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THE UPTAKE AND RELEASE OF THYROXINE AND TRI-IODOTHYRONINE BY THE PERFUSED RAT HEART

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SUMMARY

1. The uptake and release of [^{131}I]L-thyroxine and L-3,5,3′-tri-iodothyronine have been studied in the rat heart perfused by Krebs–Ringer bicarbonate solution.

2. After 30 min perfusion thyroxine equilibrates to a ‘space’ of 15 ml./g of heart (i.e. each gram of tissue accumulates thyroxine equivalent to 15 ml. of perfusion fluid). Tri-iodothyronine takes about 120 min to equilibrate to a ‘space’ of about 75 ml./g of heart. These results are independent of the concentration of the hormone between 1×10^{-5} and 1·0 μg/ml.

3. The uptake of thyroxine at 17° C is almost identical with that at 37° C.

4. Radioactive thyroxine and tri-iodothyronine are both released from the heart by two distinct exponential processes, viz. a rapid process with a half-time of 14 min and a slow process with a half-time of 60 min. About 30 % of the total thyroxine and about 83 % of the total tri-iodothyronine are present in the slow-release compartment.

INTRODUCTION

Thyroxine and tri-iodothyronine are bound to protein both in the blood and in the tissues. It is probable that the transfer of these substances from the blood to the tissues is determined mainly by the relative affinity of the two binding processes acting in competition (Robbins & Rall, 1960). In this study an examination has been made of the tissue-binding aspect of this interaction. This was done by investigating the uptake and release of thyroxine and tri-iodothyronine by the rat heart perfused by Krebs–Ringer bicarbonate solution which does not contain any binding protein.

METHODS

The method of perfusion. For several reasons conventional perfusion apparatus was found to be unsuitable for this study. First the amount of thyroxine and tri-iodothyronine adsorbed from the buffer solution onto the glassware was found to be large. Secondly, the heart needed to be mounted close to the scintillation counting device and consequently the perfusion chamber needed to be very compact. Thirdly, the volume of radioactive perfusion fluid in the vicinity of the heart and counting device needed to be as small as possible. A very simple perfusion method was finally adopted and is illustrated in Fig. 1.

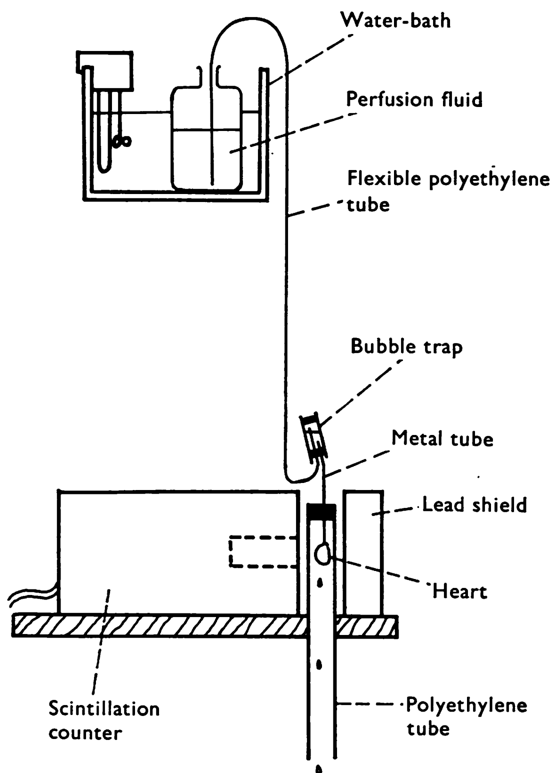

Fig. 1. A diagram of the perfusion apparatus for the rat heart.

The radioactive solutions were kept in 1 l. bottles (to maintain a low surface-area to volume ratio). Over the course of any one experiment the amount of hormone adsorbed onto the glassware was so small compared with the total amount in the fluid that it did not reduce the concentration of the hormone in solution by more than 1 %. After each experiment the glassware was cleared of radioactivity by washing with sodium hydroxide solution (10 g/100 ml.).

Perfusion fluid was gravity-fed to the heart from a height of 70 cm through a flexible polyethylene tube. The transit time for the fluid in the tube was about 15 sec. The adsorption of the hormone that occurred onto the tubing during that period was negligible.

