The Formation of Thyroid Hormones *in vivo* after the Administration of $K^{131}I$ and Thyrotropic Hormone

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(Received 14 January 1959)

Previous work upon the incorporation of intravenously injected radioactive ^{131}I into the thyroid hormones of the blood, though extensive, has been somewhat restricted since in most instances separation of the individual thyroid hormones has either not been achieved or separation was made upon samples of blood withdrawn at a single interval after injection.

For example, in studying the incorporation of ^{131}I into the serum thyroid hormones in patients suffering from hyperthyroidism and myxoedema and in the normal controls, Robbins, Rall, Becker & Rawson (1952) and Chesky, Dreese, Duboczky, Hall & Hellwig (1953) determined only the total inorganic iodine and total hormonal iodine in the blood. On the other hand, Gross & Pitt-Rivers (1951) determined the quantity and radioactivity of labelled thyroxine and tri-iodothyronine in serum from goitrous patients 24 hr. after injection of radioactive iodine. Many similar studies have been made [for reviews see Gross & Pitt-Rivers (1953) and Roche & Michel (1955)], but in only a few (cf. Taurog & Chaikoff, 1947; Tong, Taurog & Chaikoff, 1951) have attempts been made to measure the relative rates of formation of the various hormones, largely owing to the absence of sensitive methods for the separation and estimation of microquantities of iodine-containing hormones in the blood. Recently (Feuer, 1957; Szabó, Feuer & Balogh, 1957) a micromethod has been developed which permits the quantitative separation and determination of microquantities of thyroxine, tri-iodothyronine and other iodine-containing compounds in the blood and the method has been applied to the study of the rates of formation of thyroid hormones *in vivo* by measurements over a period of time after injection of ^{131}I .

Since the rate of release conditioned by various types of stress is similar, in depletion of ^{131}I of thyroid gland, to that brought about by thyrotropic hormone (Brown-Grant, Euler, Harris & Reichlin, 1954; Brown-Grant, Harris & Reichlin, 1954; Harris, 1955), the rate of release of individual hormones in animals given thyrotropin was also studied.

It has been found that the incorporation of ^{131}I into tri-iodothyronine precedes the incorporation

into thyroxine and that thyrotropin accelerates the conversion of tri-iodothyronine into thyroxine.

METHODS

Animals. Rats (200–300 g.) and rabbits (2.5–4.0 kg.) from the Laboratory stock were used in the individual experiments.

Administration of radioactive iodine and thyrotropic hormone. Radioiodine was obtained as carrier-free $K^{131}I$ from The Radiochemical Centre, Amersham, Bucks, and, after dilution with 0.9% NaCl, 0.2–0.5 ml. containing 100 μC of ^{131}I was injected into rats either through the tail vein or, under ether anaesthesia, intraperitoneally. Thyrotropic hormone (Ambinin-B Organon; 50 Junkmann-Schoeller units/ml.) was also given intraperitoneally. Rats received 0.05–0.1 ml. and rabbits received 0.5–1.0 ml. During the experiments the animals were kept in the laboratory at room temperature and were maintained on the normal diet.

At differing times after the administration of ^{131}I or thyrotropic hormone, the rats were anaesthetized and blood was removed through the dorsal aorta with the aid of a syringe. After standing for 3–4 hr. at room temperature the clotted blood was centrifuged and the serum was removed. The serum of one rat, or the combined sera of two rats (10–14 ml.) was used for fractionation of the thyroid hormones. Since the removal of this amount of blood from the rats resulted in death, separate groups of rats were used to establish each point upon the time curves. With rabbits the thyrotropic hormone was given intraperitoneally, the blood samples being taken from the marginal veins of the ears after heparinization of the animals with 1–2 ml. of heparin (Roche Products Ltd., 1000 units/ml.). Samples were taken immediately before the injection of the thyrotropic hormone and at varying times thereafter. The same animal was used to yield all the blood samples required during any one experiment.

Extraction of the thyroid hormones. Serum (3–5 ml.) was brought to about pH 3 with $N-HCl$ and butanol (2 vol.) was added. After shaking vigorously for 30 min. the butanolic phase was separated by centrifuging. This extraction was repeated three times with butanol (1 vol.). The combined butanol extracts were reduced to dryness under diminished pressure below 45°, and the residue was suspended in a volume of water equivalent to one-tenth of the volume of the serum originally taken.

