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THE BILIARY-FAECAL EXCRETION OF THYROXINE DURING COLD EXPOSURE IN THE RAT

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SUMMARY

1. The biliary-faecal route of thyroxine excretion has been examined by means of thyroxine labelled with ^{131}I in the thyroidectomized, thyroxine-maintained rat during cold exposure.

2. The rate of excretion of thyroxine into the faeces is raised in rats exposed to cold for 2 weeks and fed *ad libitum* on a high roughage diet.

3. When the faecal volumes of warm- and cold-exposed animals are made similar by controlling the roughage content of the diet the difference in their faecal thyroxine excretion rates is much reduced.

4. Warm- and cold-exposed rats eating the same amount of food excrete thyroxine into their faeces at similar rates.

5. Even when warm- and cold-exposed rats excrete thyroxine into their faeces at the same rate the cold-exposed animals still show a shorter half-life for blood thyroxine.

6. An acceleration of the rate of loss of thyroxine from the blood is demonstrable in fasting animals within 10 hr of exposure to cold.

7. Ligation of the bile-duct does not affect this acute response to cold.

8. The amount of thyroxine lost into the bile within the first 8 hr after intravenous injection is similar in warm- and cold-exposed animals. The clearance of thyroxine into the bile is however greater in the cold-exposed animal since the liver is working against a lower level of blood thyroxine.

9. An acceleration of the biliary-faecal route of thyroxine excretion is not the only process at work tending to reduce the biological half-life of thyroxine during cold exposure.

INTRODUCTION

During cold exposure the rate at which thyroxine is removed from the blood is increased (Rand, Riggs & Talbot, 1952; Bondy & Hagewood, 1952; Cottle & Carlson, 1956). Intoccia & Van Middlesworth (1959) have further

demonstrated in the rat that exposure to cold for about 2 weeks accelerates the rate at which thyroxine is excreted into the faeces. They suggested that this effect might be due to the higher faecal volume in the cold-exposed animals. Cottle (1964) has confirmed this result but also demonstrated in the cold-adapted rat a higher biliary thyroxine clearance. The mechanism by which cold exposure accelerates the faecal thyroxine excretion rate is therefore not yet resolved and in this paper an investigation of the problem is reported using thyroidectomized, thyroxine-equilibrated animals. The purpose was to determine the extent to which these two factors (biliary thyroxine clearance and faecal volume) contribute to the accelerated faecal thyroxine excretion and also to determine whether the acceleration of the biliary-faecal excretion route was the only mechanism at work tending to reduce the biological half-life of the thyroxine during cold.

METHODS

Albino rats were used in this study and male animals were used in all experiments except two (which are specified in the text). Within any one experiment all of the animals were within 30 g body wt. of one another and the average weight of rat used was about 220 g.

Thyroidectomy was performed surgically under ether anaesthesia and a fine dusting of fibrin powder was used sometimes to control bleeding. The cutting instruments used to remove the gland were straight, triangular pointed, sharp needles (size 1). Following thyroidectomy the animals were given calcium gluconate (1 g/100 ml.) (Hopkin & Williams) to drink in tap water. Either 1 or 2 days were allowed for recovery before the animals were placed on stable thyroxine therapy at the start of an experiment.

For intravenous injections an indwelling cannula was fixed in place. This consisted of a length of polythene tubing (Portex, PP. 30) drawn out to a fine tip, filled with saline and stoppered by a wire plug. The cannula was placed in the external jugular vein in the neck and fed down so that the tip just entered the thorax. A length of tubing was brought round subcutaneously to the back of the neck and an extra loop concealed under the skin of the back. At the time of injection the animal was confined in a warm small chamber, the subcutaneous loop of tubing pulled out from under the skin (this seemed quite painless to the animals), the injection given (at a dose of 0·1 ml./100 g) and washed in with 0·2 ml. of saline and the tube sealed off close to the skin by pinching it with hot forceps.

