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FURTHER STUDIES OF THE METABOLISM OF THYROXINE AND 3,5,3′-TRIIODOTHYRONINE IN THE GUINEA-PIG

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SUMMARY

1. Thyroxine labelled with ^{125}I and triiodothyronine labelled with ^{131}I have been administered simultaneously to guinea-pigs and their metabolism studied by whole body counting and measurement of radioactivity in urine, faeces, and blood.

2. The half-life of triiodothyronine in the body is about 19 hr, significantly less than that of thyroxine (41 hr).

3. After triiodothyronine administration, an unidentified iodinated compound appears in the blood which complicates the estimation of the half-life of this hormone by measurement of the radioactivity in peripheral blood.

4. A previous report, based on measurements of blood radioactivity, that the half-lives of the two hormones are similar in the guinea-pig was not confirmed.

INTRODUCTION

In an earlier study (Brown-Grant, 1963) thyroid hormones labelled in the phenolic ring with ^{131}I were administered to guinea-pigs and serial measurements were made of the excretion of radioactivity in the urine and faeces over the next few days. The percentage of the administered dose remaining in the body at different times after injection was calculated and by plotting these results semi-logarithmically an estimate of the half lives in the body was obtained. The half-life ($t_{\frac{1}{2}}$) of thyroxine (T_4) was estimated to be about 31 hr and that of 3,5,3′-triiodothyronine (T_3) about 17 hr. Studies of the early part of the blood radioactivity curves after the intravenous injection of hormone showed that T_3 left the vascular compartment very much more rapidly than did T_4. This finding was consistent with reports that a specific binding protein with a high affinity for T_4

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is present in the serum of guinea-pigs (Lybeck, 1957; Farer, Robbins, Blumberg & Rall, 1962).

In 1964, Ray & Premachandra reported their findings on the half-lives of T_3 and T_4 in the guinea-pig. They injected ^{131}I labelled hormones and made serial measurements of the blood radioactivity and found the $t_{\frac{1}{2}}$ to be 30·2 hr for T_3 and 31·3 hr for T_4. These differences were not statistically significant and they suggested that the similarity in the blood radioactivity curves might be due to the absence, or presence in extremely small amounts, of what they referred to as a 'specific thyroid hormone-binding globulin'. In an addendum to their paper they noted the disagreement between their results and those of Brown-Grant (1963) and commented on the fairly large variation between animals evident in their own results. Finally, they stated that 'in a few isolated instances, however, [^{131}I]T_3 does disappear from the body at a faster rate than [^{131}I]T_4, as determined by counting the entire body in a whole body counter,' thus introducing, without supporting data, a third type of experiment into the discussion which appears to give results indicating again that the half-lives may vary greatly between animals.

It is difficult to see how the $t_{\frac{1}{2}}$ in the blood and in the body could be so different; indeed, the use of blood radioactivity levels to estimate the half-life is based, theoretically, on the assumption that hormone in the vascular compartment is in equilibrium with hormone throughout the T_3 or T_4 distribution space of the body, or at least equilibrates with it with a time constant that is negligibly small compared with the $t_{\frac{1}{2}}$ in the body. In an attempt to find an explanation for the differences reported, further experiments have been carried out in which the influence of animal to animal variation has been reduced to a minimum by the use of thyroid hormones labelled with different radioactive isotopes of iodine so that T_3 and T_4 metabolism could be studied simultaneously in the same animal.