Separation and determination of the thyroid hormones. These were separated by chromatography on paper and the fractions estimated by methods previously described (Feuer, 1957; Szabó *et al.* 1957). Briefly, 0.1 ml. of the aqueous hormonal solution was measured on to paper

(Schleicher and Schüll no. 2043 or Whatman no. 1) and developed by the ascending technique for 18–20 hr. at room temperature with the organic phase from dioxan–butanol–aq. 2N-NH₃ soln. (1:4:5, by vol.). Samples of tri-iodothyronine and thyroxine were included in a guide strip. After development, the hormones in the guide strip were located with ninhydrin and the corresponding adjacent sections were cut from the rest of the chromatogram. These were incinerated at 450–500° in the presence of 0.5 ml. of 2N-Na₂CO₃, the ash was brought to neutrality with N-HCl and the volume adjusted to 3 ml. with water. To a portion was added: NaCl (0.8 g.), 5 mm-ferroin indicator (0.3 ml.) 0.2N-H₃AsO₃ (1.9–2 ml.) and water to 3.5 ml. The solutions were brought to 25°, 0.01N-Ce(SO₄)₂ (1.5 ml.) was added and the time taken for the colour to change from blue to red–brown was noted. The end point is fairly sharp, and the period between the addition of Ce(SO₄)₂ and the colour change bears a quantitative relationship to the amount of iodine present over the range 2×10^{-4} to 2×10^{-2} μ g. of iodine.

Fig. 1 illustrates how the method can be used with a human thyroid sample. The upper part of the figure shows the separation of the concentrated thyroid extract (1a₁) and the thyroxine and tri-iodothyronine controls (1a₂) on the paper chromatogram; the lower part (1b) gives the iodine contents of the various parts of the paper strip.

Determination of the iodine content of the whole blood. After the blood was taken, samples (0.5 ml.) were evaporated and incinerated in the presence of 0.5 ml. of 2N-Na₂CO₃ and the inorganic iodide was measured as described above.

Fig. 1. Separation and determination of thyroid hormones from human thyroid-gland extract. (a) Paper chromatogram: 1a₁, 0.1 ml. of tenfold-concentrated human thyroid-gland extract; 1a₂, 25 μ g. of thyroxine and 25 μ g. of tri-iodothyronine; developed by the ascending technique at room temperature with dioxan–butanol–aq. 2N-NH₃ soln. (1:4:5, by vol.) and located with ninhydrin. (b) Measurement of iodine content of the thyroid extract: 1, thyroxine, 2, tri-iodothyronine; 3, compound 3.

Determination of the protein-bound iodine. The blood was kept at room temperature for 3–4 hr. and, after clotting, the serum was separated by centrifuging. The proteins were precipitated from 0.5 ml. of serum with the ZnSO₄–NaOH precipitant of Somogyi. The precipitate was incinerated in the presence of 0.5 ml. of 2N-Na₂CO₃ and the inorganic iodide was measured as described above.

Measurement of radioactivity in the separated fractions. The distribution of radioactivity in the hormonal fractions, after separation by paper chromatography and location with ninhydrin, was determined by sectioning a strip 3 cm. wide at intervals of 1 cm. and measuring the radioactivity with an end-window counter. Samples were counted twice, to an accuracy of 5%.

RESULTS

Incorporation of K¹³¹I into thyroid hormones of the serum

The incorporation of ¹³¹I was studied in albino rats. Fig. 2 represents the results obtained in a typical experiment in which the radioactive iodide was administered to rats 8 hr. before blood was withdrawn. The upper part of the figure shows the position of the ninhydrin-reacting substances found on the paper chromatogram of a serum extract (2a₁) compared with known thyroid

Fig. 2. Incorporation of K¹³¹I into thyroid hormones. (a) Paper chromatogram: 2a₁, 0.1 ml. of tenfold-concentrated rat-serum extract; 2a₂, 25 μ g. of thyroxine and 25 μ g. of tri-iodothyronine controls; developed by the ascending technique at room temperature with dioxan–butanol–aq. 2N-NH₃ soln. (1:4:5, by vol.) and developed with ninhydrin. (b) Measurement of radioactivity of the serum extract: 0, inorganic iodine; 1, thyroxine; 2, tri-iodothyronine; 3, compound 3.

hormones added as markers ($2a_2$). The concentration of the ninhydrin-reacting compounds in the serum extract is very low. The lower part of the figure (b) shows the distribution of radioactivity in the chromatogram prepared from the serum extract.

