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THE EFFECT OF LOWERED ENVIRONMENTAL TEMPERATURE ON THE PERIPHERAL METABOLISM OF LABELLED THYROXINE IN THE SHEEP

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The production of thyroid hormone by the thyroid gland under the influence of the thyrotropic principle of the pituitary normally counterbalances the constant peripheral degradation of extrathyroidal hormone. Numerous reports indicate that these functions are markedly augmented during exposure of mammals to reduced environmental temperature.

The earliest evidence for the activation of the thyroid gland in the cold was based on morphological (Cramer, 1916; Mills, 1918) and chemical criteria (Seidell & Fenger, 1912; Kendall & Simonsen, 1928): the availability of ^{131}I for quantitative evaluation of thyroidal function has afforded direct confirmation (Leblond, Gross, Peacock & Evans, 1944; Ingbar, Kleeman, Quinn & Bass, 1954; Brown-Grant, 1956). Similarly, the suggestion that increased quantities of thyrotropin are elaborated by the pituitary of animals exposed to low temperatures (Wolf & Greep, 1937; Uotila, 1939; Starr & Roskelley, 1940) has recently been confirmed by direct assay (Brolin, 1946; Stevens, D'Angelo, Paschkis, Cantarow & Sunderman, 1955). To date, the evidence for an enhanced peripheral degradation of thyroid hormone in the cold is convincing, although indirect. It rests upon the observation that at low temperatures hypophysectomized animals and animals rendered athyroidal by chemical or surgical means require greater replacement dosages of thyroid hormone (Stevens *et al.* 1955; Dempsey & Astwood, 1943; Rand, Riggs & Talbot, 1952; Kassenaar, Lameyer & Querido, 1956; Bondy & Hagewood, 1952).

In the present experiments, previously described techniques (Ingbar & Freinkel, 1955*a*) were modified to assess directly for the first time the influence of environmental temperatures upon the rate of removal of extrathyroidal thyroid hormone. Thus ^{131}I-labelled L-thyroxine (^{131}I-thyroxine) has been

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given to sheep which were kept outdoors throughout the experiments and were shorn as a form of exposure to cold, in keeping with prevailing agricultural practice. Since thyroxine constitutes more than 90% of the organic iodinated compounds in the thyroidal venous effluent of sheep (Taurog, Wheat & Chaikoff, 1956), radioactive thyroxine may be employed as a valid indicator of circulating thyroid hormone in sheep. The use of sheep has also afforded a means of confirming in larger mammals the earlier reports of the influence of environmental temperatures upon the thyroid-pituitary axis.

METHODS

Animals

Four female and ten wether sheep (Clun Forest cross), weighing 25–35 kg and aged 12–14 months, were purchased from the same supplier. For one month preceding and throughout the experimental period the animals were maintained outdoors with free access to grass and water and supplementary hay. The sheep were kept on the fields of the A.R.C. Institute of Animal Physiology at Babraham, Cambridge.

Three experiments were performed: Expt. A was conducted in February during periods of environmental temperatures ranging from 20 to 50° F (− 6·7 to 10° C) and averaging 34° F (1·1° C) (Fig. 1). Expts. B and C were conducted concurrently during March, 4 weeks after the start of Expt. A. Environmental temperatures ranged from 24 to 65° F (− 3·3 to 18·4° C) and averaged 41° F (5·0° C). Daily maximum and minimum temperatures were recorded.

