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THE DISTRIBUTION AND METABOLISM OF THYROXINE AND 3:5:3′-TRIIODOTHYRONINE IN THE RABBIT

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3:5:3′-Triiodo-L-thyronine (T_3) is synthesized and secreted by the thyroid gland of many species. In most assays *in vivo* it has been found to have from five to seven times the biological potency of the major thyroid hormone L-thyroxine, (T_4), (Pitt-Rivers & Tata, 1959). Certain exceptions have been observed, however. In the chicken, Gilliland & Strudwick (1953), studying the inhibition of ^{131}I release from the thyroid, Shellabarger (1955) using the goitre prevention assay, and Newcomer (1957) studying O_2 consumption and feather growth, found the two compounds to be nearly equipotent. Brown-Grant (1955) showed that T_3 and T_4 had the same activity in suppressing thyrotrophic hormone (TSH) secretion in the rabbit as judged by inhibition of the release of ^{131}I-labelled hormone from the thyroid gland.

Until recently no satisfactory explanation was available for these differences. The recognition of the role played by specific thyroxine-binding proteins of the plasma in determining the biological half-life and the rate of entry of thyroid hormones into the tissues is a fairly recent development (Pitt-Rivers & Tata, 1959; Tata, 1960*a*; Brown-Grant, 1960). It has been demonstrated that in the chicken no specific thyroxine-binding globulin (TBG) is present in the plasma. Consequently, the affinity for and the capacity to bind T_4 is much lower in chicken than in human plasma. The major binding protein of chicken plasma is found in the albumin fraction and has identical affinities for T_3 and T_4 (Tata & Shellabarger, 1959). Thus both hormones would be expected to have the same short half-life (a value of 22·5 hr was in fact observed) and to reach the tissues at similar rates. This could explain the similarity of the biological activities in this species.

It seemed possible that a similar situation might exist in the rabbit, and the distribution and metabolism of ^{131}I-labelled T_3 and T_4 in the rabbit and the binding to serum proteins *in vivo* and *in vitro* have therefore been studied.

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METHODS

Young adult rabbits of mixed breeds (1·5–2·0 kg body weight) were used. Animals of the same sex from two litters were studied in each series of experiments and the groups were chosen so that the mean body weights were approximately the same and that equal numbers of animals from each litter received T_3 and T_4. The animals were kept in a light- and temperature-controlled (18–20° C) animal room for at least 2 weeks before and during the course of the experiments and maintained on pellets (Diet 18, Bruce & Parkes, 1947) and tap water *ad lib.* In some experiments a solution of KI in tap water (0·002 g/100 ml.) was given instead of tap water, as described under Results.

The hormones labelled with ^{131}I in the phenolic ring were obtained from Abbot Laboratories Ltd, and were shown by chromatography to be at least 85% pure. The stock solutions (in 1:2-propane-diol) were diluted with a 1:9 (v/v) mixture of rabbit plasma and NaCl solution 0·9 g/100 ml. before use and were injected intravenously in a volume of 0·5–1·0 ml. Labelled iodinated human serum albumin was obtained from the Radiochemical Centre, Amersham, England, and was diluted with 0·9% NaCl solution before injection. In each experiment an internal standard was prepared from the diluted stock solution under the same injection conditions.

Blood was drawn from the marginal vein of the ear and clotting prevented by adding a small amount of heparin powder. Plasma was obtained by centrifugation and a known volume was diluted to 10 ml. and counted to determine total plasma radioiodine content. The protein-bound radioactivity (PB ^{131}I) was determined by precipitating the plasma proteins with 5 volumes of trichloracetic acid (TCA) solution, 10 g/100 ml., resuspending after centrifuging, washing twice with 10% TCA and finally dissolving the precipitated protein in 3N-NaOH to a volume of 10 ml. and counting this solution. More than 90% of the activity appeared in the PB ^{131}I fraction when T_3 and T_4 were added to rabbit plasma *in vitro* and less than 5% when radioiodide was added *in vitro*. Tissue samples weighed after washing and gently blotting dry were also dissolved in 3N-NaOH for assay of their ^{131}I content. All counts were made with an M6 liquid counter to a statistical accuracy of ±2·5% or better. After correction for isotope decay, the results were expressed as a percentage of the administered dose by reference to the internal standard prepared at the time of injection.

The interaction of thyroid hormones with serum proteins was studied by paper electrophoresis (5 V/cm for 20 hr) in 0·05M veronal buffer, pH 8·6 (Block, Durrum & Zweig, 1955), as described elsewhere (Tata & Shellabarger, 1959). The capacity and relative affinity with which thyroxine and triiodothyronine are bound to the different binding fractions were measured (1) by paper electrophoresis at increasing concentration of added hormones and (2) by the method of displacement of radioactive thyroxine with stable triiodothyronine, as described by Robbins & Rall (1957). As was shown by Myant & Osorio (1959), rabbit serum albumin and α-globulin-type thyroxine-binding globulin cannot be well separated and the error involved in such studies is of the order of ±5–10%.

RESULTS

The distribution of T_3 and T_4 in vivo

In the first experiment eight female rabbits were used: four received T_3 (20 μc, 1·6 μg) and four received T_4 (20 μc, 2·0 μg) intravenously. Blood samples were taken at 30 min and at 6, 25, 49, 72 and 96 hr after injection. Total plasma radioactivity and PB ^{131}I were determined in the early samples. At 49 hr, PB ^{131}I was more than 95% of the total activity and only total activity was determined at 72 and 96 hr. The results are

shown in Fig. 1. As early as 30 min after injection the plasma level of T_3 was much lower than that of T_4; this difference was maintained, but from 25 to 96 hr the fractional rate of disappearance from the plasma for both compounds appeared to be the same. The biological half-life in the blood was estimated to be 27 hr.