It is seen that the spots corresponding to thyroxine and tri-iodothyronine developed with ninhydrin also correspond to areas of increased radioactivity. Thyroxine: R_F 0.45 (ninhydrin), 0.48 (activity); tri-iodothyronine: R_F 0.61 (ninhydrin), 0.645 (activity). The site of thyroxine was also checked with the aid of [^{131}I]thyroxine. In addition to these compounds, increased radioactivity was also detected at a point on the chromatogram ahead of tri-iodothyronine, indicating the existence of another iodine-containing compound, designated compound 3. The existence of such a compound has been previously demonstrated by Feuer & Vekerdly (1958). Several other authors have described unknown iodine-containing compounds with a high R_F value in the extracts of serum and thyroid of different animals (Tong *et al.* 1951; Vannotti, Cruchaud, Frei & Mahaim, 1955; Taurog, Potter & Chaikoff, 1955; Lissitzky & Roques, 1956; Taurog, Tong & Chaikoff, 1957).

The pattern of incorporation of radioactive iodine into the thyroid hormones of the blood at varying times after the administration of ^{131}I is presented in Fig. 3. In the graph only the organic ^{131}I values are shown. The total amount of this iodine value was taken to be 100% and the relative amounts of thyroxine, tri-iodothyronine and compound 3 were calculated.

It can be seen that the radioactivity of the thyroxine, which accounted for only 20% of the total activity of organic iodides at the beginning, increased markedly with time. This increase was fairly rapid in the first 8 hr., becoming slower later, and after 24–48 hr. a steady value of 70–80% was

reached. The relative amount of the radioactive tri-iodothyronine was highest (70–75%) in the first 1–1½ hr. after the administration of ^{131}I . The activity diminished with a rapidity almost equal to the rise in radioactivity of thyroxine. Later this decrease became slower and after 24–48 hr. an equilibrium value of 10–20% of the total was reached. The radioactivity of compound 3 was initially smaller than the radioactivity of thyroxine; it increased a little in 6–8 hr., and subsequently fell slowly to an equilibrium value of about 5–10% of the total.

The ranges of quantities of thyroxine, tri-iodothyronine and compound 3 in the serum of the different rats used in the above-described experiments were: thyroxine, 1.41–6.19 $\mu\text{g.}/100$ ml. of serum; tri-iodothyronine, 0.12–1.90 $\mu\text{g.}/100$ ml. of serum; compound 3, 0.15–0.91 $\mu\text{g.}/100$ ml. of serum.

It is assumed that the total iodine values in the serum remain constant under normal conditions and that the small amount of carrier-free radioactive iodine injected did not disturb this level.

In these experiments the incorporation of ^{131}I into different organic compounds has been compared with the values of ^{127}I in each fraction studied. The results are expressed in terms of specific radioactivity. These data are presented in Fig. 4. The figure shows that in the rats killed half an hour after the administration of radioactive iodide, the specific radioactivity of tri-iodothyronine was much greater than that of the other organic fractions. Its value increased in the first few hours, then diminished rapidly; 6–8 hr. after injection the decrease became slower. The specific radioactivity of thyroxine was very low at the beginning and increased throughout the period studied, finally becoming higher than that of tri-iodothyronine. The specific radioactivity of compound 3, initially higher than that of thyroxine, subsequently decreased to a lower value.

Fig. 3. Formation of thyroid hormones *in vivo* as measured in rat blood after administration of K^{131}I . For details see the Methods section. $\circ$, Thyroxine; $\bullet$, tri-iodothyronine; $\triangle$, compound 3.

Fig. 4. Relative specific radioactivity of thyroid hormones in rat blood after administration of K^{131}I . $\circ$, Thyroxine; $\bullet$, tri-iodothyronine; $\triangle$, compound 3.

Table 1. *Effect of thyrotropic hormone on the distribution of radioactive iodine in the thyroid hormones of rat blood*

Adult rats, 200–300 g. body wt., were used throughout. Details of injections and analysis are given in the Methods section.