METHODS

Animals. The guinea-pigs used were adult, albino, males and females (500–900 g body wt.) from the closed colony maintained in the Department. They were kept in temperature controlled animal rooms and fed a diet of fairly low (0·000023%, w/w) iodine content (Diet 18 from E. Dixon and Sons Ltd., Ware) *ad libitum* and received ascorbic acid in the drinking water. The uptake of radioiodine by the thyroid gland 24 hr after injection of 1 μc of $Na^{131}I$ was 9·79 ± 0·81% of the dose in six males and the injection of KSCN, (10 mg/100 g body wt., 22 hr after ^{131}I injection and 2 hr before killing) had no significant effect on the gland ^{131}I content, mean value for seven males being 9·30 ± 0·48% of the injected dose. The uptake by the thyroid was about twice as great as that of the pigmented guinea-pigs used in the previous experiments on T_3 and T_4 metabolism (Brown-Grant, 1962, 1963) and no significant amount of activity in the thyroid 24 hr after injection was present as iodide. Female pigs given 0·005% (w/v) KI solution to drink beginning 24 hr before the injection of $Na^{131}I$, showed a great reduction in uptake (0·61 ± 0·08%, mean

of six, $P < 0{\cdot}001$, as compared with a mean of $7{\cdot}95 \pm 0{\cdot}41\,\%$ in seven control pigs). This procedure was used to block recirculation of iodide from degraded thyroid hormone in some experiments as indicated in the section on Results.

Labelled hormones. These were obtained from the Radio Chemical Centre, Amersham, England. The T_4 was labelled with ^{125}I in the phenolic ring and the T_3 with ^{131}I in the 3′-position. Both were supplied in 50 % aqueous propylene glycol and were diluted with a 1:10 (v/v) mixture of guinea-pig plasma and 0·9 % (w/v) NaCl solution before injection. The hormones were not mixed before injection; animals received T_3 intramuscularly in one leg and T_4 in the other, or both were given intraperitoneally in two separate injections. On several occasions during and after the experiments portions of the diluted stock solutions were added to guinea-pig or rabbit plasma *in vitro* and the percentage of the radioactivity precipitated by the addition of 5 vols. of 5 % (w/v) trichloracetic acid (TCA) followed by one wash with 5 % TCA was determined. In the case of T_4 more than 95 % of the activity appeared in the precipitate throughout. With guinea-pig plasma, more than 85 % and with rabbit plasma, more than 90 % of the activity was in the precipitate when T_3 was added in *vitro*. After the animal experiments had been finished, T_3 was added to guinea-pig plasma and dialysed against tapwater under conditions where more than 90 % of radio-iodide added *in vitro* was lost in the first hour and more than 95 % in 2 hr. In experiments with T_3 carried out in triplicate, 6 % of the activity was lost in the first hour and no further loss occurred in the second hour. It was concluded that not more than 7 % of the activity could be present as iodide even at the termination of the experiments and that the failure to precipitate more than 85 % of the activity of T_3 by TCA was due to the low level of binding of this compound to serum proteins (see Discussion).

Measurement of radioactivity. The guinea-pigs would enter and remain still without struggling in a plastic cylinder about 7 in. (17·8 cm) in length and 4 in. (10·2 cm) in diameter, sealed at one end and with a removable cap at the other, provided with ventilation holes. The cylinder could be positioned in a reproducible manner in the counting chamber of a model 440 Armac Scintillation Detector (Packard Instrument Company). Four 10 sec counts were made at each of two settings of the pulse height analyser with an automatic scaler. Urine and faeces were also counted in the Armac Detector, the urine at a constant volume of 100 ml. and the faeces at varying volumes for which empirical correction factors were determined. The initial count soon after injection for each animal was taken as 100 % (see Experimental Procedure) and subsequent counts expressed as a percentage of the initial count after correction for isotope decay. Counts obtained from urine and faeces were compared to a standard prepared at the time of injection counted under the same geometrical conditions at the same time and expressed as a percentage of the administered dose. Blood was obtained by cardiac puncture under sodium pentobarbitone anaesthesia after which the animals were killed and in some cases the thyroid gland removed for counting. Whole blood, plasma and plasma protein bound radioactivity (determined after TCA precipitation as described above) and also thyroid gland radioactivity were measured in a well-type NaI crystal scintillation counter with pulse height analyser and automatic scaler and expressed as a percentage of the administered dose by reference to a standard prepared at the time of injection.

