*J. Physiol.* (1968), **199**, *pp.* 161–168
*With 6 text-figures*
*Printed in Great Britain*



# THE EFFECT OF SERUM ON THE UPTAKE OF THYROID HORMONES BY THE PERFUSED RAT HEART

By A. P. HILLIER
*From the Physiological Laboratory, University of Cambridge*

(*Received* 27 *May* 1968)

## SUMMARY

1. The effect of serum on the uptake of radioactive L-thyroxine and L-3,5,3′-tri-iodothyronine has been studied in the perfused rat heart.

2. With thyroxine binding protein (rat serum) in the perfusion fluid the uptake of both hormones is much reduced.

3. This inhibitory effect of serum on hormone uptake is greater with thyroxine than with tri-iodothyronine.

4. Serum in the perfusion fluid accelerates the release of accumulated thyroid hormone from the rat heart.

5. High concentrations of thyroxine increased the thyroxine space of hearts perfused by solutions containing serum.

## INTRODUCTION

About 0·1 % of the thyroxine present in blood is in a free, uncombined form; most of it is bound to specific binding proteins in the plasma (Robbins & Rall, 1960). It has been suggested that only free thyroxine is capable of diffusing into tissues and that the rate of transfer of thyroxine from the blood to the cells is mainly determined by the level of this free thyroxine (Ingbar & Freinkel, 1960).

In this study an attempt has been made to obtain direct experimental evidence to test this view by using the isolated perfused rat heart (Hillier, 1968). The uptake of L-thyroxine and L-3,5,3′-tri-iodothyronine has been studied in the presence of binding protein (rat serum) in the perfusion fluid.

## METHODS

The methods used in this investigation have been described in detail in a previous paper (Hillier, 1968). A further point, however, requires description.

Serum was collected as follows. Male rats weighing about 300 g were killed by coal gas. About 10 ml blood was taken by cardiac puncture and allowed to clot in a glass flask. Blood from several animals was pooled. The clotted blood was stored at 4° C for 18 hr to allow clot retraction and the serum was decanted off. Contaminating red cells were removed by centrifugation and the clear serum stored at −15° C.

RESULTS

*The effect of the presence of serum in the perfusion fluid on the uptake of thyroxine.* Thyroxine is both taken up and released by the perfused rat heart. When a heart is perfused by solutions containing radioactive thyroxine the hormone is gradually accumulated until after about 30 min a state of equilibrium is reached in which the rate of release equals the

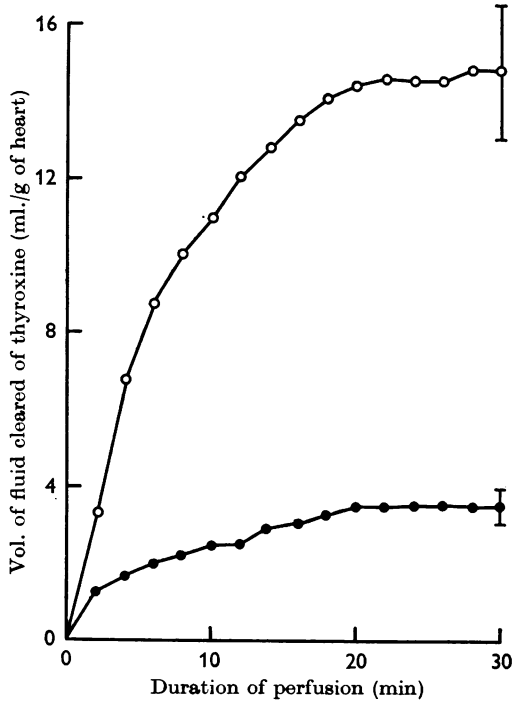


Fig. 1. The uptake of thyroxine by rat hearts over a 30 min perfusion period. Hearts perfused by solutions containing 0·25 % rat serum ●; hearts perfused by solutions containing no serum ○. There were five hearts in each group and the vertical lines indicate one standard deviation either side of the mean. The concentration of thyroxine in the perfusion fluid was $1 \times 10^{-4}$ $\mu$g/ml.

rate of uptake. In the absence of any thyroxine-binding protein in the perfusion fluid the heart, at equilibrium, accumulates thyroxine equivalent to about 15 ml. of fluid/g of heart (Hiller, 1968). Experiments were performed to investigate the effect on this system of adding thyroxine-binding protein (rat serum) to the perfusion fluid.

Ten rat hearts were perfused by solutions containing radioactive thyroxine at a concentration of $1 \times 10^{-4}$ $\mu$g/ml. In five of the experiments rat serum was added to the perfusion fluid at a concentration of 0·25 ml.

serum/100 ml. fluid. The other five hearts were perfused in the absence of serum.

