold exposed animals had excreted 9·2 % more of the dose into their urine ($P < 0·001$) and had 2·3 % less of

the dose in their blood ($P < 0{\cdot}01$) than the controls. However, in the guanethidine-treated groups the difference was reduced to 2·3% in the urine ($0{\cdot}05 > 0{\cdot}02$) and 0·1% in the blood. Guanethidine treatment therefore considerably reduced the size of the cold response.

TABLE 1. The urinary excretion of a tracer dose of inorganic radio-iodide (expressed as a percentage of the dose) in three groups of rats. There were four animals in each group

	Time after injection (hr)		
	6	12	24
Fasted, warm-exposed	34	53	60
Fasted, cold-exposed	43	54	61
Fed with Remington Diet *ad libitum* warm-exposed	59	68	89

TABLE 2. The chloride excretion over an 18 hr period in four groups of rats. The results are expressed as m-equiv of chloride per rat. There are four rats in each group

	Warm-exposed	Cold-exposed
Fasted	0·45	0·45
Fed with 10 g Remington Diet	1·67	1·70

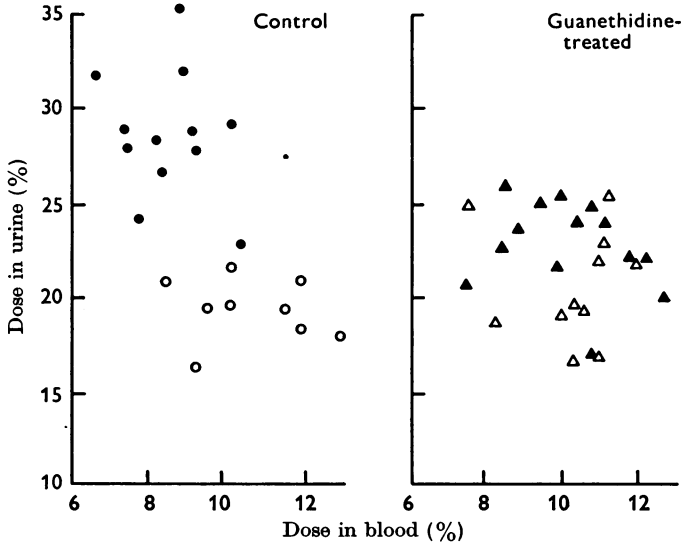

Fig. 3. The percentage of a dose of radioactive thyroxine remaining in the blood 24 hr after its injection together with the urinary radio-iodide excretion over the same period in four groups of rats. Warm-exposed rats ○ △; cold-exposed rats ● ▲. Untreated rats ○ ●; rats treated with guanethidine △ ▲.

(ii) *Bethanidine.* Forty-five male rats were used which had been thyroidectomized for 24 hr. Radioactive thyroxine was given intraperitoneally at a dose of 1·0 μg/100 g and urine collected for the next 22 hr

when blood samples were taken. Bethanidine sulphate was given intraperitoneally at a dose of 4·0 mg/100 g, 36, 24 and 12 hr before death (Boura & Green, 1965). The experimental groups were exposed to a cold environment after the radioactive injection and all of the animals allowed 15·0 g Remington Diet.

The results are given in Fig. 4. At 22 hr the intact groups were showing a large cold response, the cold-exposed animals had 9·6 % more of the dose of thyroxine in their urine ($P < 0{\cdot}001$) and had 2·5 % less in their blood

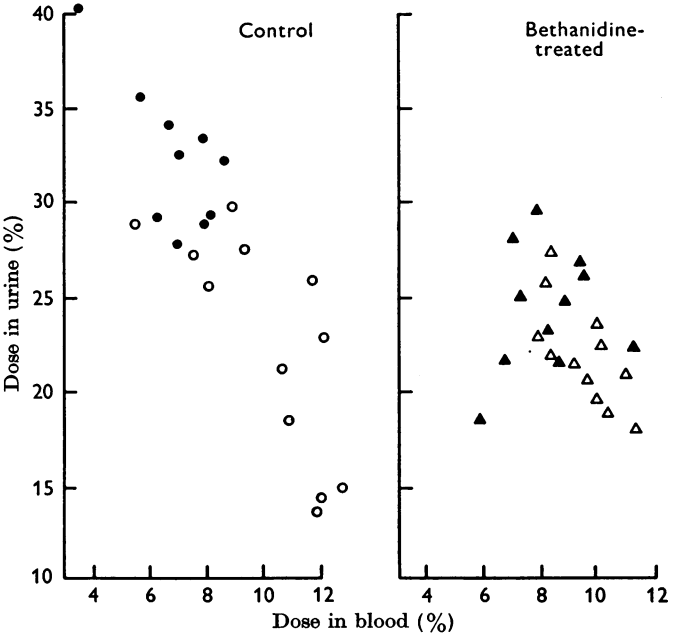

Fig. 4. The percentage of a dose of radioactive thyroxine remaining in the blood 22 hr after its injection together with the urinary radio-iodide excretion over the same period in four groups of rats. Warm-exposed rats ○ △; cold-exposed rats ● ▲. Untreated rats ○ ●; rats treated with bethanidine △ ▲.