Experiment A. L-Thyroxine labelled with ^{131}I was administered intravenously to ten unshorn and two shorn sheep. Shearing was performed with hand clippers 10 days before the start of the

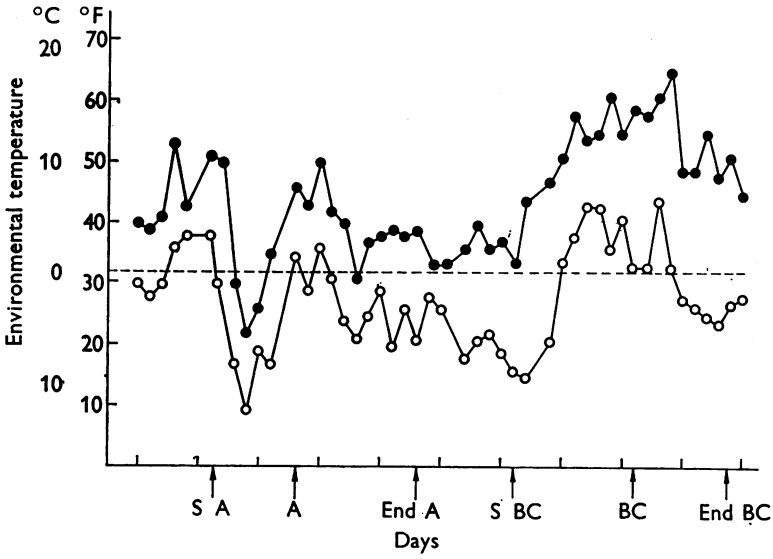

Fig. 1. Environmental temperatures during studies of thyroxine degradation by sheep. A, B and C denote the times of administration of radioactive thyroxine in Expts. A, B and C respectively; sheep shorn for Expt. A at S–A, and for Expts. B and C at S–BC; ●, maximum temp.; ○, minimum temp.

experiment. Samples of jugular venous blood were obtained daily at timed intervals for 10 days following the administration of radioactive thyroxine.

Experiment B. Five shorn and five unshorn sheep were given ^{131}I-thyroxine by intravenous injection. Each group of five animals included four of the unshorn sheep from Expt. A and one sheep which had not been studied previously. The animals were shorn with electric clippers 9 days before the start of the experiment. To correct for residual radioactivity, blood samples were taken immediately before the administration of ^{131}I-thyroxine. Thereafter samples of jugular venous blood were taken twice daily for the first 3 days, at 9 a.m. and 5 p.m., and once daily for the subsequent 6 days. Portions of plasma obtained 12 hr after the administration of ^{131}I-thyroxine were examined by electrophoresis on sheets of Whatman No. 3 filter paper in veronal buffer at pH 8·6 and at 110 V. The details of the paper electrophoretic techniques have been described by Freinkel, Dowling & Ingbar (1955). After electrophoresis the papers were radio-autographed in contact with Kodirex X-ray film in order to localize the thyroxine-binding proteins of sheep plasma.

Portions of the plasma samples obtained on the 3rd, 4th and 5th days after the administration of ^{131}I-thyroxine were pooled and analysed for protein-bound ^{127}I by the method of Barker, Humphrey & Soley (1951). The analyses were performed by Bio-Science Laboratories, 2231 S. Carmelina Avenue, Los Angeles, California, U.S.A.

At the end of Expt. B, seven of the animals were killed by humane killer; these included three shorn sheep, two unshorn sheep, and two unshorn sheep which had received a single injection of 10 U.S.P. units Thyrotropin ('Thytropar', Lot. No. P. 2308, Armour), the one at 5 hr and the other at 7 hr before being killed. In these seven animals the thyroid glands were quickly excised, weighed, and central portions from each lobe were placed in Heidenhein's Susa fixative. Histological sections 5 μ in thickness were cut from paraffin blocks and stained with haematoxylin and eosin. Mean cell heights were measured by the method of Starr & Rawson (1937).

Experiment C. The experimental animals consisted of the two remaining unshorn and the two shorn animals of Expt. A. Injection of ^{131}I-thyroxine and the collection of plasma samples were carried out as in Expt. B. In addition, the animals were given 5 ml. of a 6% (w/v) sodium iodide solution by oesophageal intubation every morning for 3 days before the start of the experiment and daily thereafter.

Animals were weighed at the beginning and end of each experiment, and at the time of autopsy in the case of those animals that died. Rectal temperatures were taken at the end of Expt. A, and at 3-day intervals during Expts. B and C.