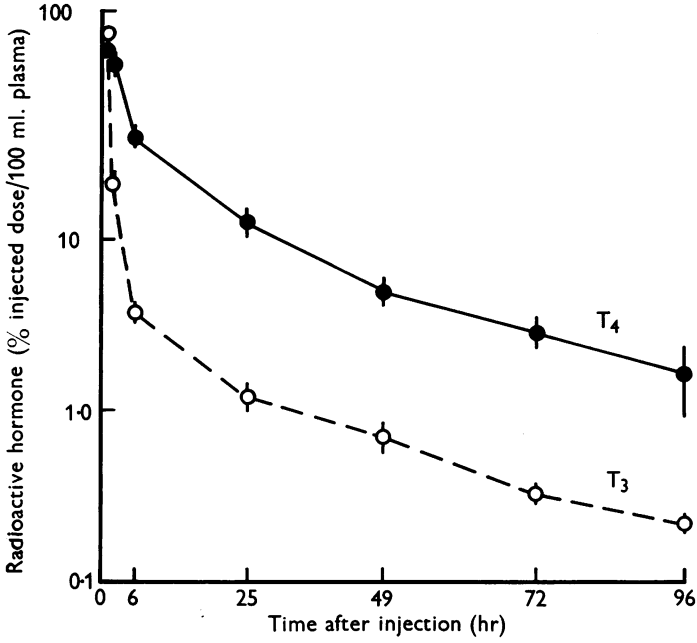

Fig. 1. The concentration of radioactive hormone in the plasma after the intravenous injection of 1·6 μg of T_3 or 2·0 μg of T_4 in rabbits. Each point is the mean value from 4 animals; the vertical bars indicate the standard errors of the means. Between 6 and 96 hr the curves are parallel, indicating similar fractional removal rates. The estimated half-life is 27 hr for both substances. Initial values at 0 hr were calculated on the basis of a complete distribution within the estimated plasma volume (Armin *et al.* 1952).

In these experiments the animals were given an 0·002 % solution of KI, beginning 2 days before and continuing throughout the period of the experiment, in order to prevent the recirculation of ^{131}I from degraded thyroid hormone. For thyroxine the extent of recirculation had previously been estimated to be only 8 % in the rabbit (Brown-Grant & Gibson, 1955). Thus the curve representing results obtained in this earlier study, in which 1 μg of labelled T_4 was injected and whole blood radioactivity was measured, was quite similar to the present results and gave a value of 25 hr for the half-life: in these earlier experiments the ^{131}I content of the thyroid gland 96 hr after injection was 5–7 % of the injected dose, whereas in the present series thyroid ^{131}I at 96 hr was 0·14, 0·26, 0·18 and 0·32 %

(mean 0·23 %) in the case of T_4 and 0·54, 0·27, 0·34 and 0·14 % (mean 0·32 %) in the case of T_3.

The fall in plasma radioactivity after the injection of T_3 was very rapid. Calculations based on the data of Armin, Grant, Pels & Reeve (1952) gave a mean value of 83 ml. for the plasma volume of the animals used in these experiments. The volume of distribution of T_4 30 min after injection was 114, 129, 109 and 89, mean value 110 ml., while the values for T_3 were 884, 951, 951 and 560, mean value 837 ml. The early stages of distribution were studied in more detail in a second series of experiments.

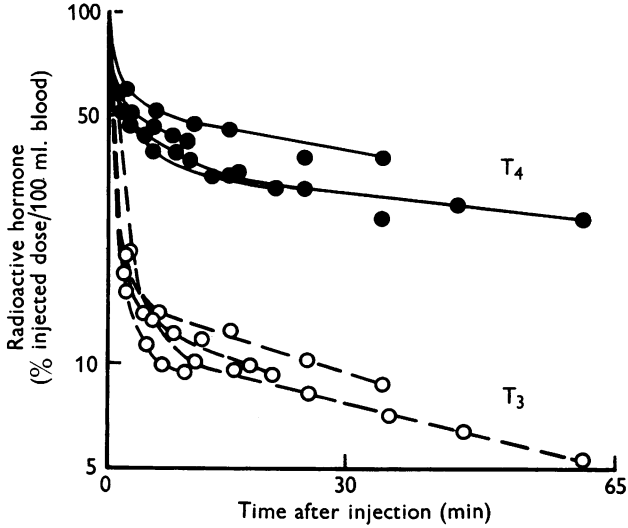

Fig. 2. The concentration of radioactivity in whole blood after the intravenous injection of radioactive T_3 or T_4 in rabbits. Each curve represents data from an individual animal. Initial point at 0 min is the calculated concentration based on a complete distribution in the estimated blood volume (Armin *et al.* 1952).

Eight female animals were used: four received T_3 (8·8 μc, 1·4 μg) and four received T_4 (6·3 μc, 1·2 μg) intravenously. Blood was collected directly into graduated tubes 1–2 min after injection and at 5–10 min intervals after this. One animal in each series was killed 10, 20, 40 and 65 min after the injection of labelled hormone by means of an overdose of intravenous sodium pentobarbitone and tissue samples were removed for the determination of ^{131}I content. The body fluids and tissues studied were whole blood, plasma, bladder urine, bile, liver, spleen, kidney, skin, muscle and thyroid. The skin sample was the tip of the ear which had not received the initial injection of hormone and the muscle sample was from the lower third of the left quadriceps femoris. Only portions of the liver and kidney were assayed, but the total weight of these organs was recorded.

The fall in blood radioactivity is shown in Fig. 2; the difference between

T_3 and T_4 is well established by 5–10 min after injection. Yet although the blood levels of the two groups are so different there is no evidence of a major difference in the total amount of hormone present in the other tissues examined, when expressed as percentage of the administered dose (Fig. 3), or of any consistent difference in the concentration (% dose/g tissue) (Table 1). The ratio of tissue to plasma ^{131}I concentration (T/P

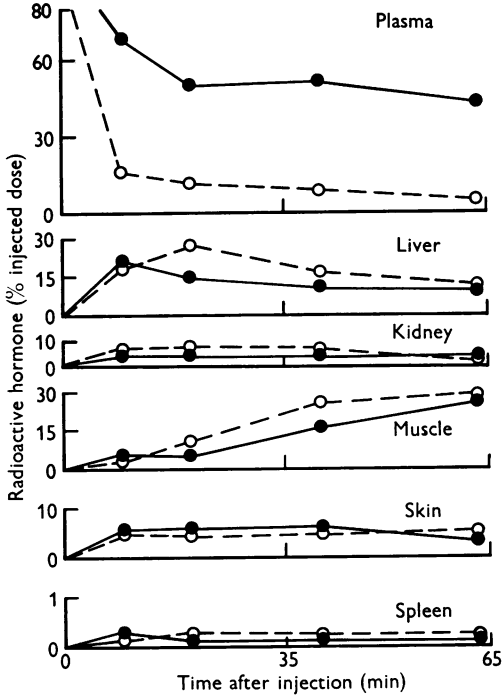

Fig. 3. The percentage of the administered dose of ^{131}I-labelled hormone found in various organs after the intravenous injection of a tracer dose of T_3 and T_4 in rabbits. The muscle mass was estimated as 50% of body weight and skin as 5% of body weight. These are values obtained before correction for hormone in the vascular compartment of each organ. ●——● thyroxine (T_4); ○- - -○ triiodothyronine (T_3).

ratio) is consistently higher for T_3 in all organs showing a significant ^{131}I content and in bile at each of the four time intervals studied (Table 1). The chemical nature of the ^{131}I in the tissues was not determined.