Both detectors respond to the gamma rays emitted by the two isotopes, ^{125}I and ^{131}I, and the magnitude of the signal is related to the energy of the radiation. By a suitable choice of analyser setting, it was possible to count pulses from the 0·36 MeV radiation of ^{131}I with no contribution from ^{125}I in the sample. In the lower energy range of the spectrum, a setting was found empirically which gave maximum efficiency for pulses from ^{125}I combined with minimum interference from pulses due to ^{131}I. A correction factor for calculating the ^{131}I contribution in the low energy window from an observed counting rate in the high energy window was determined for each instrument and the counts due to ^{125}I in the low energy window calculated by subtraction of the ^{131}I contribution from the total counts observed.

Statistical accuracy for ^{131}I was $\pm 2{\cdot}5\%$ or better; because of the varying contribution from ^{131}I, the accuracy of ^{125}I counts was usually $\pm 5\%$.

Experimental procedures. Animals were placed in individual metabolism cages 24 hr before the injection of labelled hormones. In the first experiment these were given intramuscularly and it was found in preliminary experiments that the counting rate from the animals, irrespective of position within the counter, was steady from 5 to 30 min after injection. The initial count was made 10–15 min after injection in these experiments. After intraperitoneal injection the counting rate rose slightly for the first 40 min and then remained steady for about 1 hr. Initial counts were made 60 min after injection. Subsequent counts were made 24, 48, 72 and 96 hr after the initial count. Urine and faeces were also collected at these times and isotope content measured; the cumulative excretion in urine and faeces was calculated and the residual activity in the body determined by subtraction.

Studies of the half-life in the blood were carried out on large groups of animals because of the difficulty of obtaining adequate volumes of blood from the ear vein or by repeated cardiac puncture; each animal was used only once.

Statistics. Where possible, the paired *t* test was used. Comparison of group means was by the *t* test. Not significant indicates a value for P of $> 0{\cdot}05$. Values quoted are group means $\pm$ S.E. of the mean.

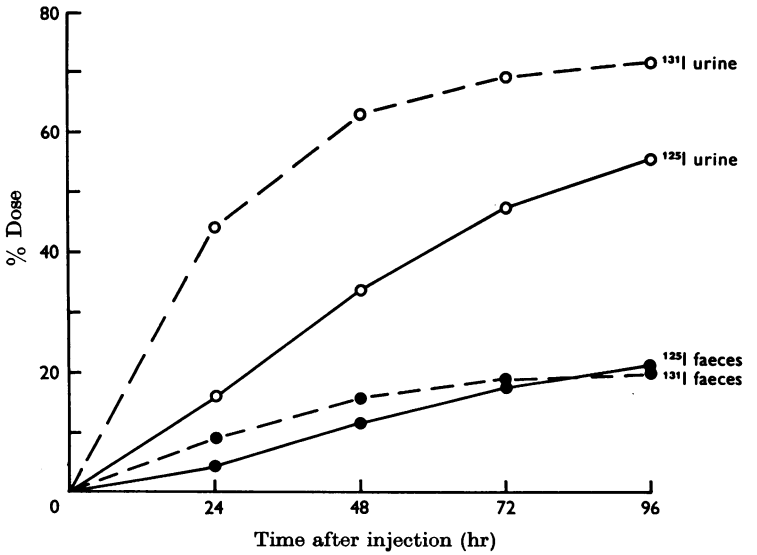

Fig. 1. The cumulative excretion of ^{125}I from T_4 and ^{131}I from T_3 in urine and faeces following the injection of labelled hormones. Each point is the mean of values for six guinea-pigs, all of whom received both hormones. ○ — ○ ^{125}I in urine, ○ - - ○ ^{131}I in urine, ● — ● ^{125}I in faeces, ● - - ● ^{131}I in faeces.