Radioactive materials

The radioactive thyroxine used in the experiments was obtained from the Radiochemical Centre at Amersham in two separate lots. It had been prepared by the iodination of L-tri-iodothyronine (Critchlow & Goldfinch, 1954) and at the time of receipt in the laboratory it was in solution in 50% propylene glycol and labelled with ^{131}I to a specific activity of 1 mc/450 μg L-thyroxine. Solutions suitable for injection were prepared by dissolving these preparations in a 9:1 mixture of 0·85% (w/v) sodium chloride and sterile sheep serum. It has been shown (Freinkel *et al.* 1955) that adsorption to glassware can be minimized by the inclusion of protein in dilute solutions of thyroxine.

At the start of each experiment, 5 ml. of the serum-saline mixture containing 55 (Expt. A) or 70μg (Expts. B and C) of L-thyroxine labelled with 120 μc of ^{131}I were given to each animal by injection into the jugular vein from calibrated syringes. Radioactive standards were prepared with mock injection techniques. The homogeneity of the labelled material at the time of injection was assessed by chromatography of an acid *n*-butanol extract of the injection mixture. The following solvents (Roche, Lissitzky & Michel, 1954) were used for one-dimensional chromatography on washed sheets of Whatman No. 1 filter paper: (*a*) *n*-butanol–acetic acid–water; (*b*) *n*-butanol–formic acid; (*c*) phenol saturated with 0·1% (w/v) aqueous ammonia, and (*d*) *n*-butanol–ammonia. Chromatography in these solvents demonstrated that radioactive matter other than thyroxine constituted less than 10% of the total administered radioactivity in Expts. A–C.

Blood samples were collected with heparin as anticoagulant, and plasma was separated by centrifuging. 1 ml. portions of plasma were pipetted directly into aluminium planchettes 2·5 cm in diameter and lined with disks of lens paper. Six drops of alkaline 1% gelatin supplemented with carrier iodide were added to the plasma within the planchettes. Dried planchettes were counted with a mica end-window Geiger–Mueller tube. Sufficient counts were observed to reduce the probable error of the measurement to less than 1%. Plasma radioactivity was related to an injection standard of comparable geometry and self-absorption characteristics.

Representative portions of plasma were precipitated with trichloroacetic acid and with $ZnSO_4$-$Ba(OH)_2$. More than 90% of the circulating radioactivity in both shorn and unshorn sheep was precipitated with plasma proteins at all times after the administration of ^{131}I-thyroxine. Plasma radioactivity was therefore not corrected for inorganic components and the total observed counts, expressed as percentage of administered radioactivity per litre of plasma, were plotted directly on semi-logarithmic paper. The time required for the concentration of circulating radiothyroxine to decrease by 50% (i.e. half time, $t\frac{1}{2}$) was determined by standard graphic analysis. Statistical analyses were made according to the methods described by Fisher (1948).

RESULTS

Disappearance curves of ^{131}I-*thyroxine.* In Expts. A and B the disappearance of radioactivity from the blood could be described in terms of two components: an initial rapid decline which included the values obtained after 24 hr and until 90–120 hr following the injection of ^{131}I-thyroxine (slope 1), and a second slower component which became manifest after 90–120 hr (Fig. 2) and upon which all subsequent values for plasma could be projected (slope 2). In Expt. A the half-life values for slope 1 were 24 and 28 hr in the two shorn animals (Table 1) and averaged 37 hr (S.D. $\pm$ 4·5) in the ten unshorn sheep: $t\frac{1}{2}$ for slope 2 in the shorn animals were 58 and 54 hr and averaged 54 hr (S.D. = 5·1) in the unshorn animals. In all ten unshorn sheep the circulating radioactivity expressed as percentage of administered ^{131}I-thyroxine per litre of plasma exceeded the values obtained in the shorn animals at all times.