In view of the marked differences in the plasma concentrations of T_3 and T_4 even at these short periods after injection, considerable differences in the ^{131}I content of the organs examined were expected but not observed. The possibility that T_3 might be concentrated in some organ or tissue other than those examined in these first experiments was investigated in male rabbits. Four rabbits were injected with T_3 (6 μc, 0·6 μg) and four

TABLE 1. The concentration of labelled hormone (% injected dose/g tissue) and the tissue/plasma (T/P) concentration ratio for selected organs at varying intervals after the intravenous injection of T_3 and T_4 in rabbits. Values not corrected for hormone in the vascular compartment

Hormone	Time after injection (min)	Liver		Spleen		Kidney		Skin		Muscle		Thyroid		Bile	
		%/g	T/P	%/g	T/P	%/g	T/P	%/g	T/P	%/g	T/P	%/g	T/P	%/g	T/P
T_3	10	0·302	1·43	0·076	0·36	0·420	1·98	0·056	0·26	0·004	0·02	0·040	0·20	0·240	1·13
T_4	10	0·258	0·26	0·370	0·37	0·358	0·36	0·149	0·15	0·058	0·06	0·060	0·06	0·040	0·04
T_3	20	0·331	2·37	0·113	0·64	0·562	4·02	0·052	0·37	0·015	0·10	0·094	0·67	0·193	1·37
T_4	20	0·207	0·32	0·057	0·09	0·430	0·66	0·071	0·11	0·008	0·01	0·084	0·01	0·062	0·95
T_3	40	0·370	2·54	0·175	1·26	0·618	4·43	0·060	0·39	0·036	0·26	0·240	1·71	0·140	1·01
T_4	40	0·225	0·29	0·083	0·11	0·590	0·77	0·096	0·13	0·013	0·02	0·186	0·21	0·068	0·09
T_3	65	0·180	2·47	0·105	1·44	0·352	4·77	0·054	0·74	0·024	0·33	0·168	2·18	0·053	0·72
T_4	65	0·189	0·44	0·067	0·16	0·257	0·59	0·052	0·12	0·057	0·13	0·200	0·40	0·400	0·91

with T_4 (5 μc, 0·5 μg) and killed 30 min later. In addition to the organs examined in the previous study, the ^{131}I content of the adrenal gland, the heart, the genital tract, stomach, small intestine, large intestine, lungs, diaphragm, tongue, salivary glands, pancreas and thymus was determined. In addition, multiple samples of liver, skin and muscle rather than single samples were assayed. None of the organs examined had a strikingly higher content of ^{131}I after the injection of T_3 as compared with the content

TABLE 2. Distribution of ^{131}I 30 min after the intravenous injection of labelled T_3, T_4 and iodinated albumin. Each value (% administered dose) is the mean of results from four rabbits. Total muscle content calculated on the basis of a muscle mass equal to 50 % of the body weight and blood content on the estimated blood volume from the data of Armin *et al.* (1952). Values for albumin are % dose in each organ and the calculated value for the volume of plasma in each organ

	Direct		Corrected		Iodinated albumin	
Organ	T_3	T_4	T_3	T_4	(% dose)	(ml. plasma)
Adrenal	0·049	0·020	0·047	0·010	0·180	0·113
Bile	0·170	0·150	0·168	0·139	0·027	0·013
Genital tract	0·328	0·441	0·285	0·226	0·388	0·244
Heart	0·572	0·523	0·567	0·317	0·443	0·278
Kidneys	5·885	6·295	5·56	4·65	2·92	1·83
Large intestine	8·83	9·49	8·43	6·285	3·92	2·45
Liver	23·4	18·1	22·7	12·1	10·675	6·69
Lungs	1·18	1·05	0·961	0·280	1·19	0·752
Muscle	27·1	26·4	25·4	15·7	15·75	9·895
Skin	6·01	7·19	5·645	4·33	3·30	2·07
Small intestine	9·51	9·18	7·17	4·32	3·025	1·91
Spleen	0·134	0·110	0·130	0·056	0·130	0·082
Stomach	5·37	5·01	4·57	2·74	3·255	2·04
Salivary glands	0·319	0·237	0·309	0·180	0·094	0·059
Thymus	0·048	0·035	0·046	0·026	0·105	0·066
Thyroid	0·102	0·263	0·090	0·203	0·002	0·001
Blood	14·2	53·5	14·2	53·5	—	—
Total	103	138	96·3	105	—	—

after the injection of labelled T_4 (Table 2). No evidence was obtained to support the hypothesis that the T_3 that had left the vascular compartment of the body was concentrated preferentially in any organ of the body.

The measurements of the ^{131}I content in a wider range of tissues enabled a more complete 'accounting' of the injected radioactivity to be carried out. As shown in Table 2, when the total % recoveries for T_3 and T_4 were calculated on the assumption that muscle accounts for 50 % of the body weight (Gross, Ford, Symchowicz & Horton, 1957), and on the basis of the plasma volumes given by Armin *et al.* (1952), the values obtained were 103 and 138 % respectively of the injected dose. Errors in the estimated muscle mass or plasma volume could not account for such a discrepancy. It seemed likely that the error arose from a duplication in the measurement of the radioactivity in the vascular compartment of the body, due to the

inclusion of ^{131}I in the blood in the total hormone content of the different organs measured.