Expt. no.	Compound measured	¹³¹ I (as percentage of total hormonal radioactivity)			
		After 6 hr.		After 24 hr.	
		Without thyrotropin	With thyrotropin	Without thyrotropin	With thyrotropin
1	Thyroxine	46.8	65.6	57.6	55.6
	Tri-iodothyronine	49.5	32.0	40.0	42.2
	Compound 3	4.7	2.7	2.4	2.3
2	Thyroxine	38.6	72.0	76.8	85.8
	Tri-iodothyronine	54.2	24.2	13.2	8.5
	Compound 3	7.2	3.8	7.2	5.6
3	Thyroxine	26.2	61.8	55.7	63.0
	Tri-iodothyronine	46.8	24.8	29.7	22.3
	Compound 3	27.0	13.4	15.7	14.8
4	Thyroxine	51.1	81.0	62.3	66.6
	Tri-iodothyronine	31.0	15.5	33.6	27.4
	Compound 3	17.9	3.7	3.9	5.7
5	Thyroxine	57.9	71.2	66.0	63.6
	Tri-iodothyronine	41.0	22.8	23.8	27.5
	Compound 3	4.9	6.7	9.2	8.8
6	Thyroxine	38.7	70.0	70.0	76.2
	Tri-iodothyronine	52.0	27.6	26.7	20.7
	Compound 3	9.3	2.7	3.2	3.1

Effect of thyrotropic hormone on new synthesis of thyroid hormones

In these experiments male albino rats were used. The animals were distributed into different groups, two or three animals constituting a group. One group was given radioactive iodine alone, other groups were given either thyrotropic hormone and radioactive iodine together or the iodine 24 hr. after the injection of the hormone. After suitable periods the radioactivity of the thyroxine, tri-iodothyronine and other iodine compounds in the blood was determined.

The data are presented in Table 1. The percentage radioactivity of thyroxine was higher and that of tri-iodothyronine was lower 6 hr. after the previous injection of thyrotropic hormone, compared with the control values, which suggested that the formation of thyroxine was accelerated by the thyrotropic hormone.

Effect of thyrotropic hormone on the secretion of the thyroid hormones

In further experiments the action of thyrotropin on the quantities of the thyroid hormones secreted was studied in adult rabbits. Fig. 5 represents the change of the protein-bound iodine of rabbit serum at different times after the administration of the thyrotropic hormone. The protein-bound iodine content of the blood increased rapidly after the injection of thyrotropic hormone and reached a steady value in about 24 hr.

Fig. 5. Change of the iodine content of rabbit serum after the administration of thyrotropic hormone. Curves are the results of two experiments on individual animals.

When the administration of the thyrotropic hormone was repeated between 10 and 20 days after the initial injection, in most cases the opposite phenomenon was observed, the injection of the hormone resulting in a lower protein-bound iodine content of the blood. A third injection 10–20 days after the second once again lowered the levels of protein-bound iodine. Table 2 shows the iodine content of the whole blood and the protein-bound iodine in serum after the first, second and third injection of thyrotropic hormone.

In several experiments the hormones were extracted from the serum, separated and quantitatively determined. The results are summarized in Table 3. Parallel with the change in protein-bound iodine, thyroxine showed the most marked change of all the thyroid hormones. Its quantity in

Table 2. *Effect of thyrotropic hormone on the total and protein-bound iodine of rabbit blood*

Rabbits, 2.5-4.0 kg. body wt., were given thyrotropin as stated. Analysis was done as described in the Methods section.

Expt. no.	No. of injections	Thyrotropin injected (units)	Time of sampling after injection (hr.)	Period between injections (days)	Iodine content ($\mu\text{g./100 ml. of blood}$)			
					Whole blood		Protein-bound iodine	
					Without thyrotropin	With thyrotropin	Without thyrotropin	With thyrotropin
1	1	50	24	0	15.4	18.2	4.7	9.6
	2	50	6	16	19.1	10.2	4.8	4.2
2	1	25	24	0	7.2	13.7	1.4	4.9
	2	25	24	13	21.0	11.9	6.9	5.5
3	1	25	24	0	15.6	16.9	3.6	4.4
	2	50	24	10	24.9	19.6	4.8	4.4
4	1	50	24	0	16.1	18.2	4.2	6.9
	2	50	24	14	21.6	8.4	9.3	5.8
5	1	50	24	0	2.3	4.9	0.6	1.6
	2	50	24	9	18.5	15.3	7.1	8.3
6	1	50	24	0	5.2	6.8	3.6	4.4
	2	50	24	14	9.3	6.2	4.8	5.2
	3	50	24	15	28.0	17.6	8.5	9.6
7	1	50	24	0	13.2	11.1	3.6	4.4
	2	50	6	10	21.6	8.4	6.8	3.2
	3	50	24	18	16.2	10.5	5.0	2.7
8	1	50	24	0	23.4	28.1	5.7	8.0
	2	50	24	17	28.6	20.3	7.7	4.8
	3	50	24	12	9.0	7.6	8.0	2.7

the serum increased after the first injection of thyrotropin and decreased after the second or third injection. The amount of tri-iodothyronine and compound 3 altered only slightly.