The temperature of the water-bath was maintained at 43° C. Because of the cooling that occurred during the passage of the fluid through the tubing and bubble-trap, the temperature

on reaching the heart was about 37°–38° C. The heart took about 2 min to equilibrate to this temperature when transfer was made from perfusion fluid at room temperature (23° C) to that at 37° C. In the results it will be seen that the uptake processes being studied were hardly affected at all by changes in temperature between 17° and 37° C. Consequently the thermostatic regulation used in this perfusion method, although rather simple, was more than adequate.

The heart was contained within a polyethylene tube (diameter 1·6 cm). The relative humidity inside the chamber reached 100 % within about 1 min of the start of perfusion. Fluid passing through the heart dropped down through the open bottom of the chamber and away from the scintillation counting device.

The radioactivity of the heart was measured by a well-type scintillation counter (Panax). The upper lead shield of the 'castle' was removed and the castle mounted on its side. The heart chamber was positioned in front of the well so that the heart was in the position for most efficient counting. A further lead shield was then placed around the heart chamber to protect the exposed end of the castle.

A typical experiment measuring thyroxine uptake was performed as follows. A few specks of heparin B.P. were dropped into a syringe and about 0·5 ml. of water and 30 mg (0·5 ml.) of pentobarbitone sodium (Nembutal) were drawn into it. A rat was injected intraperitoneally with this mixture. After about 3 min the heart was quickly removed and placed in a beaker of perfusion fluid at 15° C. The perfusion cannula (made from a syringe needle blank size 0) was then tied into the aorta and the heart perfused by fluid at room temperature for 1 min to wash out contained blood. Perfusion was then started using the radioactive solution at 37° C. The radioactivity of the heart was determined thereafter by counting for 1 min periods every 2 min. At the end of the experiment the heart was perfused by ordinary fluid for 30 sec to wash out the radioactive solution in the heart chambers. The perfusion was then stopped and a final determination made of the radioactivity of the heart *in situ*.

The heart was then removed, blotted dry, weighed, and its radioactivity compared with that of a 2 ml. sample of the perfusion fluid using a well-type scintillation counter (Panax). This allowed the radioactivity of the heart to be expressed in terms of millilitres of perfusion fluid. Using this value and also the value for the final radioactivity of the heart *in situ* the uptake of thyroxine over the whole perfusion period could be expressed in terms of millilitres of perfusion fluid cleared of thyroxine.

No correction was made for the radioactive fluid contained within the heart chambers and vascular compartment since this was probably less than 0·3 ml./g of heart and the uptakes obtained were usually equivalent to 15 ml. of perfusion fluid or more.

In experiments where thyroxine release was studied the following procedure was adopted. The heart was perfused with radioactive thyroxine solution at room temperature for 5 min. It was then perfused for 1 min. with ordinary fluid at 37° C to wash out the radioactive solution from the heart chambers. The perfusion was then stopped for 1 min whilst the radioactivity of the heart was determined (and this value taken as 100 %). Perfusion by ordinary fluid at 37° C was then continued and the radioactivity of the heart determined thereafter at 2 min intervals. The results were expressed as a percentage of the initial value.

The perfusion fluid. The Krebs–Ringer bicarbonate solution used for perfusion was prepared by the method described by Umbreit, Burris & Stauffer (1957), except that only half of the amount of calcium chloride recommended there was used and glucose (100 mg/100 ml.) was added.

During its preparation the perfusion fluid was filtered free of dust using Whatman No. 1 filter paper. No further filtering was found to be necessary. Bubbles in the perfusate were trapped just before they reached the heart. With these precautions a steady perfusion rate of about 5 ml./min.g of heart was maintained for about an hour. After 3 hr the rate dropped to about 3–4 ml./min.g. In control experiments (see Results) it was found that this rate was still sufficient to allow normal thyroxine uptake. Most experiments lasted less than an hour.