($P < 0{\cdot}01$) than the controls. In the bethanidine-treated groups, however, these differences were reduced to 2·7 % in the urine ($0{\cdot}05 > P > 0{\cdot}02$) and 0·9 % in the blood ($0{\cdot}05 > P > 0{\cdot}02$). Bethanidine treatment therefore reduced the size of the cold response.

In another experiment the effect of bethanidine on the deiodination of tri-iodothyronine was investigated. The conditions were exactly similar to those in the thyroxine experiment except that the dose of tri-iodothyronine used was 0·1 μg/100 g. Because of the limited supply of radioactive material only the two cold-exposed groups were prepared.

The results are given in Fig. 5. At 22 hr the bethanidine-treated animals

had 11·3 % less of the dose in their urine ($P < 0{\cdot}001$) and had 0·6 % more in their blood ($P < 0{\cdot}01$).

Relating to these observations two control experiments were performed. Firstly, the effect of these blocking agents on the urinary excretion of a tracer dose of radio-iodide was determined in cold-exposed animals. No evidence was obtained suggesting a difference between the control and

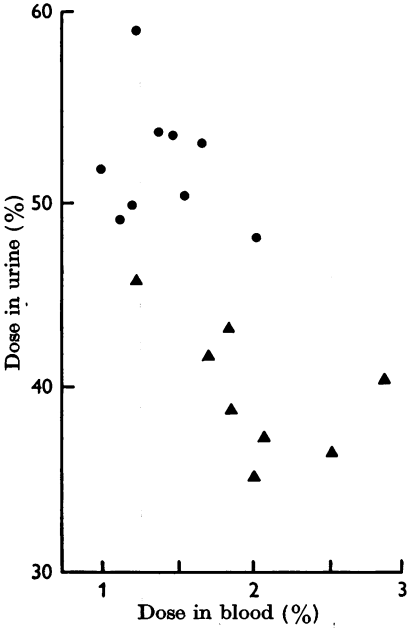

Fig. 5. The percentage of a dose of radioactive tri-iodothyronine remaining in the blood 22 hr after its injection, together with the urinary radio-iodide excretion over the same period in two groups of cold-exposed rats. Untreated rats ●; rats treated with bethanidine ▲.

treated groups. Secondly, because these blocking agents have some local anaesthetic action the effect of another local anaesthetic on the thyroxine deiodination during cold was examined. Cocaine hydrochloride injected subcutaneously during the cold-exposure period, at a rate of 0·3 mg/100 g/12 hr had no action on thyroxine deiodination as estimated by the urinary radio-iodide excretion.

Thyroxine deiodination and the urinary catecholamine excretion during cold—a correlation

Leduc (1961) found that in the rat cold exposure caused a sudden large increase in the urinary noradrenaline excretion from the post-ganglionic sympathetic nerves and a much smaller increase in the excretion of

adrenaline from the adrenal medulla. (During the first day of cold exposure about 90 % of the total urinary catecholamines is noradrenaline.)

In the present experiment the total urinary catecholamine was measured and used as an index of sympathetic activity. In addition the urinary radio-iodide excretion following a tracer dose of radio-thyroxine was determined and this was used as an index of thyroxine deiodination. These two quantities were measured together in the same animal to see if there was any correlation between them.

Intact male rats were used and given a subcutaneous injection of radioactive thyroxine at a dose of 0·1 μg/rat. Urine was collected for the next 30 hr and urinary radioactivity and catecholamine excretion were determined. Some of the rats were cold exposed during the collection period and all were fasted.

The result is given in Fig. 6. The cold-exposed animals had 6·7 % more of the dose of thyroxine in their urine ($P < 0{\cdot}001$) and excreted more catecholamine (1·70 μg/100 g; $P < 0{\cdot}001$). In the cold-exposed group there was a positive correlation between thyroxine deiodination and catecholamine excretion ($r = 0{\cdot}53$; $P < 0{\cdot}002$). There was no such correlation in the warm-exposed animals. This result was therefore in accord with the hypothesis that the enhanced deiodination during cold exposure is caused by greater sympathetic nervous activity.