DISCUSSION

The experiments described above have shown that after the intravenous or intraperitoneal injection of radioactive iodine into the rat, the radioactive iodine was rapidly incorporated into the tri-iodothyronine fraction; this compound was formed first and contained the highest proportion of the total radioactivity shortly after the injection. Later on, the proportion of radioactivity increased in the thyroxine fraction (cf. Fig. 3).

Current hypotheses about the mechanism of formation of thyroxine have been summarized (Gross & Pitt-Rivers, 1953; Roche & Michel, 1955) and are two: (a) thyroxine is formed by the coupling of two molecules of di-iodotyrosine (cf. Harington, 1944), and thereafter tri-iodothyronine by dehalogenation of thyroxine; (b) tri-iodothyronine is first formed by the coupling of one molecule of monoiodotyrosine to one molecule of di-iodotyrosine, thyroxine being formed from tri-iodothyronine by addition of an atom of iodine.

Although Pitt-Rivers (1948) had succeeded in demonstrating the formation of a thyroxine derivative *in vitro* by coupling two di-iodotyrosine derivatives, subsequent work (Gross & Pitt-Rivers,

1953; Roche & Michel, 1955) has not yielded evidence sufficient to establish this as a major route of synthesis of thyroxine. In a re-examination of this type of mechanism Pitt-Rivers & James (1958) have shown that *N*-acetyl-DL-di-iodotyrosyl-L-glutamic acid was converted into the corresponding thyroxine derivative with a 35% yield.

Dehalogenation of thyroxine to tri-iodothyronine has been studied in many tissues. Gross & Leblond (1951) have demonstrated that [^{131}I]thyroxine was dehalogenated *in vivo* to tri-iodothyronine. Albright, Larson & Trist (1954) and Tata (1958) have shown that slices of kidney and homogenates of brain and skeletal muscle can dehalogenate thyroxine. However, such deiodination has not been found to occur in the thyroid gland (Roche, Michel, Lissitzky & Michel, 1951; Roche, Michel, Michel & Lissitzky, 1951), which leads to the suggestion that tri-iodothyronine is formed directly from a mono- and di-iodotyrosine rather than by dehalogenation of thyroxine. Attempts to confirm this suggestion by Gross & Pitt-Rivers (1953) have been unsuccessful.

It is thus seen that the two hypotheses of thyroxine formation have been derived from experiments conducted largely *in vitro*, whereas the present experiments were carried out while hormone formation was proceeding largely undisturbed. Inspection of Figs. 3 and 4 shows that shortly after the administration of radioactive

iodine the proportion and the relative specific radioactivity of tri-iodothyronine is many times greater than that of thyroxine, indicating that synthesis of tri-iodothyronine precedes synthesis of thyroxine. These data seem to be in good agreement with the precursor-product relationship described by Zilversmit, Entenman & Fishler (1943).

In previous experiments measurements of the distribution of radioactivity in the hormones of the thyroid gland over a period of 24 hr. after the injection of ^{131}I yielded results similar to those in Fig. 3 (Feuer & Vekerd, 1958); this indicated that the pattern of release into the blood is similar to the pattern of formation in the gland. However, similar results could be obtained if thyroxine was not uniformly distributed in the thyroid gland and

if the tri-iodothyronine arose from a small but highly labelled pool. Though this has not been directly demonstrated, evidence exists which suggests that incomplete mixing of iodine derivatives may occur under certain circumstances (Triantaphyllidis, 1958*a, b*). In these experiments the uptake of radioactive iodine to the gland was inhibited by administration of sodium perchlorate 6 hr. after injection of ^{131}I . Four days later measurement of the levels of radioactive and non-radioactive iodine showed that 55% of ^{131}I and only 27% of ^{127}I had been secreted during this period. From the different speeds of secretion of the isotopes it was concluded that secretion of the iodine derivatives of the gland was not a uniform process.

Table 3. *Distribution of the protein-bound iodine between the thyroid hormones of rabbit serum after the administration of thyrotropic hormone*

Details and methods are as given in Table 2.