RESULTS

The $t_{\frac{1}{2}}$ of T_3 and T_4 in guinea-pigs receiving iodide. As far as possible, the conditions of the experiments of Brown-Grant (1963) were duplicated. Six male pigs received iodide to block recirculation of radio-iodide and the hormones $[^{125}I]T_4$, 3 μc, 0·21 μg and $[^{131}I]T_3$, 1·5 μc, 0·11 μg were injected

intramuscularly. The whole body counts were made 10–15 min after injection and then daily up to 96 hr and the cumulative excretion of radioactivity in urine and faeces was measured. The percentage of the injected dose of each isotope remaining at 24, 48, 72 and 96 hr was determined by whole body counting and by calculation from the cumulative excretion and was compared by the paired t test. The retention of ^{125}I, the isotope labelling T_4, was significantly greater at each time ($P < 0{\cdot}01$ or less).

The pattern of excretion of radioactivity is shown in Fig. 1. Urinary excretion of ^{131}I from T_3 was greater at all time intervals ($P < 0{\cdot}01$) in the paired t test. Faecal excretion of ^{131}I was higher ($P < 0{\cdot}01$) at 24 and 48 hr, but the faecal excretion of ^{131}I and ^{125}I at later stages did not differ significantly.

Both the whole body counts (because of possible errors in the initial count) and the cumulative excretion (because of failure to achieve complete collection of urine or faeces) are liable to systematic errors. The means of the values for the percentage remaining in the body determined by the two methods were used to calculate the group mean and standard error and these results are shown in Fig. 2. Rough estimates of the $t_{\frac{1}{2}}$ in the body can be made from these graphs; the value for T_4 is about 40 hr and for T_3 about 20 hr.

The $t_{\frac{1}{2}}$ of T_3 and T_4 in animals not receiving iodide. In the study of Ray & Premachandra (1964) the hormones were injected intraperitoneally and in most cases no attempt was made to block recirculation of iodide from degraded hormone. In order to reproduce the conditions of Ray & Premarchandra's experiments as closely as possible, six male pigs were injected with $[^{125}I]T_4$ (3·0 μc, 0·3 μg) and $[^{131}I]T_3$ (2·0 μc, 0·3 μg) intraperitoneally and received tap water to drink.

Intraperitoneal injection is unsatisfactory in the guinea-pig because of the danger of injection into the lumen of the large gut. This probably occurred in two of these animals; one animal excreted 35·6 % of the dose of T_3 in the faeces in the first 24 hr compared to a mean value of $12{\cdot}0 \pm 1{\cdot}3$ % for the other five. A second animal excreted 61·7 % of the dose of T_4 in the faeces in the first 24 hr compared with a mean value of $6{\cdot}7 \pm 0{\cdot}7$ % for the other five. Results for these hormones in these two animals were discarded, leaving in this experiment four animals in which both T_3 and T_4 metabolism could be studied, plus two additional animals in whom either T_3 or T_4 metabolism could be studied. The same statistical tests were carried out as in the first experiment. Comparisons by the paired t test were made for four animals and by a comparison of group means for five animals. The pattern of excretion of radio-iodide was very similar to that shown in Fig. 1. Excretion of radio-iodide in the urine was significantly greater at all time intervals for ^{131}I from T_3 than for ^{125}I from T_4 ($P < 0{\cdot}05$)

and faecal excretion was higher ($P < 0{\cdot}02$) at 24 hr, but not at later time intervals. Retention of isotope for the four animals providing data for both hormones was higher ($P < 0{\cdot}01$) for ^{125}I at all time intervals as measured by whole body counting and up to 72 hr ($P < 0{\cdot}05$), but not at 96 hr, by calculation. The curves shown in Fig. 2 are based on the mean retention determined by the two methods in five animals. Deviation from a single exponential curve is greater than in the first experiment, especially for T_3, but approximate $t_{\frac{1}{2}}$ for the first 48 hr were 42 hr for T_4 and 18 hr for T_3.