Correction for radio-iodide contamination of the radioactive hormone solutions. The solutions of radioactive thyroxine and tri-iodothyronine were contaminated with 5–10 % radio-iodide. The degree of contamination was determined by paper chromatography using butanol:acetic acid:water (78:10:12) as solvent and Whatman 3 mm paper. In control experiments it was found that radio-iodide was hardly accumulated at all by rat hearts. The volume of fluid cleared of radio-iodide was only about 0·3 ml. and it remained constant with time. Consequently determinations of thyroxine clearance using radioactive material contaminated with 10 % radio-iodide would give values 10 % too low. For this reason, for each sample of radioactive hormone used the radio-iodide contamination was determined and a subsequent correction made to the results to eliminate this error.

Animals. In all experiments male albino rats weighing about 150 g were used.

Extraction procedure. Extraction and anlysis of the radioactive materials in the perfused hearts were performed as follows. The heart was minced with scissors and ground by pestle and mortar in 2 ml. of water containing about 10 μg thyroxine, 10 μg tri-iodothyronine and 0·5 mg propyl thiouracil. This mixture was shaken with 8 ml. butanol, centrifuged, and the butanol phase decanted off. To this extract 2 ml. of chloroform and about 5 drops of ammonia (sp.gr. 0·88) were added. The mixture was shaken, centrifuged, and the aqueous layer (about 0·3 ml.) removed and applied directly to the chromatography paper (Whatman 3 mm). The solvent used was *tert.*-amyl alcohol saturated with 2·5 N-NH_4OH. The chromatogram was prepared by a descending method. The distribution of radioactivity was determined by cutting the dried chromatogram into centimetre strips and counting each strip individually.

Radioactive material. [^{131}I]L-Thyroxine and L-3,5,3′-tri-iodothyronine and inorganic ^{131}I were obtained from the U.K. Atomic Energy Authority's Radiochemical Centre, Amersham. About 3 % of the total radioactivity of the thyroxine solution was due to contamination with tri-iodothyronine and vice versa. Stable L-thyroxine (Na salt) and stable L-3,5,3′-tri-iodothyronine were obtained from Koch–Light.

RESULTS

The uptake of thyroxine and tri-iodothyronine at low concentrations. When perfusion was begun with either thyroxine or tri-iodothyronine at a concentration of 1×10^{-5} μg/ml. the heart cleared the hormones from the perfusate at a rate of about 1·5 ml./min.g of heart. With thyroxine this clearance rate rapidly declined until a steady uptake value was reached in which the heart had cleared 15·1 ml. perfusate/g of heart (this value can be regarded as the thyroxine 'space'). The uptake of tri-iodothyronine took about 120 min to reach equilibrium at a value of 73 ml./g of heart. It will be seen from later experiments that the hearts during perfusion were also releasing the accumulated radioactive hormone. At the steady state the rate of accumulation must equal the rate of release. A typical experiment measuring these uptake processes is illustrated in Fig. 2.

In another experiment hearts were perfused for 30 min with tracer amounts of thyroxine or tri-iodothyronine. The radioactive materials were then extracted and analysed by chromatography. In the hearts perfused with thyroxine about 95 % of the total radioactivity was accounted for by thyroxine itself and about 3–4 % by tri-iodothyronine (due mainly to contamination in the original solution). There were only traces of inorganic

radio-iodide. In the hearts perfused with tri-iodothyronine there was an analogous distribution; 95 % of the radioactivity was due to tri-iodothyronine and about 4 % to thyroxine.

The effect of low temperature on the uptake process. The uptake of both thyroxine and tri-iodothyronine was almost unaffected by performing the perfusion at 17° C instead of at the normal 37° C. The experiment illustrated in Fig. 3 shows that at the lower temperature the final equilibrium position was unchanged and was reached at only a slightly slower rate.