Expt. no.	No. of injections	Thyrotropin injected (units)	Time of sampling after injection (hr.)	Period between injections (days)	Compound measured	Iodine content ($\mu\text{g./100 ml. of blood}$)	
						Without thyrotropin	With thyrotropin
1	1	25	24	0	Thyroxine	1.23	2.06
					Tri-iodothyronine	0.10	0.12
					Compound 3	0.08	0.09
					Sum	1.38	2.27
	2	25	24	13	Thyroxine	1.95	1.08
					Tri-iodothyronine	1.13	1.46
					Compound 3	1.96	1.26
					Sum	5.08	3.80
	2	25	24	0	Thyroxine	2.00	2.20
					Tri-iodothyronine	1.15	1.46
					Compound 3	0.84	1.10
					Sum	3.99	4.76
	2	50	24	10	Thyroxine	1.98	1.09
					Tri-iodothyronine	1.22	0.84
					Compound 3	1.44	0.96
					Sum	4.54	2.89
3	1	50	24	0	Thyroxine	2.02	2.35
					Tri-iodothyronine	1.38	1.76
					Compound 3	1.10	1.00
					Sum	4.50	5.11
	2	50	24	14	Thyroxine	4.00	1.91
					Tri-iodothyronine	3.58	3.08
					Compound 3	1.44	1.74
					Sum	9.02	6.73
	4	50	24	0	Thyroxine	2.13	2.30
					Tri-iodothyronine	1.14	1.46
					Compound 3	0.86	0.98
					Sum	4.13	4.74
	2	50	6	10	Thyroxine	3.02	0.68
					Tri-iodothyronine	0.96	0.41
					Compound 3	0.97	0.09
					Sum	4.95	1.18
	3	50	24	18	Thyroxine	2.59	1.45
					Tri-iodothyronine	1.30	0.29
					Compound 3	0.80	0.49
					Sum	4.69	2.23

These experiments are not considered to invalidate the present conclusions. Thus the secretion of the total iodine only was studied during a relatively long period, and not the incorporation of iodine into the individual hormone during shorter intervals. Also, inhibition of sodium perchlorate involves a change in the normal function of the thyroid gland. In the present work the metabolism of the gland was not disturbed in this manner.

The process of formation of thyroid hormones was accelerated by thyrotropin. Thus 6 hr. after the injection of thyrotropic hormone the radioactivity of thyroxine was higher and that of tri-iodothyronine was lower than the values in control animals (Table 1). It is considered that the results show that thyrotropic hormone has an effect not only on the secretion of the thyroid hormones but also on the transformation of tri-iodothyronine into thyroxine.

Repeated injections of thyrotropin into rabbits at intervals of 10–20 days decreased the levels of protein-bound iodine after each injection subsequent to the first, which produced the well-known increase (see Table 2). Reasons for this phenomenon are not known. In other animals, such as the guinea pig, repeated daily injections of thyrotropin consistently produce the expected response (cf. Adams & Purves, 1955). It is possible that the continuous high level of blood thyroxine persisting after the initial dose renders the thyroid in the rabbit less responsive to further endogenous thyrotropin, or that thyrotropic hormone exerts a peripheral effect on thyroid-hormone metabolism in addition to its action on the thyroid gland, but in the absence of further data such suggestions must remain hypothetical.

SUMMARY

1. The formation of thyroid hormones *in vivo* has been studied in the blood of different animals with the aid of a new micromethod.

2. After an injection of radioactive iodine into rats, it was found that the radioactivity of tri-iodothyronine initially exceeded that of thyroxine, later becoming much less. It is thought that tri-iodothyronine is a precursor of thyroxine.

3. Six hours after the injection of thyrotropic hormone, together with radioactive iodine, the radioactivity of the blood thyroxine was much higher, and that of tri-iodothyronine much lower, than that of the controls. Thyrotropic hormone has an accelerating effect on the transformation of tri-iodothyronine into thyroxine.

4. After an injection of thyrotropic hormone into rabbits, the protein-bound iodine of the blood increased. Second or third injections at intervals of 10–18 days produced a decrease.

I am very grateful to the Ford Foundation for support during a part of these investigations and wish to thank Professor H. McIlwain for his help and interest during the progress of this work, Dr P. J. Heald for help in preparation of the paper and Miss M. McKernan for skilful technical assistance.

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