The possibilities of repeating the experiments under anaesthesia and terminating them by exsanguinating the animals or of perfusing the animals immediately after death were considered but rejected on the grounds that either procedure would almost certainly lead to gross changes in the distribution of the labelled hormone. Instead, the fraction of the plasma volume contained in each organ as prepared for assay in these experiments was determined from a study of the distribution of the radioactivity after the injection of a small dose of radio-iodinated human serum albumin. The dose injected was 20 μc (830 μg of protein) in a volume of 0·25 ml. The animals were killed 30 min later. From the plasma level of radioactivity, plasma volumes of 29, 33, 35 and 33 ml./kg body weight were calculated. The ^{131}I content of each organ, as a percentage of the injected dose, was determined and the plasma content (^{131}I content of organ divided by ^{131}I content of 1 ml. plasma) was calculated. The mean results are given in Table 2. From the calculated plasma content of each organ, and the known plasma level of radioactivity in each animal that received labelled hormone, it was possible to calculate a corrected organ content of labelled hormone for each animal representing the percentage of the injected dose present after allowing for the amount contained in the vascular compartment. When this was done, it was clear that the tissue content of T_3 was consistently higher than that of T_4 (Table 2), the difference being observed in all organs examined except the thyroid. When the corrected organ contents plus blood content were added together, an average value of 96 % of the T_3 and 105 % of the T_4 injected was accounted for.

At these physiological levels there is no indication that the early stages in the distribution of T_3 and T_4 in the rabbit differ from those observed in the rat or man (Pitt-Rivers & Tata, 1959). The behaviour of the two hormones is consistent with the hypothesis that a specific binding protein(s) with a higher affinity for T_4 than for T_3 exists in the blood of rabbits as of other mammals. This question was examined directly.

Interaction of thyroid hormones with rabbit serum proteins

By paper electrophoresis in the presence of veronal buffer (Tata & Shellabarger, 1959) both thyroxine and triiodothyronine were found to be bound to two major rabbit serum protein fractions: (*a*) an α-globulin (localized between α_1-globulin and albumin) thyroxine-binding globulin (TBG), and (*b*) albumin. In Table 3 it is seen that these two fractions accounted for over 90 % of protein-bound hormonal ^{131}I, whether the hormones were endogenously labelled (36 hr after injection of radioiodide)

or injected or mixed *in vitro*. In a separate experiment it was found that endogenously labelled thyroid hormone consisted of about 90–95% of organic ^{131}I as thyroxine and about 2–6% as triiodothyronine, between 24 and 110 hr after administration of ^{131}I-iodide to a normal rabbit.

Despite the qualitative similarity of thyroid hormone binding, there were differences at the quantitative level when thyroxine-binding in rabbit serum was compared to that in sera of other mammals. The capacity

TABLE 3. Distribution of radioactivity in TBG and albumin of rabbit serum in the presence of ^{131}I-labelled thyroxine (T_4) and triiodothyronine (T_3) under different conditions. The proteins were separated by paper electrophoresis in veronal buffer at pH 8·6

Experimental condition	Labelled hormone present	% ^{131}I distributed in	
		TBG	Albumin
Endogenously labelled*	T_4 T_3	74	21
30 min after injection†	T_4	71	23
	T_3	53	40
Added *in vitro*‡	T_4	68	26
	T_3	46	48

* Serum of rabbit, 48 hr after injection of 50 μc of carrier-free ^{131}I-iodide. † As in Fig. 1. ‡ (0·01 μg T_4 and T_3)/ml. serum.

TABLE 4. Capacity of rabbit serum TBG to bind radio-active thyroxine and triiodothyronine as determined by paper electrophoresis in veronal buffer at pH 8·6

Substance added	Amount (μg/ml.)	% ^{131}I distributed in		
		TBG	Albumin	Other*
L-Thyroxine	0·01	68	26	6
	0·05	52	41	7
	0·20	29	60	11
	0·50	10	76	15
3:5:3′-triiodo-L-thyronine	0·01	46	48	6
	0·05	23	65	12
	0·20	7	74	19
	0·50	5	78	17

* Includes β- or γ-globulins or free hormone.

of rabbit serum TBG to bind thyroxine and triiodothyronine is low (Table 4). Assuming that 90% of the average rabbit protein-bound iodine (PBI) value of 5·0 μg/100 ml. plasma (Myant, 1958) is thyroxine, it will be seen that doubling of thyroxine concentration causes a significant degree of saturation of serum TBG. Under similar conditions hardly any radio-active thyroxine would be displaced to the albumin zone of human serum (Robbins & Rall, 1957; Tata, 1960*a*). With triiodothyronine the saturation of TBG was achieved more readily. On the other hand, the capacity (as distinct from 'affinity') of rabbit serum albumin was extremely high; a significant displacement of ^{131}I from this protein (to other protein fractions)

could only be detected when a level of 5μg thyroxine/ml. was exceeded. In this respect it is similar to human and rat serum albumin examined in veronal buffer.

The relative binding affinities for thyroxine and triiodothyronine, determined by the progressive displacement as labelled T_4 by the addition of stable T_3 (Rabbins & Rall 1957) vary with the amount of radioactive thyroxine present (Table 5). At low concentrations of added radioactive T_4 (0·01 μg/ml.), when most of it was bound to TBG (see Table 4), triiodothyronine was bound about three times less firmly in rabbit serum.

TABLE 5. Relative affinities of rabbit serum TBG (whole serum) and serum albumin for thyroxine and triiodothyronine as determined by the method of Robbins & Rall (1957); compared on the basis of thyroxine = 100

Sample	Amount of radioactive thyroxine present (μg/ml.)	Relative affinity for triiodothyronine
Whole serum	0·01	40
	0·05	71
	0·50	82
Rabbit serum albumin	0·01	88
	0·05	95
	0·50	90

However, when these measurements were made at higher concentrations, when more T_4 was bound to the albumin (see Table 4), the relative affinities approached the same value. That rabbit serum albumin binds the two hormones with almost the same affinity is seen in Table 5 from experiments performed on isolated rabbit serum albumin.