The results are illustrated in Fig. 1. The total amount of thyroxine taken up was only about 25 % of the control value.

In another experiment rat hearts were perfused for 90 min by solutions containing radioactive thyroxine at a concentration of $1 \times 10^{-4}$ $\mu$g/ml. During the middle 30 min period the perfusion fluid contained 1·0 % rat serum.

The result of a typical experiment on one heart is illustrated in Fig. 2. Over the first 30 min period the heart almost reached an initial equilibrium

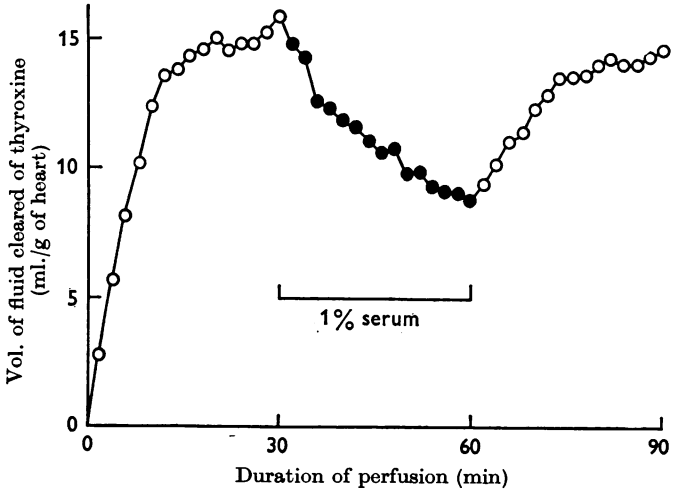


Fig. 2. The uptake of thyroxine by a single rat heart over a 90 min period. During the middle 30 min period (indicated by the filled circles and time mark) the perfusion fluid contained 1 % rat serum. At other times there was no serum in the perfusion fluid. The concentration of thyroxine was $1 \times 10^{-4}$ $\mu$g/ml.

level of thyroxine uptake. When serum was added to the fluid, however, there was a net loss of thyroxine from the heart. On changing tack to the original solution the thyroxine uptake was restored to its previous value.

*Comparison of thyroxine and tri-iodothyronine.* In another experiment hearts were perfused for 30 min by solutions containing radioactive thyroxine ($1 \times 10^{-4}$ $\mu$g/ml.) and the amount of the hormone taken up was measured. This was done at several different concentrations of serum; four determinations were made at each concentration. The thyroxine uptake at any given concentration was then expressed as a percentage of the uptake in the absence of serum. A similar experiment was also performed using L-3,5,3′-tri-iodothyronine.

The results are illustrated in Fig. 3. The uptake of both hormones was

very markedly affected by the presence of serum in the perfusion fluid. A large reduction in uptake occurred between 0 and 1·0% serum for thyroxine and between 0 and 2·0% serum for tri-iodothyronine. With both hormones the addition of serum in excess of 3% caused relatively little change in uptake. At all concentrations serum was more effective in inhibiting the uptake of thyroxine compared with tri-iodothyronine.

*The effect of high concentrations of thyroxine on thyroxine uptake in the presence of serum.* In the absence of binding protein in the perfusion fluid

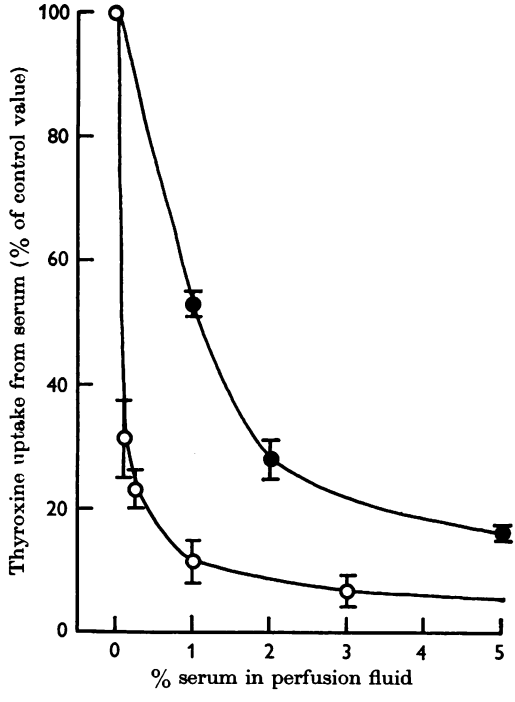


Fig. 3. The uptake of thyroxine and tri-iodothyronine at different concentrations of serum. The uptake at any given concentration is expressed as a percentage of the uptake in the absence of serum. Each point represents the mean of four separate experiments and the vertical lines indicate one standard deviation either side of the mean. Thyroxine ○; tri-iodothyronine ●. The concentration of both hormones in the perfusion fluid was $1 \times 10^{-4}$ $\mu$g/ml.

thyroxine clearance by the heart is independent of the concentration of the hormone (Hillier, 1968). Here, experiments were performed to determine the effect of different concentrations of thyroxine on the hormone uptake in the presence of serum.