In Expt. B the $t\frac{1}{2}$ values for slope 1 averaged 24 hr (S.D. = 1·2) in the five shorn animals and 37 hr (S.D. = 1·0) in the unshorn (cf. average $t\frac{1}{2}$ in humans is 6·7 days; Ingbar & Freinkel, 1955*a*); these results being statistically different ($P < 0{\cdot}01$). Differences of comparable statistical significance could not be demonstrated for slope 2; the $t\frac{1}{2}$ values were 46 hr (S.D. = 5·2) and 49 hr (S.D. = 4·1) in the shorn and unshorn groups respectively. However, the levels of circulating radioactivity in the two groups in Expt. B were not significantly different for the first 20 hr following the administration of ^{131}I-thyroxine.

Since some of the animals in Expt. B had also been studied in Expt. A, the reproducibility of the slopes and the effects of exposure to cold could be assessed on an individual as well as on a group basis. Slope 1 remained constant in the four animals which were not shorn between the experiments: the $t\frac{1}{2}$ values were 35, 32, 40 and 40 hr during Expt. A and 37, 38, 38 and 36 hr during Expt. B respectively. In contrast the slopes were significantly

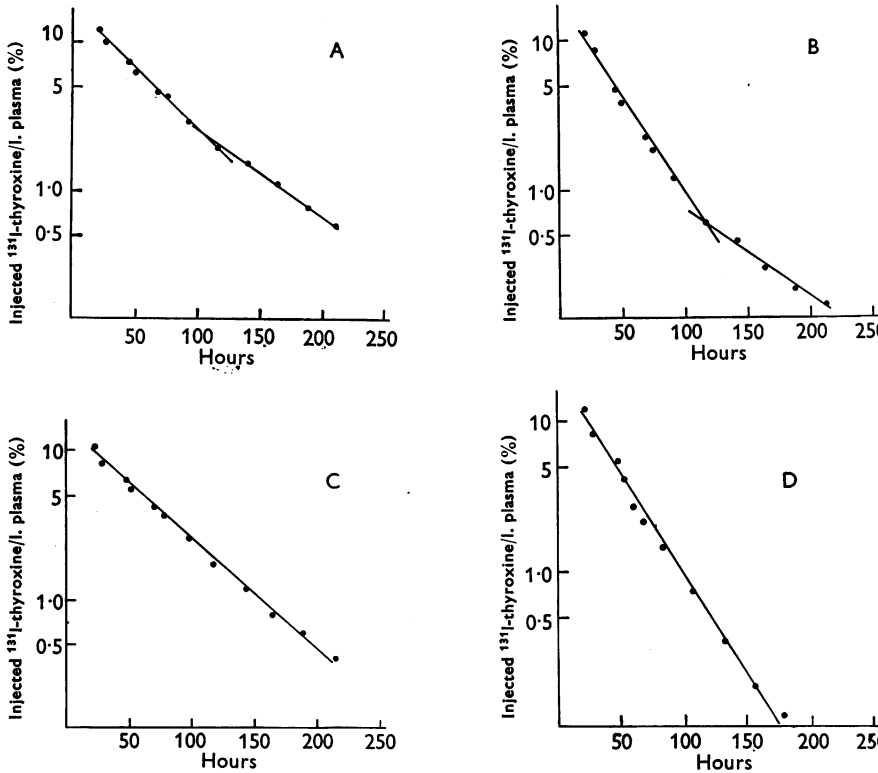

Fig. 2. The disappearance of circulating radioactivity following the administration of ^{131}I-thyroxine. The results are expressed as percentage of the amount injected per litre of plasma at the time indicated, plotted on semi-logarithmic paper. *A*, sheep 136, unshorn; *B*, sheep 140, shorn; *C*, sheep 134, unshorn and dosed with iodide; *D*, sheep 132, shorn and dosed with iodide.

TABLE 1. Half-lives of the injected ^{131}I-thyroxine in Expts. A–C, for both 1st and 2nd slopes. Shearing carried out in preparation for either Expt. A or B as indicated. S, shorn; U, unshorn.