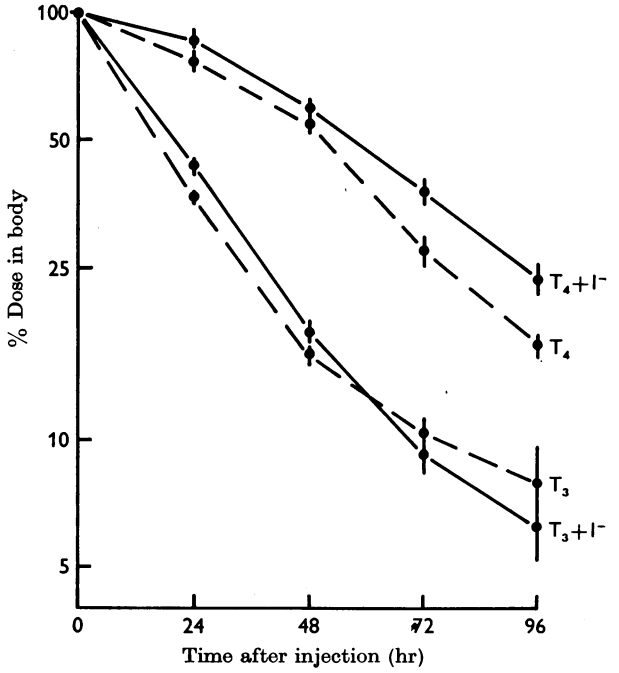

Fig. 2. The percentage of the administered dose of radioactivity remaining in the body at various times after the injection of labelled T_3 and T_4 in guinea-pigs. Values for each pig are the mean percentage remaining as determined by both whole body counting and by calculation from the cumulative excretion. Points are the mean ± S.E.M. (indicated by vertical bands) for six pigs in the first experiment in which animals were given KI to block recirculation for iodide and the mean of five in the second experiment when KI was not given.

Radioactivity in the blood after T_3 and T_4 administration. A total of twenty animals not receiving iodide were injected intraperitoneally with [^{125}I]T_4 (3·0 μc, 0·3 μg) and [^{131}I]T_3 (2·25 μc, 0·33 μg) and four animals were killed at 6, 24, 48, 72 and 96 hr after injection. The isotope content of blood and plasma and the TCA precipitable activity of plasma were measured and the results are shown in Fig. 3.

The first point to be noted (which is obscured in Fig. 3 by the use of a logarithmic scale) is the very much higher level of T_4 (approximately tenfold). The second is that while the fraction of plasma radioactivity precipitable by TCA is high, 90 % or more in all samples for T_4, this is not so for T_3. Initially, TCA precipitable activity averaged 63 % at 6 hr, presumably because an appreciable part of the ^{131}I was present as iodide. From 24 hr onwards, more of the activity is precipitable (96 %, 100 and 93 % at 48, 72 and 96 hr) than was the case when T_3 was added to plasma *in vitro* (85 %). The third point is that although accurate estimation of half-lives in blood is not possible, the blood or plasma curves for ^{125}I from T_4 and ^{131}I from T_3 are not grossly different as are the whole body curves in Fig. 2. An estimate of about 30 hr for both radioactivity curves is quite reasonable and is consistent with the estimates of the biological decay of blood radioactivity made by Ray & Premachandra (1964).