Bile collections were made as follows. The cannula consisted of a long length of polythene tubing (Portex PP. 30), drawn out fairly fine at both ends. One end of the cannula was inserted into the bile duct as high up to its bifurcation as possible (to avoid damage to the pancreatic ducts which enter the bile duct in its distal section). The other end of the cannula was inserted into the bile duct just below the first insertion but was fed in the opposite direction down into the lumen of the intestine. These lengths of tubing were secured to the wall of the abdominal cavity where they penetrated it and the loop formed by them was brought subcutaneously round to the back of the neck and secured to the skin there. Bile flowed freely through the cannula into the intestines. In one or two cases obstructions developed which were readily detected by poor bile flow when the cannula was divided at the back of the neck, and also by blanching of the yellow bile pigment in the stagnant bile in the part of the cannula (which was exposed to light) at the neck. Animals in which there was any obstruction were not used. By this method patent ducts were found even after 10 days although in the experiments reported here no animal was used which had been cannulated for more than 24 hr. When bile was to be collected the tube at the neck was

divided and fitted with 6 in. (15·2 cm) lengths of fine metal tubing (syringe needle blanks size 15) and further polythene tubing put on the ends of these. Collection was therefore possible through the proximal limb of the cannula (since the collecting tube was about 12 in. (30·5 cm.) below the animal the bile was collected under a slight negative pressure) and reinjection through the distal limb. The animals, when confined to a small cage, readily tolerated having the tubing attached to them for many hours.

Cold exposure was performed in a small cold-room which was kept at about 4 ± 2 °C. The lighting was not controlled but generally it was illuminated for about 8 hr during the day. The room received a faint natural illumination. The animals were removed for about 20 min each morning for cleaning and injections. Wire cages were used which also had wire bottoms, the faeces and urine being dropped on to sawdust underneath. Each compartment was about 7 in. × 8 in. (17·8 × 20·3 cm) and usually one animal was kept in each; in a few cases however, two animals were placed together. Protection was given against draughts by using either cardboard or polythene sheeting around the cages (this did not affect the air temperature around the animals).

The control animals were kept at room temperature which was maintained at about 22 ± 2 °C. These animals are described in the results as warm-adapted or warm-exposed.

When collection of faeces was to be made metabolism cages with wire bottoms and plastic funnels with metal grids to trap the faeces were used. Before the faecal radioactivity was assayed the pellets were washed free of urine in tap water. In one experiment faecal volume was measured by packing the faeces down tightly into a 25 ml. measuring cylinder after they had been washed and blotted dry.

The percentage of a dose of radioactivity remaining in the blood was determined as follows. The animals were killed by administration of coal gas and the thorax was rapidly opened. A 2 ml. blood sample was taken from the right atrium, weighed and its radioactivity determined. In order to obtain a value for total blood radioactivity a value for the total blood weight is required. This was taken as 7 g blood/100 g body wt. (Wang, 1959). This value was used for both the warm- and cold-exposed rats since changes in blood volume and haematocrit on cold exposure are very small, a few per cent (Baker, 1960).

Assay of the protein-bound radioactivity was made as follows. Animals were killed with coal-gas and about 8 ml. of blood was removed by cardiac puncture, heparinized and centrifuged. One millilitre of plasma was taken, its radioactivity determined and it was then injected into 9 ml. of trichloroacetic acid (T.C.A.) (Hopkin & Williams, 20 g T.C.A./100 ml. water). The mixture was shaken well, centrifuged and the radioactivity of the supernatant (iodide) assayed and also that of the precipitate (thyroxine) after it had been redissolved in sodium hydroxide solution (20 g/100 ml.).

Animals were fed on Oxoid Diet 41 B (Oxo Ltd.) *ad libitum*. In some experiments Remington's Low-Iodine Diet (Nutritional Biochemicals Corp.) was given and mixtures of this with cellulose powder (Hopkin & Williams, Whatman B quality, coarse grade). The proportion of cellulose varied between 0 and 40 % by weight.

[^{131}I]Thyroxine was obtained from the U.K. Atomic Energy Authority's Radiochemical Centre, Amersham. It was analysed chromatographically in order to determine the degree of radio-iodide contamination. The solvent used was butanol:acetic acid:water (78:10:12) (reagents from Hopkin & Williams) and the paper was Whatman 3 mm. An ascending method was used and after 24 hr the separation of thyroxine and radio-iodide was complete. The radio-iodide usually accounted for about 6 % of the total radioactivity and never more than 9 %. Correction was not made for this and so a small error is introduced when comparison is made between two different experiments. In any one experiment the same thyroxine solutions were used throughout.