The metabolism and excretion of T_3 and T_4

The rapid loss of T_3 from the vascular compartment of the body during the first 6 hr after injection and the finding of a higher general tissue uptake of T_3 than of T_4 are consistent with the observations made on the protein-binding of the two hormones in the serum. However, the observation that from 24 hr after injection to 96 hr the half-lives in the blood are essentially the same, although the levels are very different, raises the question as to the relative half-lives in the body as a whole. As a whole-body γ-counting apparatus for animals of this size was not available, the rate of excretion of ^{131}I in the urine and faeces after the injection of labelled hormone was determined, and the amount remaining in the body calculated and plotted on a semi-logarithmic scale. The animals were kept in individual metabolism cages and given 0·002% KI solution to drink, beginning 48 hr before the injection of hormone and continuing throughout the experi-

mental period. Urine was collected at 8 hr after injection and urine and faeces at 24 hr and then daily.

Four rabbits were injected intravenously with 6·6 μc (0·4 μg) of T_3 and four with 5·0 μc (0·2 μg) of T_4. The percentage of the dose retained in the

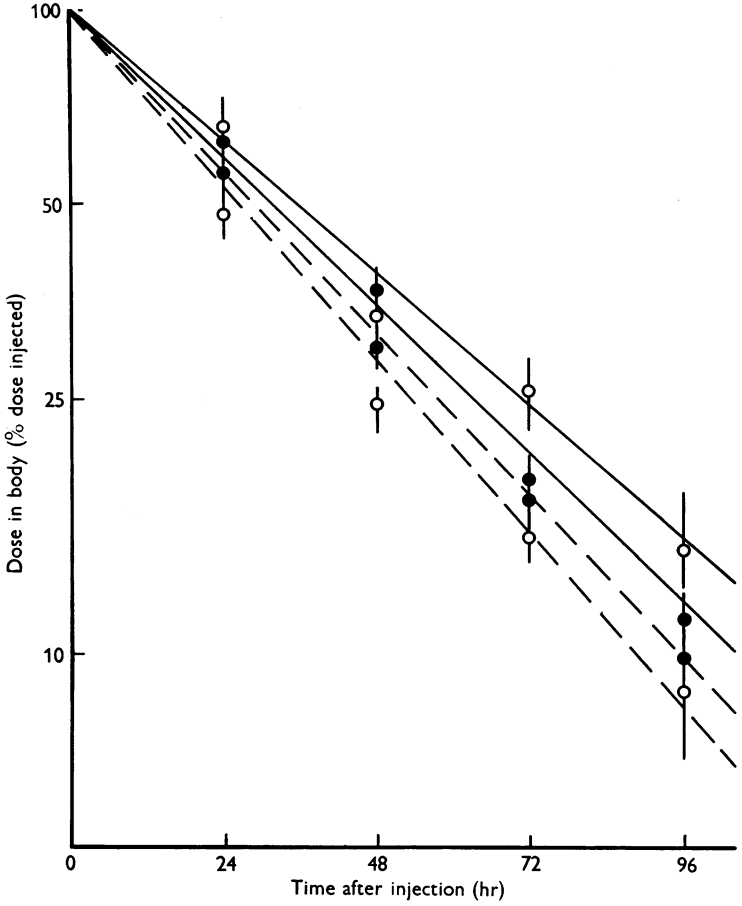

Fig. 4. The % retention of ^{131}I in the body after the administration of a tracer (0·5 μg) or a carrier dose (50 μg) of labelled T_3 and T_4. Each point is the mean of values from 4 rabbits; vertical bars indicate standard errors of the means. O- - -O tracer T_3; O——O carrier T_3; ●- - -● tracer T_4; ●——● carrier T_4.

body at different times is shown in Fig. 4, and the pattern of urinary and faecal excretion is shown in Figs. 5 and 6. The estimated half-lives for T_3 and T_4 are 26 and 27 hr respectively. In both instances more of the radioactivity appears in the urine than in the faeces. The level of ^{131}I in whole-blood samples is shown in Table 6.

The experiment was repeated with a larger dose of hormone (50 μg) in

order to study the excretion of an excess of T_3 and T_4. The retention of activity within the body is shown in Fig. 4. The mean half-lives, determined graphically, were 34 hr for T_3 and 29 hr for T_4, and thus a slight difference between the mean rate of excretion of T_3 and T_4 in physiological doses, or when given in excess, was observed. The pattern of excretion, however, differed markedly when an excess of hormone was injected. The

TABLE 6. Whole blood ^{131}I levels (% dose/ml.) after the injection of tracer (0·5 μg) or carrier (50 μg) doses of labelled T_3 and T_4. Each value is the mean ± S.E. of mean or results from four rabbits. Value at 0 hr is based on a complete distribution in the estimated blood volume (Armin *et al.* 1952)

Time (hr)	T_3		T_4	
	Tracer	Carrier	Tracer	Carrier
0	1·20 ±0·13	1·08 ±0·17	1·29 ±0·21	1·09 ±0·14
2	0·068±0·010	0·102±0·002	0·343±0·024	0·334±0·039
24	0·012±0·001	0·031±0·008	0·078±0·001	0·093±0·005
48	0·004±0·001	0·009±0·002	0·034±0·001	0·042±0·003

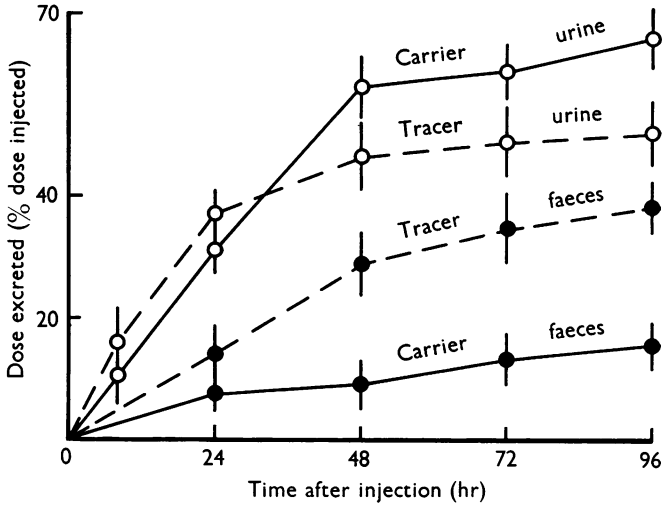

Fig. 5. The cumulative excretion of ^{131}I in urine and faeces after the intravenous injection of a tracer or a carrier dose of labelled T_3. Each point is the mean of values from 4 rabbits; vertical bars indicate the standard errors of the means.

proportion of the activity lost in the urine (as iodide) was increased with respect to the faecal excretion (Figs. 5 and 6). The excretions for the initial periods of 8 and 24 hr were somewhat lower in both cases, but the final urinary excretion was significantly higher. This delay in excretion is of interest with respect to the slightly longer half-lives observed when an excess of hormone is injected and the raised level of ^{131}I in whole blood samples (Table 6).