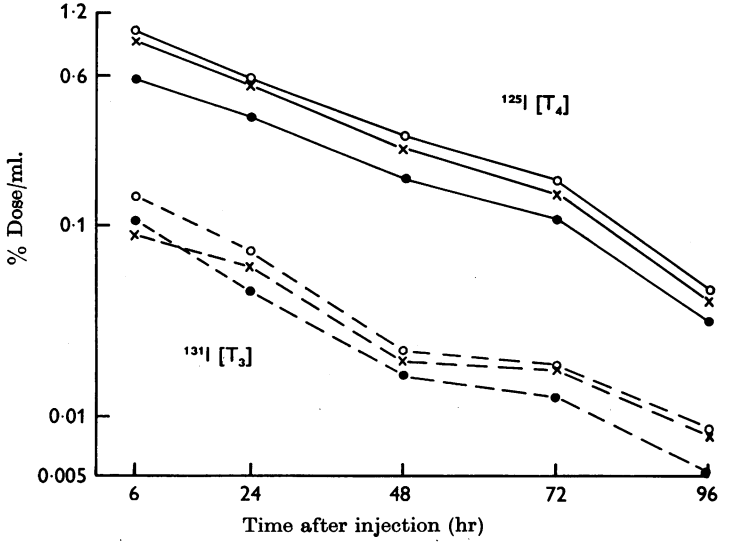

Fig. 3. Blood, plasma, and plasma TCA-precipitable radioactivity at different times after the injection of [^{125}I]T_4 and [^{131}I]T_3 in guinea-pigs. Each point is mean of values for four animals. ● Blood, ○ plasma, × TCA precipitable plasma activity.

The second half of the ^{131}I blood curve is somewhat less steep than the initial portion and the whole body curves for T_3 also turn up from 48 hr onwards, especially when iodide is not given (Fig. 2). A possible recirculation of iodide via the thyroid after the rapid deiodination that occurs in the first 24 hr after injection was considered. Mean thyroid content of ^{125}I from T_4 was $2{\cdot}27 \pm 0{\cdot}39$ % and of ^{131}I from T_3 was $3{\cdot}88 \pm 0{\cdot}29$ % in four animals killed 72 hr after injection and $3{\cdot}99 \pm 0{\cdot}44$ % and $4{\cdot}25 \pm 0{\cdot}19$ % in four killed at 96 hr.

A group of six male pigs receiving KI solution to drink and a second group of six receiving tap water were injected intramuscularly with 4·0 μc (1·0 μg) of [^{131}I]T_3. Half-life for T_3 in both groups was about 19 hr as determined by whole body counting and by calculation from the measured excretion of isotope. Three animals from each group were killed at 48 hr and at 96 hr after injection. Whole blood, plasma and TCA precipitable activity in the two groups were quite similar at both 48 and 96 hr in both groups (Table 1). The values are close to those shown in Fig. 3, and the

TABLE 1. Radioactivity in blood and plasma (as % injected dose/100 ml. plasma) and the fraction of plasma radioactivity precipitable by TCA (% protein bound) in guinea-pigs 48 and 96 hr after the injection of [^{131}I]T_3. Group *A* received KI, Group *B* did not

	Group *A*			Group *B*		
	Blood	Plasma	Protein bound (%)	Blood	Plasma	Protein bound (%)
48 hr	1·7	2·2	89·3	1·9	3·1	87·2
	2·4	3·5	88·1	2·3	3·6	96·2
	1·9	2·7	88·2	1·9	2·7	85·9
96 hr	0·4	1·0	96·6	0·8	1·1	100·0
	0·8	1·2	100·0	0·7	1·4	92·8
	0·7	1·1	95·5	0·6	1·2	97·1

fall between 48 and 96 hr is similar in the two experiments. However, thyroid contents at 48 and 96 hr for the iodide treated group were 0·37 ± 0·07 and 0·34 ± 0·11 %, while the control group gave values of 4·30 ± 0·27 and 3·19 ± 0·28 %, significantly higher ($P < 0·01$). The ^{131}I-labelled material in the blood between 48 and 96 hr after injection, showing better than 95 % precipitably by 5 % TCA in both groups cannot be T_4 released from the thyroid in view of the gross differences in uptake.