Measurements of radioactivity were made with a well-type scintillation counter (Panax) and sufficient counts were recorded to reduce the error in counting to less than 1 %. All counting was done with constant geometry using 2 ml. samples (in some experiments all the

samples counted were 1 ml.). Faecal radioactivity was measured by packing dried pellets down into the plastic counting pots until they occupied a volume of 2 ml.

Stable thyroxine (sodium salt, Light and Co.) and radioactive thyroxine were stored in 5% rat plasma (5 ml. plasma in 95 ml. of sodium chloride solution 0·9 g/100 ml) in polythene containers at −10 °C. The solutions of stable thyroxine were made up by first dissolving the solid in about 1 ml. of warmed 0·1 N sodium hydroxide, then diluting the solution to near its final volume, adding the rat plasma and making up to final volume. Under these storage conditions over the period of any one experiment the degree of radio-iodide contamination of the radiothyroxine was found to rise by only about 1%.

Tests of significance of the results were made by Student's t test and all results are given as a mean ± one standard deviation.

RESULTS

The excretion of thyroxine in the warm-adapted and cold-adapted rat fed ad libitum. Intoccia & Van Middlesworth (1959) have shown that the faecal thyroxine excretion rate is raised in intact animals exposed to cold for about 2 weeks. Their animals were not thyroidectomized and the hyperthyroidism induced by cold may have accounted for their result. In order to eliminate this possibility an experiment was performed using thyroidectomized, thyroxine-maintained rats.

Thirty-four thyroidectomized male rats were used and were given stable thyroxine by daily subcutaneous injections at a rate of 3 μg/100 g/day for 14 days. This is approximately the rate at which the rat secretes thyroxine during cold (Purves, 1964). The experimental animals were kept in the cold room from the beginning of the experiment. Radioactive thyroxine was given intravenously at a dose of 0·5 μg/100 g on the final day (when they were not given their daily stable thyroxine replacement). The injection was made through an indwelling polythene cannula which had been put in place 24 hr before. Groups of animals were killed at varying times after the injection (four animals per group except at 24 hr when there were five) and faeces were collected during the radioactive period.

The animals fed *ad libitum* on Oxoid Diet 41 B which has a high roughage content. On that diet over the 24 hour period the controls produced 3·7 ± 1·0 g faces/100 g and the experimental animals 9·8 ± 1·5 g/100 g. The greater faecal thyroxine excretion which accompanied this higher faecal weight in the cold-exposed animals is shown in Fig. 1. Figure 2 shows the change in the blood radioactivity over the 24 hr period, demonstrating a more rapid fall in the cold-adapted animals.

Two questions arose from this observation. First, what was the mechanism of the greater faecal thyroxine excretion and secondly, was the shorter half-life of blood thyroxine in the cold-exposed animal due entirely to the greater rate of loss of the hormone via the biliary-faecal route, or were other mechanisms at work? The experiments reported below attempted to answer these questions.

Comparison of the faecal excretion and blood concentration of thyroxine in warm- and cold-adapted animals at similar faecal volumes. Van Middlesworth (1957) has shown faecal volume to be a factor in determining the faecal thyroxine excretion rate and it was possible that the higher faecal thyroxine excretion of the cold-exposed animals was due to their higher

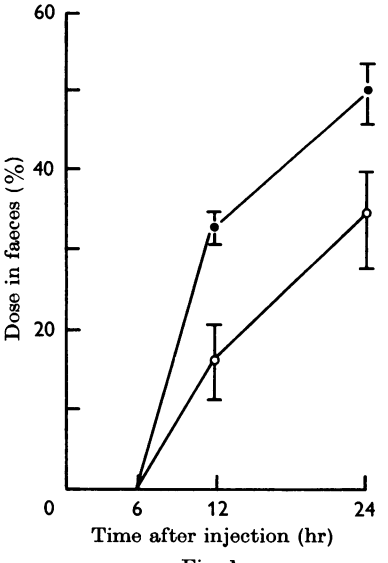

Fig. 1

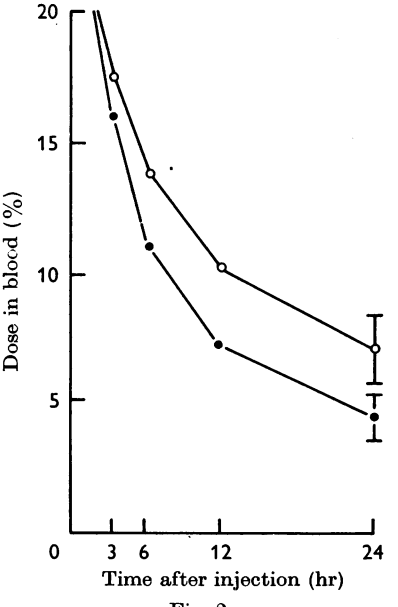

Fig. 2

Fig. 1. The faecal excretion of radioactivity following an intravenous injection of radioactive thyroxine in two groups of rats fed *ad libitum* on a high roughage diet. Warm-adapted rats ○; cold-adapted rats ●. The vertical lines indicate one standard deviation either side of the mean.