The effect of catecholamines on the deiodination of thyroxine

Several workers have found that in the rat adrenaline injections accelerate the rate at which thyroxine is lost from the blood (Williams, Jaffe & Kemp, 1949; Botkin & Jensen, 1952; Eskelson, Firschein & Jensen, 1954). Adrenaline in the rat also accelerates the *in vivo* deiodination of thyroxine (Kallman & Starr, 1959; Escobar & Morreale, 1963; Galton, 1965). Kallman & Starr (1959) have also demonstrated an effect of adrenaline on the deiodination of tri-iodothyronine and Galton (1965) has found that *in vivo* treatment of mice with adrenaline or noradrenaline increases the deiodinating activity of liver homogenates. Two further experiments on this subject are reported here.

Ten male rats were thyroidectomized and five were given adrenaline in oil intraperitoneally at a rate of 100 μg/100 g body wt./day for 4 days (each 100 μg of adrenaline was contained in 0·05 ml. peanut oil). Two hours before the last injection radioactive thyroxine was given intraperitoneally at a dose of 0·1 μg/100 g and the urine collected for the next 24 hr. During the collection period the animals were fasted. Over that period the adrenaline-treated group excreted 8·5 % more of the dose into the urine ($P < 0{\cdot}02$).

In another experiment the effect of noradrenaline was investigated. Five

male rats weighing about 250 g each were thyroidectomized and given thereafter daily intraperitoneal injections of thyroxine at a rate of 4·0 μg/rat/day. Three days after the beginning of this treatment the animals were placed in individual metabolism cages and urine collections were made at

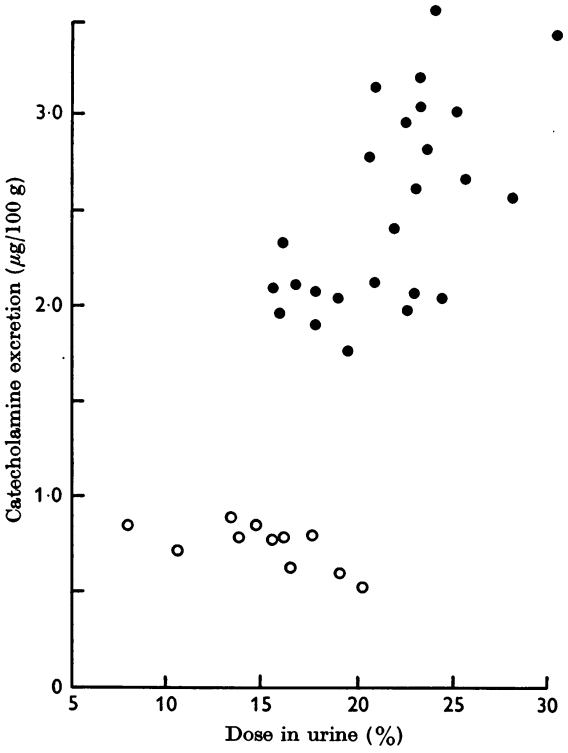

Fig. 6. The total urinary catecholamine excretion (expressed as noradrenaline) together with the urinary excretion of radio-iodide following injection of radioactive thyroxine in two groups of rats. The collection period was 30 hr. Warm-exposed rats ○; cold-exposed rats ●.

24 hr intervals over the next 7 days. On two consecutive days noradrenaline in oil (100 μg/0·05 ml. peanut oil) was given intraperitoneally in a dose of 200 μg/100 g 2 hr after the thyroxine injections. The animals were fed throughout on Remington Diet *ad libitum*.

The result is illustrated in Fig. 7. The noradrenaline injections raised the urinary radio-iodide excretion. Noradrenaline is certainly less potent than adrenaline (Galton, 1965) and in both cases large doses are necessary.

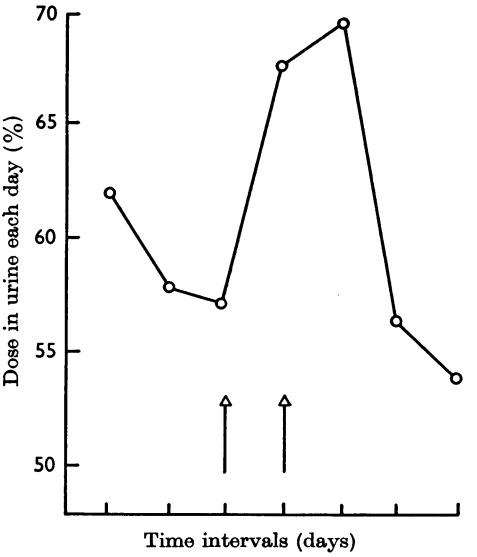

Fig. 7. The mean daily urinary radio-iodide excretion by a group of five rats receiving radioactive thyroxine by daily injections. The animals received thyroxine for 3 days before being placed in the metabolism cages. The time scale is in days. Noradrenaline in oil was given intraperitoneally on two consecutive days at a dose of 200 μg/100 g body wt.