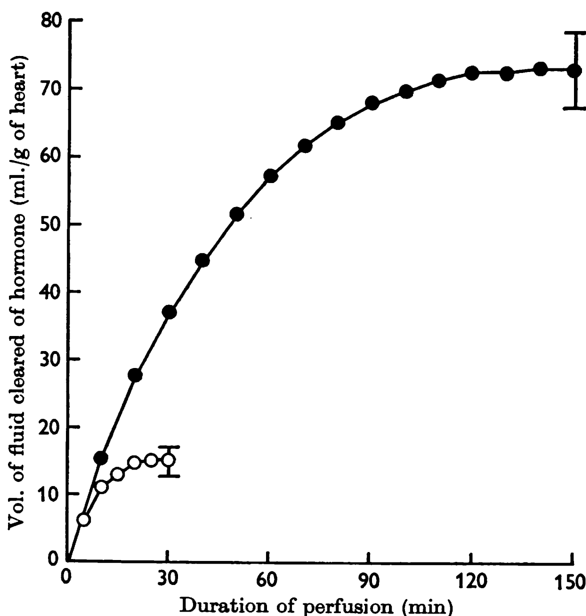

Fig. 2. The uptake of radioactive thyroxine and tri-iodothyronine by the perfused rat heart, plotted against the duration of perfusion. There were six animals in each experiment and the vertical lines indicate one standard deviation either side of the mean. Thyroxine ○; tri-iodothyronine ●. The concentration of both hormones in the perfusion fluid was 1×10^{-5} μg/ml.

The effect of variations in the perfusion rate. In a control experiment the clearance of tri-iodothyronine was measured at varying perfusion rates (obtained by alterations in the perfusion pressure). It was found that at flow rates in excess of 3·0 ml./g of heart this clearance was independent of flow rate. Below this value the clearance tended to decline. In the initial stages of a perfusion both hormones were cleared at a rate of about 1·5 ml./min.g and the perfusion rate was about 5·0 ml./min.g. The hearts were thus extracting about 30 % of the hormone passing through in the perfusate.

The uptake of thyroxine and tri-iodothyronine at high concentrations. When

the uptakes of the two hormones were examined at a very much higher concentration (1·0 μg/ml.) the same properties were shown. The initial rate of clearance and the final 'space' to which the hormones equilibrated were unchanged. Experiments using concentrations in excess of 1·0–10 μg/ml. were difficult since the thyroid hormones were found to be insoluble under physiological conditions at these concentrations. A typical experiment showing uptakes at high concentration is illustrated in Fig. 4 (only the initial part of the tri-iodothyronine uptake curve is shown).

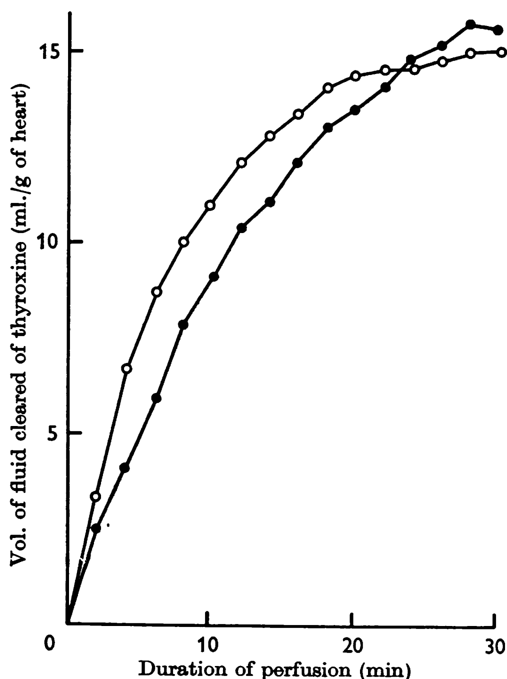

Fig. 3. The effect of temperature on the uptake of thyroxine by the heart. The uptake of radioactive thyroxine by the perfused rat heart, plotted against the duration of perfusion. There were four experiments at each temperature (37° C ○; 17° C ●). The concentration of thyroxine in the perfusion fluid was 1×10^{-5} μg/ml.

The release of accumulated radioactive thyroxine and tri-iodothyronine. In these experiments rat hearts were loaded with radioactive hormones over a 5 min period at low concentrations (1×10^{-4} μg/ml.). Then the decline in the radioactivity of the hearts was measured as they were perfused by ordinary non-radioactive perfusion fluid. For both thyroxine and tri-iodothyronine, the radioactivity of the heart decayed exponentially, showing two distinct release processes. The fast process had a half-time of about 14 min and the slow process a half-time of about 60 min.

By extrapolating the slow-release curve back to zero time an estimate was obtained for the relative proportions of hormone present in the fast- and slow-release compartments. For thyroxine about 70 % was present in the fast-release compartment and for tri-iodothyronine about 17 %. Similar release curves were found when the initial loading was continued for 30 min rather than for five or when loading was done at high concentrations of hormone (1·0 μg/ml.) rather than at tracer levels.