Fig. 2. The percentage of a dose of radioactive thyroxine remaining in the blood at varying times after its intravenous injection. Warm-adapted rats ○; cold-adapted rats ●. The vertical lines indicate one standard deviation either side of the mean.

faecal volumes. If this were so the effect ought to be abolished by elimination of the differential faecal volume. Further, if the shorter half-life of blood thyroxine in the cold-exposed animals was due to its greater loss in the faces, then elimination of the difference in faecal thyroxine excretion ought to eliminate the difference in the blood thyroxine half-lives. These two points were investigated.

Faecal volumes were controlled by feeding a highly refined low-roughage diet (Remington Diet) diluted with varying proportions of refined cellulose powder (to act as roughage). Using this procedure the faecal volumes of the control animals could be brought up to the level of the experimentals and both groups could be compared over a wide and similar range.

Twenty-eight male rats were thyroidectomized and given stable thyroxine subcutaneously by daily injections at a rate of 3·0 μg/100/g day for 14 days. Radioactive thyroxine was given subcutaneously at a dose of 1·0 μg/100 g on the final day (when they did not receive their stable thyroxine replacement). Faeces were collected for the next 24 hr and at the end of that period blood samples were taken. The experimental groups were kept in the cold throughout. The special Remington/Cellulose mixtures were fed *ad libitum* for 6 days before killing of the animals. The concentration of cellulose used varied between 0 and 40 % by weight.

Figure 3 shows that over the whole range of faecal volumes observed the controls had more radioactive thyroxine remaining in their blood 24 hr after its injection. In Fig. 4 the faecal thyroxine excretion is plotted against the faecal volume. It is apparent that the marked difference in faecal thyroxine excretion observed previously (Fig. 1), is here very much reduced when comparison of warm and cold-exposed animals is made at similar faecal volumes. Figure 5 shows more clearly that even when both animals were excreting similar amounts of radioactivity into their faeces the cold-exposed animal still had less thyroxine remaining in its blood at 24 hr. This latter point indicates that the shorter half-life of thyroxine in the blood of cold-adapted rats fed *ad libitum* was at most only partially dependent upon their higher faecal thyroxine excretion.

Comparison of the faecal excretion of thyroxine in warm- and cold-adapted animals at similar faecal volumes. In this experiment a second method was used to allow comparison of warm- and cold-adapted animals at similar faecal volumes; the food consumption of the cold-exposed rats was restricted down to the level of the controls causing both to produce the same amount of faeces. Under these conditions the faecal thyroxine excretion rates were compared.

Six thyroidectomized male rats were given stable thyroxine by daily subcutaneous injections at a dose of 3·0 μg/100 g for 14 days. The experimental animals were kept in the cold throughout. Radioactive thyroxine was given subcutaneously at a dose of 3·0 μg/100 g on the final day (when the stable thyroxine injection was omitted) and faeces were collected for the next 3 days. Food intake was restricted to 15·0 g Oxoid Diet/100 g/day and was given in small regular amounts so that both groups ate similar amounts and at similar rates. The food restriction was begun 24 hr before the radioactive injection and continued to the end of the experiment. On this diet both groups produced the same amount of faeces.

Under these conditions there was no difference in the faecal excretion of radioactivity between the two groups (Fig. 6). The faecal thyroxine excretion rates of cold- and warm-adapted animals were therefore again similar when compared at similar faecal volumes.