Two further metabolic experiments were carried out, the results of which are relevant to the discussion of the previous results. The excretion of iodide was studied to determine whether the iodide solution had effectively blocked the uptake of iodide by the thyroid gland and whether there was any significant contamination of the faeces by urinary radioiodide. Four rabbits received 7·5 μc of carrier-free ^{131}I intravenously. Urinary excretion was rapid and complete; the mean cumulative excretion

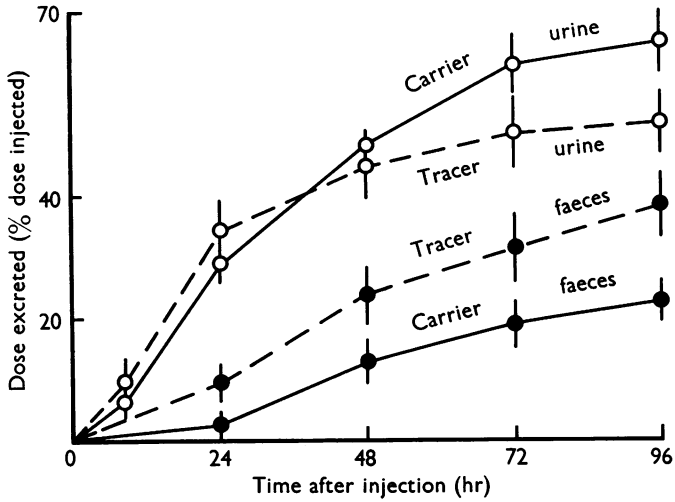

Fig. 6. The cumulative excretion of ^{131}I in urine and faeces after the intravenous injection of a tracer or a carrier dose of labelled T_4. Each point is the mean value from 4 rabbits; vertical bars indicate the standard errors of the means.

was 93·2 ± 1·9, 99·3 ± 0·9 and 100·1 ± 1·0 % (S.E. of mean of 4 determinations) respectively at 24, 48 and 72 hr after injection. Only 0·6 % of the radioactivity was found in the faeces in the first 24 hr and less than 0·2 % in the 24–48 hr period after injection.

During these experiments the animals were kept in metabolism cages that allowed them to move about and to continue their normal behaviour as regards refection. Only hard faecal pellets were obtained in the daily collection. The possibility that the administration of an excess of thyroid hormone might lead to increased refection, with a greater degree of enterohepatic recirculation of hormone and a higher probability of de-iodination rather than faecal excretion as the eventual means of disposal from the body, had to be considered. It seemed likely that any form of restraining device would prove to be a considerable 'stress' to the animals and that this might itself alter the rate or pattern of thyroid hormone metabolism. An indirect method was therefore preferred.

Seven rabbits were kept in metabolism cages for 3 days before the

experiments began. They were offered a known weight of pellets in excess of their daily intake in non-spill hoppers and their daily intake measured to ± 2 g. The faecal material was collected daily and weighed to ± 2 g. A known volume of iodide solution in excess of their daily intake was available and the fluid intake and urine volume were measured daily to within 5 ml. The animals were also weighed daily to ± 10 g. The pattern of solid and fluid intake and output and of weight gain were followed for

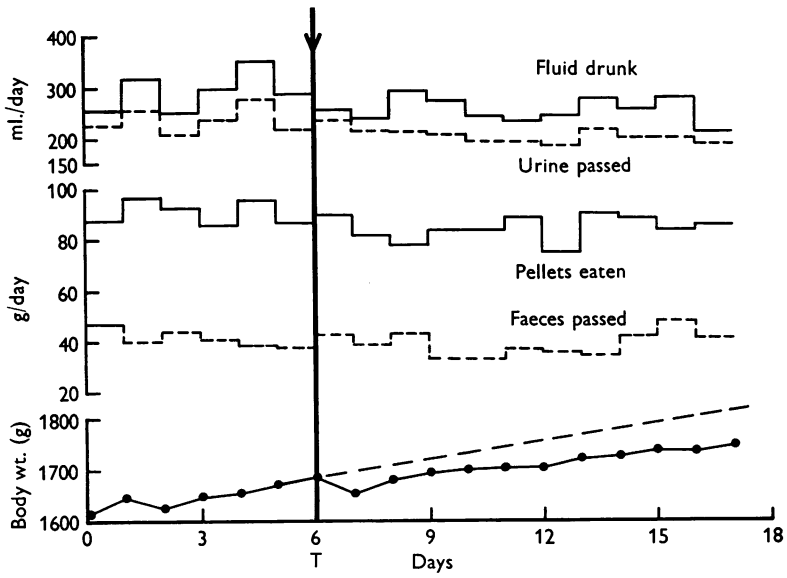

Fig. 7. The effect of 50 μg T_4 intravenously on fluid intake, urine output, food intake, faecal output and on body weight. Each value given is the mean result from 7 rabbits. Note the slight decrease in food intake without significant decrease in faecal mass after the injection of 50 μg T_4 intravenously at T.

6 days. Each animal was then injected intravenously with 50 μg of L-thyroxine in a volume of 0·25 ml. and the study continued for a further 11 days.

Figure 7 shows the results obtained. Following the injection of 50 μg of T_4 there was no obvious change in fluid intake or in urine volume; the excess of fluid drunk over urine collected remained the same. Mean food intake was slightly, but not significantly ($P > 0{\cdot}05$) below the pre-injection level for 6 days after injection (before, 91 ± 2 g/day (S.E. of mean of 42 measurements); after, 87 ± 3 g/day). The faecal mass was not significantly changed (40 ± 2 compared to 37 ± 2 g/day) despite the slight decrease in food intake, which does not suggest that any change in the direction of increased refection had occurred. The steady increase in body weight (pre-injection average 12 g/day) ceased for 3 days but was then

resumed, although the predicted weight was not regained within the 11-days post-injection period of the experiment. There was no support for the hypothesis that a greater degree of enterohepatic recirculation due either to increased refection or associated with a more efficient absorption of intestinal contents resulted from the injection of a large dose of thyroid hormone and could be responsible for the greater proportion of hormone deiodinated under these conditions.