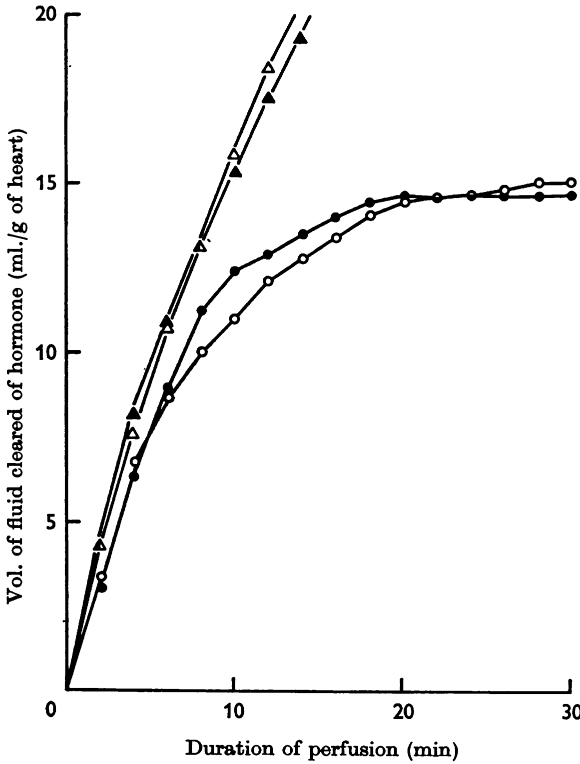

Fig. 4. The uptake of thyroid hormones at high concentration. The uptake of radioactive thyroxine and tri-iodothyronine by the perfused rat heart, plotted against the duration of perfusion. There were four experiments for each hormone at each concentration. Thyroxine (○) and tri-iodothyronine (△) at a concentration of 1×10^{-5} μg/ml. Thyroxine (●) and tri-iodothyronine (▲) at a concentration of 1·0 μg/ml.

DISCUSSION

Thyroxine and tri-iodothyronine are both accumulated and released by the perfused rat heart. During their perfusion both hormones reach a state of equilibrium in which the rate of release equals the rate of accummulation. This process is independent of the concentrations of the

hormones in the perfusate and is hardly affected by changes in temperature. These results suggest that a simple partition of the hormones is occurring between the perfusion fluid and some component within the heart in which the hormones are relatively much more 'soluble'. This component must be of very large capacity since it is not saturated by perfusion with fluid containing hormones at concentrations of 1·0 μg/ml.

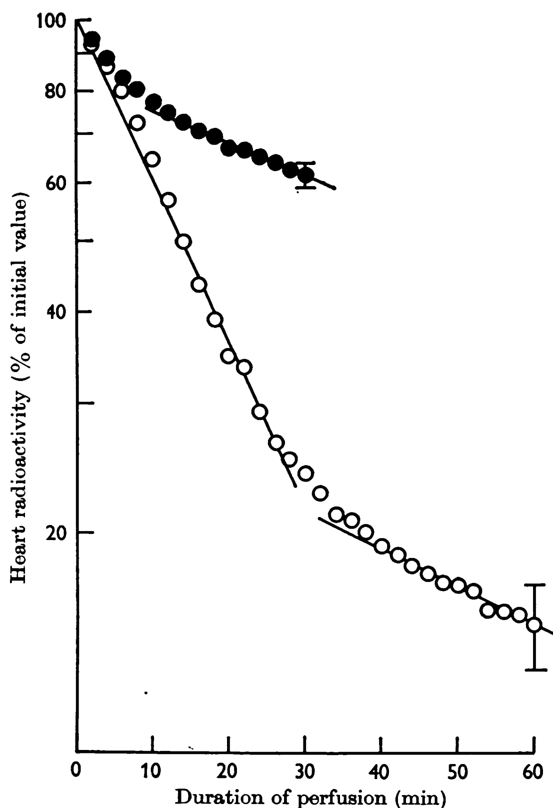

Fig. 5. The release of radioactive thyroxine and tri-iodothyronine by the perfused rat heart, plotted against the duration of perfusion. The concentration of radioactivity remaining in the heart at any time is expressed as a percentage of the initial radioactivity. The release curve is plotted on a semi-log scale. There were six animals in each experiment and the vertical lines indicate one standard deviation either side of the mean. Thyroxine ○; tri-iodothyronine ●.