The effect of acute exposure to cold on the rate of loss of thyroxine from the blood in fasting animals. The results so far suggested that a major cause of the differential faecal thyroxine excretion between warm- and cold-exposed animals was their difference in faecal volume but that the cold response in terms of blood thyroxine (acceleration of its loss from the blood) was independent of a differential faecal thyroxine excretion. If this latter

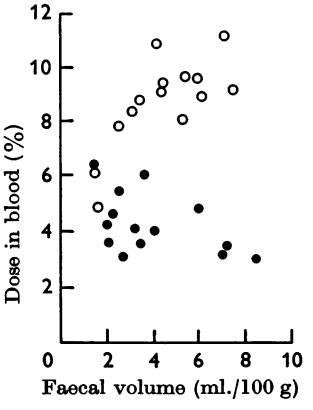

Fig. 3

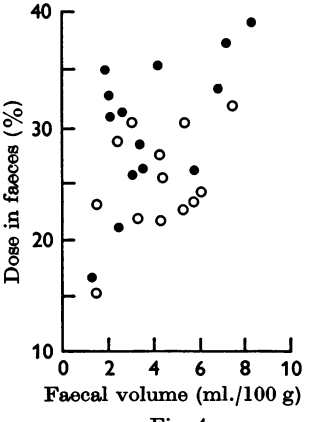

Fig. 4

Fig. 3. The percentage of a dose of radioactive thyroxine remaining in the blood 24 hr after its injection together with the volume of faeces produced over the same period. The animals were allowed to feed *ad libitum* on diets consisting of varying proportions of Remington Diet and cellulose powder in order to control the bulk of the faeces produced. Warm-adapted rats ○; cold-adapted rats ●.

Fig. 4. The percentage of a dose of radioactive thyroxine excreted into the faeces over the first 24 hr following its injection, together with the volume of faeces produced over the same period. These results are from the same experiment reported in Fig. 3. Warm-adapted rats ○; cold-adapted rats ●.

suggestion were correct then a cold response ought to be demonstrable in fasting animals. This point was investigated. In addition, an attempt was made to determine how soon after the onset of cold exposure a cold response could be demonstrated.

Twenty-seven animals of both sexes were used. Thyroidectomy was performed and radioactive thyroxine given intraperitoneally 6 hours later at a dose of 1·0 μg/100 day. Eighteen hours later blood samples were taken under ether anaesthesia by cutting off the tip of the tail and bleeding about 2 % of the blood volume. The blood radioactivity was then assayed. At varying time intervals later a second blood sample was taken by the same method and the second result expressed as a percentage of the first. During the interval between the two samples the experimental animals were cold-exposed and all of the animals were fasted.

The results in Fig. 7 demonstrate that a cold response in terms of blood thyroxine is given by fasting animals and that the effect develops within the first 12 hr.

In a control experiment it was found that 24 hr after an injection of radiothyroxine between 85 and 90% of the plasma radioactivity was protein bound and precipitated by trichloroacetic acid. This proportion was not changed if the animals were cold-exposed over the 24 hr period.

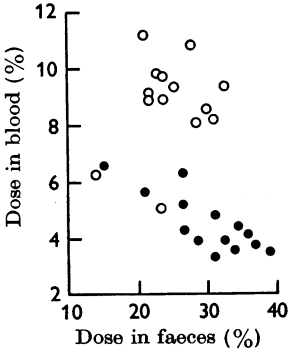

Fig. 5

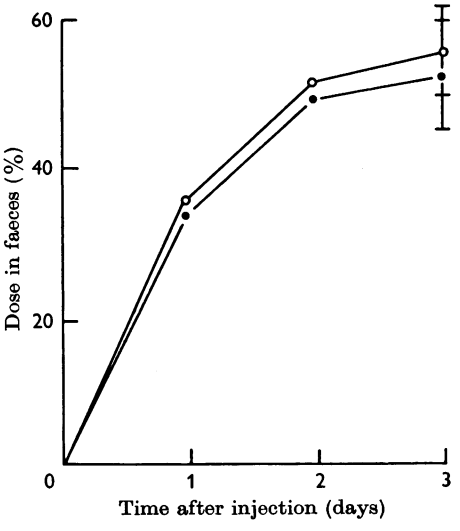

Fig. 6

Fig. 5. The percentage of a dose of radioactive thyroxine in the faeces over the first 24 hr following its injection, together with the percentage of the dose remaining in the blood at 24 hr. Warm-adapted rats ○; cold-adapted rats ●. These results are from the same experiment reported in Figs. 3 and 4.