DISCUSSION

In the present experiments on rabbits the half-life of T_3 and T_4 in the blood after the initial rapid fall in blood level during the phase of distribution was studied. The half-life of both compounds after a tracer dose was estimated from the data of Fig. 1 to be 27 hr. This is close to the value of 25 hr for T_4 obtained previously by Brown-Grant & Gibson (1955). The finding of indistinguishable half-lives for T_3 and T_4 in the rabbit is in contrast to observations in other mammals. Values of 6–11 days for T_4 and 2–3 days for T_3 in man and of 16–19 hr and 4–10 hr respectively in the rat have been reported by several groups of workers (Pitt-Rivers & Tata, 1959).

These are species in which the biological potencies of T_3 and T_4 are strikingly different. Tata & Shellabarger (1959), however, found identical half-lives (22·5 hr) in the chicken, in which the hormones are equipotent. Pipes, Premachandra & Turner (1959) obtained values of 1·99 and 2·47 days respectively for T_3 and T_4 in dairy cows, and it had been shown previously that the effects of T_3 and T_4 on the milk yield of cows in declining lactation was very similar (Bartlett, Burt, Folley & Rowlands, 1954). In the rabbit, too, T_3 and T_4 are about equipotent in suppressing pituitary thyrotrophic hormone secretion (Brown-Grant, 1955).

Tata & Shellabarger (1959) have suggested that these two phenomena may be connected with one another and be related, in birds, to the absence of a specific TBP from the serum. This was not found to be the case in the rabbit. Studies on the binding of thyroid hormones to the serum proteins of the rabbit during electrophoresis in barbiturate buffer showed that qualitatively the pattern of binding as well as the demonstrably higher affinity of the TBP for T_4 than for T_3 were as in other mammals and different from the bindings in birds (Robbins & Rall, 1957; Tata & Shellabarger, 1959; Tata, 1960*a*).

The results from studies of the distribution in the tissues of physiological doses of T_3 and T_4 during the first hour after injection are consistent with these observations on serum protein binding. The injected T_3 leaves the blood very rapidly as compared with T_4 (Fig. 2). When organ contents

are compared, after correction for the hormone contained in the vascular compartment, it is clear (Table 2) that the T_3 content of each of the wide variety of tissues examined is higher than that of T_4. However, there is no evidence that during the first hour after injection the pattern of distribution of T_3 and T_4 between organs is grossly different (Fig. 8). In particular, the liver content of T_3 as compared with T_4 is not grossly different from the

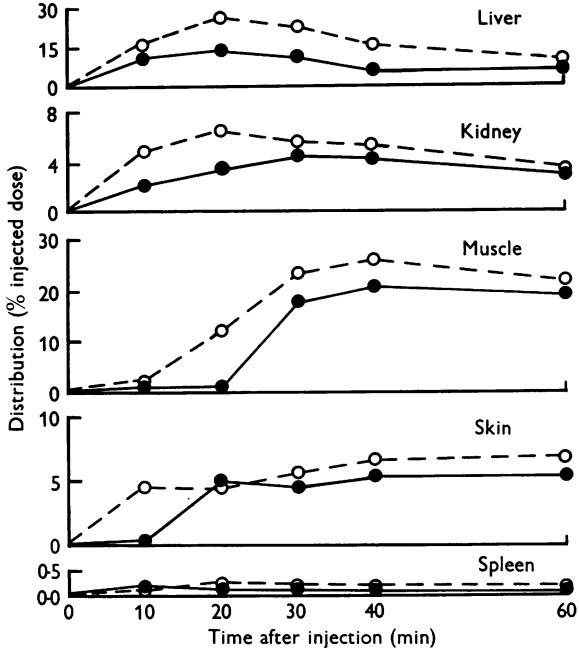

Fig. 8. The distribution of T_3 and T_4 in rabbit tissues 10–65 min after the intravenous injection of a tracer dose. The values given are % of the administered dose after correction for hormone in the vascular compartment, as explained in the text. Except for the values at 30 min, which are mean of results in 4 animals, each point is based on results from a single animal. Note that the general pattern of distribution is the same for both T_3 and T_4, though the values for T_3 are consistently higher. O---O T_3; ●——● T_4.

relative T_3 and T_4 contents of, say, the adrenal or the salivary gland or of muscle (Table 2). There is no evidence to support the suggestion of Gross *et al.* (1957) that the passage of T_3 through the liver or kidney and the formation of some conjugate other than the glucuronide is a necessary preliminary to the entry of T_3 into the other tissues of the body. Gross's compound X (the unidentified T_3 conjugate) was not detected on our chromatograms of plasma obtained after T_3 injection. Further, significant tissue levels of ^{131}I have been observed in animals injected with labelled T_4 at a time when no labelled T_3 was found on chromatograms of plasma.

The method used for the determination of the relative biological activities of T_3 and T_4 in the rabbit (Brown-Grant, 1955) depended on the maintenance of a raised level of hormone in the blood. When the level of hormone was raised in the electrophoresis experiments, it was seen that rabbit TBG has a very limited binding capacity for T_4. Table 4 illustrates the saturation of TBP and the displacement to albumin. Even with endogenously labelled hormone at normal plasma levels, a significant proportion of the activity was localized in the albumin zone (Table 3). These findings suggest that rabbit blood contains very little TBG compared with other mammals. The high values for thyroxine-binding capacity reported by Myant & Osorio (1959) may be due to a failure to achieve a comparable degree of separation of albumin and TBP in the two groups of experiments.

Accepting that the binding capacity of rabbit TBG is low, even a relatively small increase in serum hormone level would result in a large fraction of the hormone being bound to serum albumin, which has roughly equal but lower affinities for both T_3 and T_4. Under these circumstances the rate of disappearance of T_4 from the blood might be greatly increased, perhaps even approaching that of T_3 and the half-lives of both compounds might be much shorter. The findings of Balfour & Tunnicliffe (1960) that during electrophoresis in buffer other than barbiturate a significant proportion of the T_4, even at physiological plasma levels, travels with the pre-albumin fraction may necessitate a reconsideration of this hypothesis. The T_3-binding capacity of rabbit pre-albumin is not known, but it has been established that human thyroxine-binding pre-albumin (TBPA) does not bind T_3 at all (Ingbar, 1958; J. R. Tata, X. Y. Widnell & Y. Z. Grazner, unpublished).