Tissue proteins have been described which are able to bind thyroxine (Tata, 1958). It is therefore possible that the thyroxine-absorbing component in the rat heart is protein in nature.

It has been noted that *in vivo* the rate of transfer of tri-iodothyronine from the blood into the tissues is greater than the transfer rate for thyroxine

(Brown-Grant & Tata, 1961). This effect has been attributed to a weaker plasma protein binding for tri-iodothyronine. Results presented above, however, suggest that another factor may be partially responsible, namely, a greater tissue-binding capacity for tri-iodothyronine.

Experiments, in which the release of thyroid hormones from hearts was measured, demonstrated the existence of two separate 'compartments' both of which accumulate thyroid hormones. These two compartments were characterized by the difference in the rates at which they released the accumulated hormone. One compartment (from which release was rapid) had affinities for thyroxine and tri-iodothyronine which were about the same. The slow-release compartment, however, showed a markedly greater affinity for tri-iodothyronine.

Tommaselli, Gravina & Roche (1965) injected radioactive thyroxine and tri-iodothyronine into rats and examined their localization in the heart by autoradiography. They found that the conducting Purkinje tissue accumulated about 3 times as much hormone (per unit volume) as the normal contractile myocardial fibres. This difference could provide the basis for the two compartments mentioned above; this suggestion is, however, unlikely. There is a marked difference between thyroxine and tri-iodothyronine in the relative 'sizes' of the two compartments (70 % of the thyroxine is in the fast-release compartment and only 17 % of the tri-iodothyronine). Tommaselli *et al.* (1965) found no such difference in the distribution of the two hormones between the normal myocardial fibres and the Purkinje fibres.

It is probable that the two compartments represent two separate binding-protein systems on or in the cell. The compartment from which release is most rapid might possibly represent binding sites responsible for the initial accumulation of thyroxine from the blood. These sites could be on the surface of the cells or in the cytoplasm. Tommaselli *et al.* (1965) in their study of the radioautographic localization of thyroid hormones in the heart found evidence for intranuclear accumulation of thyroxine and tri-iodothyronine. Possibly the slow-release compartment represents hormone found within the nucleus.

L-3,5,3′-Tri-iodothyronine has greater hormonal activity than L-thyroxine and it has been suggested that thyroxine must first be converted to tri-iodothyronine before it can exert metabolic effects (Escobar Del Rey & Morreale De Escobar, 1964; Barker, 1964). It is possible that the much greater affinity for tri-iodothyronine shown by the heart tissue (in the slow-release compartment) might provide an explanation for these effects.

REFERENCES

BARKER, S. B. (1964). Physiological activity of thyroid hormones and analogues. *The Thyroid Gland*, vol. I, ed. PITT-RIVERS. R. & TROTTER, W. R. London: Butterworths.

BROWN-GRANT, K. & TATA, J. R. (1961). The distribution and metabolism of thyroxine and 3:5:3'-tri-iodothyronine in the rabbit. *J. Physiol.* **157**, 157–170.

ESCOBAR DEL REY, F. & MORREALE DE ESCOBAR, G. (1964). The peripheral deiodination of thyroid hormones and some metabolic implications. *Proc. 2nd int. Congr. Endocr.* Part II, pp. 1151–1167.

ROBBINS, J. & RALL, J. E. (1960). Proteins associated with thyroid hormones. *Physiol. Rev.* **40**, 415–465.

TATA, J. R. (1958). A cellular thyroxine binding protein. *Biochim. biophys. Acta* **28**, 91–97.

TOMMASELLI, A., GRAVINA, E. & ROCHE, J. (1965). Physiological disposition of thyroid hormones in the heart of the rat. *Thyroid Research*, ed. CASSANO, C. & ANDREOLI, M. pp. 382–393. London: Academic Press.

UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1957). *Manometric Techniques*. Minneapolis: Burgess.