Fig. 6. The percentage of a dose of radioactive thyroxine excreted over the first 3 days following injection. The animals were restricted to 15 g Oxoid Diet/100 g/day. Vertical lines indicate one standard deviation either side of the mean. Warm-adapted rats ○; cold-adapted rats ●.

The biliary excretion of thyroxine during cold exposure. Cottle (1964) has demonstrated that in intact cold-acclimated rats the biliary thyroxine clearance is raised. The extent to which this phenomenon was important in accelerating the removal of thyroxine from the blood under the present conditions was investigated in two experiments.

(i) Examination of animals with ligated bile-ducts. Eighteen female animals were used which had been thyroidectomized for a day. Radioactive thyroxine was given subcutaneously at a dose of 0·5 μg/100 g, the bile-ducts having been ligated 2 hr previously. The experimental animals

were placed in the cold immediately after injection. Thirty-hours later the blood radioactivity was assayed.

At 30 hr (Fig. 8) the control group had 4·1 % more of the dose of thyroxine remaining in the blood than the experimental group ($P < 0{\cdot}001$). This result showed that a cold response could be demonstrated in animals with ligated bile ducts—where the biliary route of thyroxine excretion had been entirely eliminated.

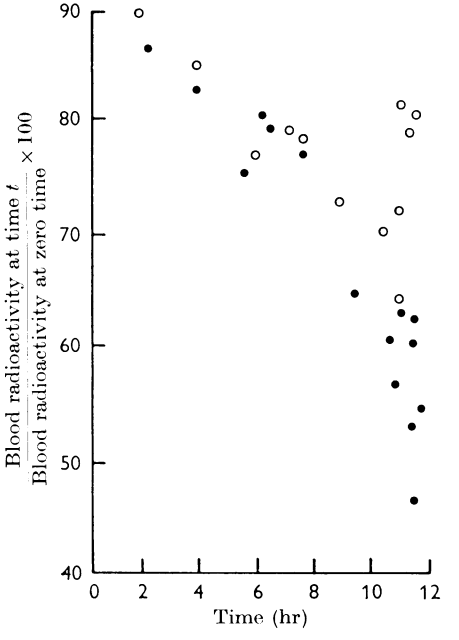

Fig. 7

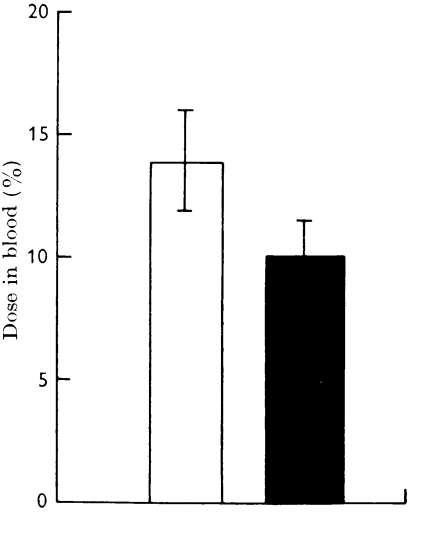

Fig. 8

Fig. 7. The decay of blood radioactive thyroxine over a 12 hr period. The blood radioactivity at any time is expressed as a percentage of the concentration at zero time (the beginning of the exposure period). The animals were fasted. Warm-exposed rats ○; cold-exposed rats ●.

Fig. 8. The percentage of a dose of radioactive thyroxine remaining in the blood 30 hr after its injection in animals with ligated bile ducts. Vertical lines indicate one standard deviation either side of the mean. Open column, warm-exposed animals; shaded column, cold-exposed animals.

(ii) Measurement of the biliary excretion of thyroxine. Ten male rats were thyroidectomized and given stable thyroxine by subcutaneous injection at a rate of 3·0 μg/100 g/day for the next 14 days. Indwelling, recirculating bile cannulae (see Methods) and venous cannulae were inserted in place 24 hr before the radioactive thyroxine injection which was given at a dose of 1·0 μg/100 g. No stable thyroxine was given on the day of the

radioactive injection. Bile was collected immediately in hourly fractions, its radioactivity assayed and then reinjected via the distal limb of the cannula. This procedure was continued for 8 hr and blood samples were then taken at the end of that period.