DISCUSSION

In this paper evidence is presented in support of the view that exposure to cold accelerates the rate of thyroxine deiodination and that this effect is caused by increased activity of the sympathetic nervous system. There are however difficulties in this theory.

The blocking agents, guanethidine and bethanidine, are non-toxic, potent inhibitors of noradrenaline release from sympathetic nerve endings (Boura & Green, 1965). However, their mechanism is not fully understood and some side effect may have accounted for the above results. These agents did not, however, produce a general depression in thyroxine deiodination since they only affected the extra deiodination induced by cold. This suggested that their inhibitory effect was not due to a general toxic action on thyroxine metabolism and that the 'resting rate' of deiodination was not under 'tonic sympathetic stimulation' to any great extent. Further, the animals withstood treatment with these drugs very well and their appearance and behaviour made them indistinguishable from untreated animals. Perhaps an important reason for this is that guanethidine and bethanidine do not affect the release of adrenaline from the adrenal medulla (Boura & Green, 1965), so that this supply of cate-

cholamine was available to the animals during exposure to cold. In the absence of further evidence it would seem reasonable to conclude that these drugs produced their inhibitory effect by virtue of their ability to block the release of noradrenaline from the sympathetic nerve endings.

The doses of catecholamine required to accelerate thyroxine deiodination are large—most workers have used 100 μg/100 g. How does this compare with the amounts secreted by the rat during cold? Over the first day of cold exposure the total urinary catecholamine excretion is about 2·0 μg/100 g. This value is in agreement with the findings of Leduc (1961). Since about 3 % of an injected or infused dose of noradrenaline is excreted into the urine (Leduc, 1961), 2 μg in the urine represents a secretion or overspill rate into the blood of about 60 μg/100 g. Estimate of the secretion rate actually within the tissues is difficult but it clearly must be in excess of 60 μg/100 g. It is important also to note that, of the injected amine, only a fraction will ultimately arrive at those sites where it can influence thyroxine metabolism. Large doses of catecholamine are therefore necessary in order to attempt to mimic the intense degree of sympathetic activity in the tissues during cold exposure. It is important to note that the major sites of noradrenaline release during cold exposure—liver and skeletal muscle (Leduc, 1961) are also important sites of thyroxine deiodination (Tata, 1964) and probably also of non-shivering thermogenesis (Depocas, 1960).

For several reasons the adrenal medulla is unlikely to play a major part in accelerating thyroxine deiodination during cold exposure. Firstly, Leduc (1961) noted that about 90 % of all the catecholamine which was secreted or which escaped into the blood during acute cold exposure was noradrenaline and that cold was a much more potent stimulant to noradrenaline than to adrenaline secretion. Secondly, investigations on the role played by catecholamines during cold in the mobilization of food materials (Brodie, Maikel & Stern, 1965) and the stimulation of thermogenesis (Hsieh & Carlson, 1957) strongly emphasize the dominant role played by noradrenaline as opposed to adrenaline. Thirdly, in experiments reported above (in which a considerable reduction in the size of the cold response was observed) the blocking agents used prevent only the release of noradrenaline from the peripheral sympathetic nerve endings and do not impede the secretion of adrenaline from the adrenal medulla (Boura & Green, 1965). Further, experiments were also performed (but are not reported here) which failed to demonstrate any difference in ability between intact and chronically adrenal-demedullated animals to produce a thyroxine cold-response.

The mechanism by which catecholamines enhance thyroxine deiodination is not clear. Galton (1965) has shown that they do not affect the renal

excretion of inorganic iodide. Rall (1966) has suggested that their effects on thyroxine metabolism may be secondary to their cardio-vascular actions. This, however, is unlikely in view of the large doses required to demonstrate any action and also because their effects on thyroxine persist long after the circulatory changes have subsided (Galton 1965). The observation that in mice treatment with adrenaline or noradrenaline *in vivo* can increase the deiodinating activity of liver homogenates suggests a possible action on the enzymes of deiodination (Galton 1965). A full understanding of this problem must await further investigations.

It has been suggested that the deiodination of thyroxine is in some way related to its hormonal action (Escobar & Morreale, 1963). The demonstration of enhanced thyroxine deiodination during cold exposure possibly indicates the existence of a mechanism which may, to some extent, be able to regulate the peripheral utilization of the hormone.

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