Rat hearts were perfused for 30 min with solutions containing radioactive thyroxine at a concentration of $1 \times 10^{-4}$ $\mu$g/ml. This allowed the

system to reach equilibrium. Then the hearts were changed to a perfusion solution containing the same amount of radioactive hormone together with stable thyroxine at a concentration of 10 $\mu$g/ml. Both perfusion solutions were made up to contain 0·25 % rat serum.

The result of a typical experiment on one heart is illustrated in Fig. 4. In the first solution the heart reached an initial equilibrium. When the heart was changed to the solution containing the stable thyroxine there was a further increase in the amount of radioactive hormone taken up.

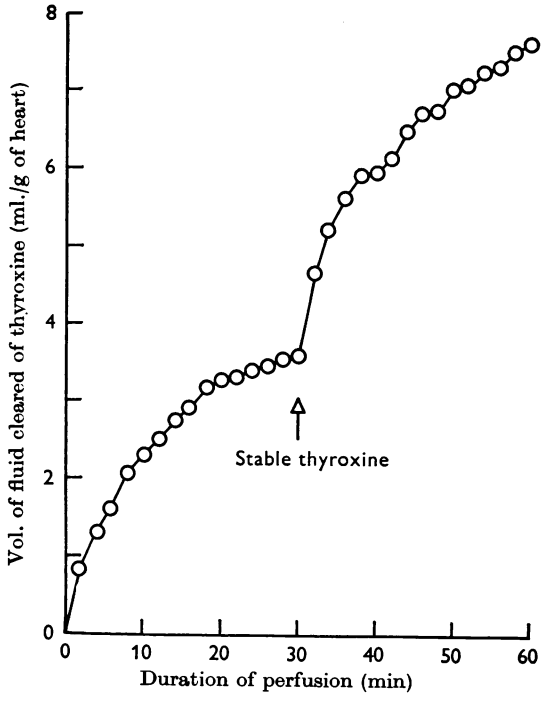


Fig. 4. The uptake of thyroxine by a single rat heart over a 60 min period. At 30 min (indicated by the arrow) the perfusion solution was changed from one containing radioactive thyroxine at a concentration of $1 \times 10^{-4}$ $\mu$g/ml. to one containing in addition stable thyroxine at 10 $\mu$g/ml. Both solutions contained 0·25 % rat serum.

When similar experiments were performed in the absence of binding protein there was no increase in uptake on transferring to the second solution.

In another experiment a dose–response curve for this effect was established. The experiments were performed in an exactly similar way to that already described. The percentage increase in thyroxine uptake was measured for various different concentrations of stable thyroxine.

The results are illustrated in Fig. 5. Each point represents a single determination on one heart. The dilution of serum used in this experiment was 1:400 and at this degree of dilution the concentration of thyroxine normally found in blood would be about $1 \times 10^{-4}$ $\mu$g/ml. It can be seen from Fig. 5 that the effect is demonstrable only at thyroxine concentrations higher than are normally found in the blood. Further, the effect shows signs of becoming maximal at concentrations of about $1 \times 10^{-1}$ $\mu$g/ml. At these high levels the thyroxine uptake by the heart is equivalent to

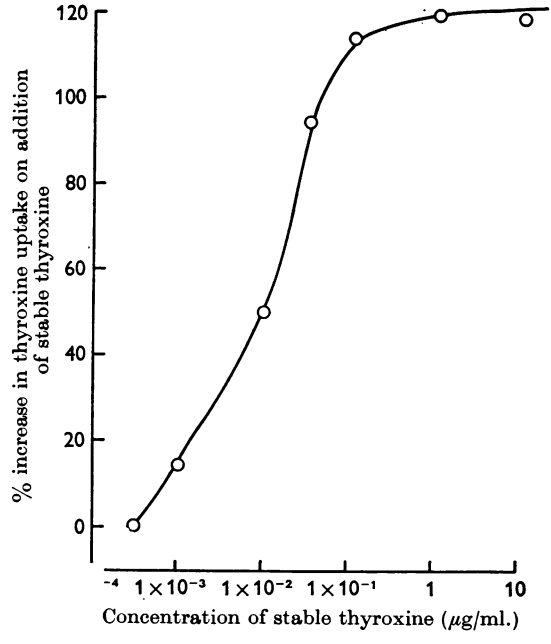


Fig. 5. A dose–response curve for the effect illustrated in Fig. 4. The percentage increase in thyroxine uptake on changing to the solution containing stable thyroxine is plotted against the concentration of stable thyroxine in the second solution. Each point represents one separate experiment.

about 7 ml. perfusion fluid/g. This is less than half of the uptake obtained in the absence of serum. Consequently the levelling off of the curve at high concentrations cannot be attributed to saturation of the uptake process of the heart.