DISCUSSION

The half-lives of T_3 and T_4 determined either by whole body counting or by calculation from the urinary and faecal excretion of radioactivity differ significantly in the guinea-pig. Estimated $t_{\frac{1}{2}}$ for animals receiving KI to block recirculation of radio-iodide were 20 and 40 hr for T_3 and T_4 respectively. The value for T_3 is close to the $t_{\frac{1}{2}}$ of 17 hr previously reported (Brown-Grant, 1963) but that for T_4 is rather longer—40 hr as opposed to 31 hr. When recirculation of iodide is not blocked estimated half-lives were 18 and 42 hr. The pattern of excretion of radioactivity (Fig. 1) shows that the major pathway of metabolism of both hormones is deiodination.

Ray & Premachandra (1964), on the basis of measurements of the radioactivity in blood after injection of labelled T_3 and T_4, reported that the

half-lives of the two hormones were the same—about 30 hr. It is impossible to reconcile this with the very different half-lives for the two hormones obtained by other methods. Their experiments were repeated, as nearly as possible under the conditions chosen by them, and in essence their experimental observations were confirmed. If the initial count of blood or plasma radioactivity 24 hr after injection of labelled hormones is taken as the starting point, then the total level of activity in blood or plasma over the next few days does decrease at about the same exponential rate for both the isotope used to label T_4 and that used to label T_3 (Fig. 3). A reasonable estimate of the half-life of the activity in blood over this period would be about the 30 hr reported by them. However, what was either not noted or not reported by Ray & Premachandra is that the starting levels at 24 hr are grossly different, about 0·7 % of the injected dose/ml. for T_4 but only about 0·05 % of the dose/ml. for T_3. The subsequent changes represent the metabolism of about 80 % of the injected dose of T_4 but at 24 hr after injection only about 40 % of the T_3 remains in the body and by 48 hr, only about 15 %. If the activity in the vascular compartment represented labelled hormone and if this were in equilibrium with hormone in the extravascular compartment, it is difficult to see why the activity in the blood should not fall at the same rate as the radio-iodide content of the whole body, yet in the case of T_3 it clearly does not. The possibility that the labelled component that persists in the blood after radioactive T_3 administration is thyroxine, secreted by the thyroid after recirculation of radio-iodide originally present in T_3 appears to be eliminated by the observation that it is present despite treatment with a large dose of KI or, in the experiments of Ray & Premachandra, 1-methyl-2-mercapto-imidazole, to block recirculation. An alternative explanation is that during the rapid and extensive de-iodination of T_3 that takes place in the first 24 or 48 hr after injection, a small amount of the iodine released becomes associated with some tissue component, possibly a protein, and that some of this iodinated material reaches the blood where it has a comparatively long half-life. Trans-iodination of proteins during the de-iodination of thyroid hormones by tissue homogenates *in vitro* is well documented (Tata, 1960*a*, *b*) and Ford, Corey & Gross (1957) detected an iodinated material, possibly protein in nature, remaining at the point of application in their chromatographic system, in tissue extracts from guinea-pigs receiving labelled T_3 *in vivo*. A combination of decreasing amounts of radioactivity representing T_3 with a short half-life and increasing amounts of an iodoprotein with a long half-life could give rise to an over-all decay curve of radioactivity in plasma with an apparent half-life of about 30 hr after T_3 administration, as observed by Ray & Premachandra (1964), and in the present experiments and erroneously interpreted by them as indicating a

long half-life for T_3, comparable to that of T_4. Evidence in favour of this view is the high percentage of the plasma radioactivity that is precipitable by TCA from 48 hr onwards after T_3 administration (Fig. 3 and Table 1), a finding that is not consistent with the activity being present as T_3. Biochemical investigation of the nature of the residual activity in plasma after T_3 administration is likely to be difficult; the absolute amount of T_3 that can be given is limited by physiological considerations to microgram or submicrogram amounts and the amount of isotope by the fact that the specific activity of the labelled T_3 is limited by the danger of chemical changes induced by self-irradiation (Tata, 1959).

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