The results are shown in Fig. 9. Over the 8 hr period both the warm- and cold-exposed rats secreted about the same amount of thyroxine into the bile; the differences was not significant ($0{\cdot}3 > P > 0{\cdot}2$). However, in the

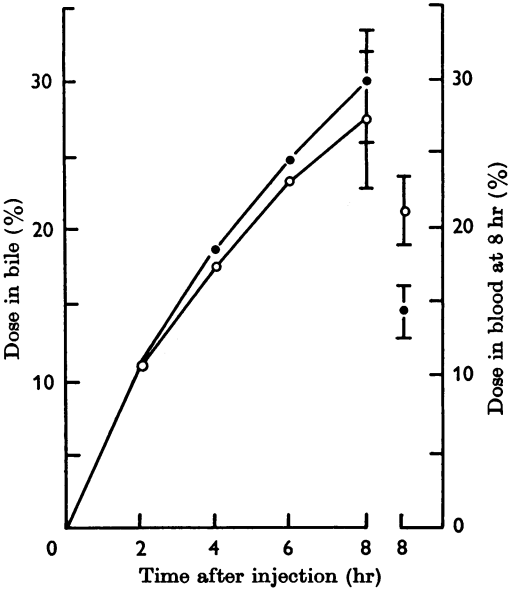

Fig. 9. The biliary excretion of radioactivity following an intravenous injection of radioactive thyroxine over the first 8 hr together with the percentage of the dose of thyroxine remaining in the blood at the end of the 8 hr period. Vertical lines indicate deviation either side of the mean. Warm-adapted rats ○; cold-adapted rats ●.

blood at the end of the 8 hr period the cold-exposed animals had very much less thyroxine remaining ($P < 0{\cdot}1$). In terms of clearance therefore there was a marked difference between the two groups. The biliary thyroxine clearance in the warm-exposed animals was 0·797 ml./hr and in the cold-exposed animals was 1·28 ml./hr.

DISCUSSION

Intoccia & Van Middlesworth (1959) demonstrated in intact rats that exposure to cold for about 2 weeks caused an acceleration in the rate at which thyroxine was excreted into the faeces. This result has been con-

firmed in thyroidectomized, thyroxine-maintained animals. How is this effect brought about?

In intact cold-acclimated rats the biliary thyroxine clearance is raised (Cottle, 1964; Cottle & Veress, 1966). Hyperthyroidism also increases the biliary thyroxine clearance (personal observation, unpublished). The effect of cold, however, is not secondary to a cold-induced hyperthyroidism since it is present in thyroidectomized, thyroxine-maintained animals. Observations presented above suggest that under these conditions the absolute amount of thyroxine lost into the bile is similar in warm- and cold-exposed rats but that the higher clearance in the cold animals is due mainly to the liver working against a lower level of blood thyroxine, the lower level of blood thyroxine being due to accelerated loss of the hormone from the blood, by routes other than the biliary-faecal route (Hillier, 1968).

The mechanism by which the biliary thyroxine excretion is raised is not yet clear. It may in part reflect an increased ability of the liver to conjugate and secrete thyroxine (Cottle, 1966) or reflect a reduction in the plasma binding of the hormone (Cottle & Veress, 1966).

Although the amounts of thyroxine secreted into the bile were similar in the above experiments, the cold exposed rats feeding *ad libitum* on a high roughage diet still excreted thyroxine into their faeces at a faster rate than did the controls. This effect was reduced by adjusting the roughage content of the diet so that the two groups were compared at similar faecal volumes or by feeding the two groups the same amount of food. This suggests that the mass of material passing along the intestines is important in determining the rate at which thyroxine appears in the faeces.

The extent to which this rapid passage can impede thyroxine reabsorption is not clear. Cottle (1966) has noted that raising the roughage content of the diet can sufficiently increase the faecal thyroxine loss such that the urinary route of thyroxine excretion (deiodination) is depressed. This strongly suggests that reabsorption can be reduced by high faecal bulk. The importance of faecal bulk in determining the faecal thyroxine excretion has been discussed by Pitt-Rivers & Tata (1959).

Experiments reported in this paper, using thyroidectomized, thyroxine-maintained rats given [^{131}I]thyroxine suggest that during cold a higher biliary clearance is able to maintain the biliary thyroxine output against a lower level of blood thyroxine and that the higher faecal bulk accelerates the hormone's passage into the faeces, probably also reducing its reabsorption. In spite of these effects, however, it is also clear that the increase in the biliary faecal route of excretion is not the only factor tending to reduce the biological half-life for thyroxine during cold exposure.

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