The possibility that differences in the firmness of binding to serum proteins at raised plasma hormone levels could affect the relative rates of distribution and metabolism of the two hormones in the rabbit was examined in experiments in which a tracer (0·4 or 0·2 μg) or a carrier (50 μg) dose of hormone was administered and the urinary and faecal excretion studied. Blood levels at 2, 24 and 48 hr after injection of a tracer dose were very close to those seen in the previous experiment; after a carrier dose the level of T_4 was approximately the same as in the tracer experiment and still considerably greater than that of T_3, although the level of this compound was above that seen when a tracer dose was given (Table 6). This is consistent with the half-lives in the body determined from the cumulative urinary and faecal excretion. The half-lives of tracer and carrier T_4 were very similar (27 and 29 hr respectively) but the T_3 experiments gave a value of 26 hr for the tracer dose and a rather longer half-life (34 hr) for carrier T_3. Again there appears to be no change in the

initial distribution of the hormones, as judged by the 2 hr level in the blood, or in the biological half-life in the body that can be related to the pattern of protein binding or that can explain the similarity in biological activity.

An alternative explanation may be available from a consideration of the pathways by which the rabbit disposes of an excess of thyroid hormone. It appears to differ in this from the rat. In the rat a large dose of thyroxine is eliminated to a greater extent in the faeces than is a small, physiological one, owing to an increased rate of biliary secretion as the plasma level of thyroxine is raised (Myant, 1957). In contrast, the biliary clearance of T_3 in the rat is already maximal at normal plasma levels and is unchanged as this level is increased. An animal such as the rat, in which biliary clearance and faecal excretion is the main means of dealing with an excess of hormone, should be better able to handle an excess of T_4 than of T_3. This is in fact found to be the case; Maclagan & Wilkinson (1954) showed that, whereas the percentage urinary excretion of ^{131}I (as iodide) after the administration of increasing doses of T_3 and T_4 in the rat remained constant, the percentage excreted in the faeces at 24, 48 and 120 hr after injection increased markedly as larger doses of T_4 were given, but remained constant after giving T_3, despite similar increases in the dose administered. Thus the biological half-life of T_4 decreases with increasing doses, but not that of T_3.

An apparently greater biological potency for T_3 could well arise from these differences. The rabbit does not appear to behave at all like the rat in dealing with an excess of thyroid hormone. In confirmation of the previous findings of Brown-Grant & Gibson (1955), when a large dose of labelled T_4 is administered to rabbits, it is the proportion of the ^{131}I excreted in the urine that is increased by comparison with the pattern of excretion seen after giving a tracer dose (Fig. 6). Similarly, an increase in urinary and a decrease in faecal excretion is seen when the metabolism of a large dose of T_3 is compared with that of a tracer dose (Fig. 5). Again, in contrast to the findings in the rat, the biological half-life of T_4 is not decreased when the dose is increased and in the case of T_3 the half-life after a large dose may even be slightly lengthened.

The explanation suggested for the finding that T_3 and T_4 are equipotent in birds, namely that there is no specific inter-α-globulin TBG and that consequently both hormones reach the tissues at equal rates, is not applicable to the similar equipotency observed in the rabbit. At least when the two hormones are compared in an assay depending on the maintenance of an excess of hormone in the body, their equipotency appears to result from the manner in which they are metabolized. Whereas in the rat the process of biliary secretion appears to be the limiting factor in the metabolism of the hormones, in the rabbit deiodination by the tissues seems to

be the most important means of dealing with an excess of hormone. The tissues of the rabbit contain a thyroxine dehalogenase which is capable of dealing with far larger amounts of both T_3 and T_4 than are normally available as substrate (Tata, 1960*b*). The equipotency of T_3 and T_4 may be related to the existence of a large reserve capacity for dealing with an excess of either hormone in the rabbit in contrast to the limited capacity of the major excretory pathway for T_3 in the rat.

The over-all effectiveness of the biliary-faecal excretory pathway for thyroid hormones in the rabbit is low. Whether this is the result of a low rate of biliary secretion or the high efficiency of reabsorption, either on the first passage through the gut or as a result of refection is not known. The relatively short half-life suggests that the first explanation is more probable.

SUMMARY

1. The distribution of a physiological (2 μg) dose of ^{131}I-labelled T_3 and T_4 in the rabbit has been studied.

2. At 30 min after injection the volume of distribution was 837 ml. in the case of T_3 and 110 ml. in the case of T_4 as compared with an estimated plasma volume of 83 ml.

3. When the hormone content of a wide variety of tissues was determined 30 min after injection it was found that the T_3 content was always higher than that of T_4.

4. No evidence for a specific concentration of T_3 in any organ was obtained; the pattern of distribution followed that of T_4. The liver and muscle were quantitatively the most important sites.

5. The distribution of normal and increased amounts of T_3 and T_4 was studied by paper electrophoresis of serum in barbiturate buffer. Rabbit serum, like that of other mammals, contains a specific α-globulin thyroxine-binding globulin with a higher affinity for T_4 than for T_3. The capacity of this protein is less than that of the TBG of human plasma.

6. The half-lives of T_3 and T_4 in the plasma between 25 and 96 hr after injection were estimated to be 27 hr. The half-life of a similar physiological dose was determined by measuring the rate of loss from the body in the urine and faeces and was found to be 26 hr for T_3 and 27 hr for T_4. When an excess of hormone (50 μg) was injected, the half-lives determined in this way were 34 and 29 hr respectively.

7. A difference in the pattern of excretion after a large dose of either hormone was noted. Urinary excretion was increased, suggesting that a greater degree of deiodination was occurring after the injection of an excess of hormone.

8. The pattern of binding to the serum proteins does not explain the

anomalous equipotency of T_3 and T_4 in the rabbit. This may be related to the observation that an excess of hormone is dealt with by deiodination.

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