*The effect of serum on the release of tri-iodothyronine.* Perfused rat hearts gradually release accumulated thyroid hormone into the perfusate (Hillier, 1968). In an experiment an attempt was made to determine whether the presence of serum in the perfusion fluid had any effect on the rate of this release process.

Twelve hearts were loaded with radioactive tri-iodothyronine by per-

fusing them for 5 min with a solution containing the hormone at a concentration of $1 \times 10^{-3}$ $\mu$g/ml. The perfusion was then continued with fluid containing no tri-iodothyronine. In six experiments rat serum was added to the perfusion fluid at a concentration of 1·0 %. The gradual decline in heart radioactivity over the next 30 min was then determined.

The results are illustrated in Fig. 6. The release of tri-iodothyronine was more rapid from those hearts perfused by the 1 % serum.

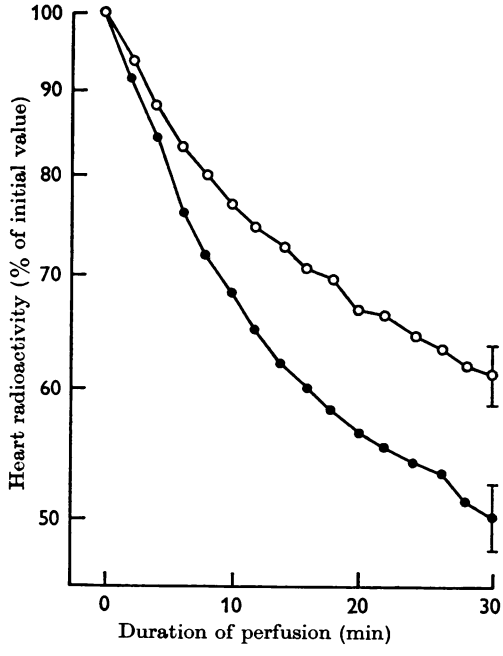


Fig. 6. The release of radioactive tri-iodothyronine from rat hearts perfused by ordinary buffer solution ○ and solution containing 1 % rat serum ●. The radioactivity of the heart at any time is expressed as a percentage of the initial radioactivity. There were six hearts in each group and the vertical lines indicate one standard deviation either side of the mean.

## DISCUSSION

In this paper direct experimental evidence is presented in favour of the theory that the extent to which thyroxine is taken up by the tissues is determined in part by the degree to which the hormone is bound onto proteins in the plasma.

Several workers have noted that tri-iodothyronine is bound less firmly onto the plasma proteins than is thyroxine (Tata, 1964; Robbins & Rall, 1960). This effect probably explains the relatively greater inhibitory action of serum on thyroxine uptake illustrated in Fig. 3.

The increase, by high concentrations, of the thyroxine space of hearts perfused with serum is similar to an effect observed by Osorio & Myant (1963). They found that large doses of stable thyroxine enhance the biliary clearance of the hormone. These effects are very probably due to saturation of the thyroxine-binding sites in the plasma or serum resulting in an increase in the proportion of the hormone in the free, unbound form. Since this phenomenon is demonstrable only at a very high thyroxine concentration it is probably of no physiological significance.

It was observed that serum in the perfusion fluid enhanced the release of tri-iodothyronine from rat hearts. The most probable interpretation of this effect is that a small fraction of the hormone lost into the intravascular fluid is later reabsorbed and that the serum, by trapping this released hormone, impedes its subsequent reabsorption. In this way overall release would be enhanced.

## REFERENCES

HILLIER, A. P. (1968). The uptake and release of thyroxine and tri-iodothyronine by the perfused rat heart. *J. Physiol.* **199**, 151–160.

INGBAR, S. H. & FREINKEL, N. (1960). Regulation of peripheral metabolism of thyroid hormones. *Recent Progr. Horm. Res.* **16**, 353–383.

OSORIO, C. & MYANT, N. B. (1963). Effects of salicylate on the biliary excretion of thyroxine in rats. *Endocrinology* **72**, 253–258.

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

TATA, J. E. (1964). Distribution and metabolism of thyroid hormones. In *The Thyroid Gland*, vol. I, ed. PITT-RIVERS, R. & TROTTER, W. R. London: Butterworths.