	Shearing		1st slope, $t\frac{1}{2}$ (hr)		2nd slope, $t\frac{1}{2}$ (hr)	
Sheep no.	Expt. A	Expt. B or C*	Expt. A	Expt. B or C*	Expt. A	Expt. B
131	U	S	46	25	60	47
133	U	S	42	26	56	44
135	U	S	31	23	50	48
140	U	S	36	24	54	53
125	—	S	—	24	—	39
136	U	U	35	37	51	52
137	U	U	32	38	50	49
138	U	U	40	38	61	45
139	U	U	40	36	59	44
124	—	U	—	38	—	53
132	S	S*	24	23*	58	—
141	S	S*	28	25*	54	—
134	U	U*	34	40*	44	—
142	U	U*	38	38*	55	—

* 4 sheep used in Expt. C.

different in each of the four sheep which were shorn between Expts. A and B; the $t\frac{1}{2}$ values which had been 46, 42, 31 and 36 hr in Expt. A were reduced to 25, 26, 23 and 24 hr respectively in Expt. B.

In Expt. C when 300 mg of non-radioactive sodium iodide was administered daily, the rate of disappearance of circulating radioactivity could be described in terms of a single exponential. Half-time values for this function were 23 and 25 hr in the two shorn sheep and 40 and 39 hr in the unshorn animals. For the same animals $t\frac{1}{2}$ values for slope 1 in Expt. A had been 24, 28, 34 and 38 hr respectively.

TABLE 2. Weight of the thyroid glands and their mean cell heights in the sheep killed at the end of Expt. B. During the experiment three sheep were shorn (S) and four unshorn (U)

Sheep no.	Weight of thyroid (g)	Mean cell heights (μ)	S.D.
133 S	1·47	11·7	2·7
135 S	1·67	8·8	1·7
140 S	1·90	10·1	2·0
136 U	1·54	5·5	1·5
137 U	1·48	5·8	1·4
138 U	1·08	6·7*	1·8
139 U	1·69	7·2†	1·7

* 10 u. (U.S.P.) thyrotropin administered 5 hr before killing.
† 10 u. (U.S.P.) thyrotropin administered 7 hr before killing.

Thyroid weights and mean cell heights. In the seven sheep which were killed at the end of Expt. B the thyroid weights had not been significantly affected by shearing or by the acute administration of thyrotropin (Table 2). The mean cell heights were 11·7 μ (S.D. = 2·7), 8·8 μ (S.D. = 1·7) and 10·1 μ (S.D. = 2·0) in the three shorn animals as opposed to 5·5 μ (S.D. = 1·5) and 5·8 μ (S.D. = 1·4) in the unshorn and 6·7 μ (S.D. = 1·8) and 7·2 μ (S.D. = 1·7) in the two unshorn animals given 10 U.S.P. units thyrotropin 5 and 7 hr respectively before being killed. Differences between mean cell heights of the three shorn and four unshorn sheep were highly significant ($P < 0{\cdot}01$) (Fig. 3).

Protein-bound iodine (PBI). During Expt. B portions of the plasmas collected on the 3rd, 4th and 5th days were pooled and analyses of the protein-bound iodine were performed. The results (Table 3) show that the average values for protein-bound ^{127}I are 7·7 μg/100 ml. plasma (S.D. = 0·9) for the shorn animals and 6·6 μg/100 ml. plasma (S.D. = 0·8) for the unshorn sheep; the differences being of doubtful statistical significance ($P < 0{\cdot}1$).

Thyroxine-binding protein. In confirmation of the findings of Freinkel, Dowling & Ingbar (unpublished) the circulating protein-bound radioactivity in both shorn and unshorn sheep was principally associated with a single plasma component which migrated with the α-globulins during paper electrophoresis at pH 8·6. The addition of unlabelled L-thyroxine to the plasma before electrophoresis in concentrations of 5 μg/ml. effected displacement of most

of the radioactivity on to the albumin (Fig. 4). Quantitative comparisons of the thyroxine-binding capacities of shorn and unshorn sheep plasma were not made.

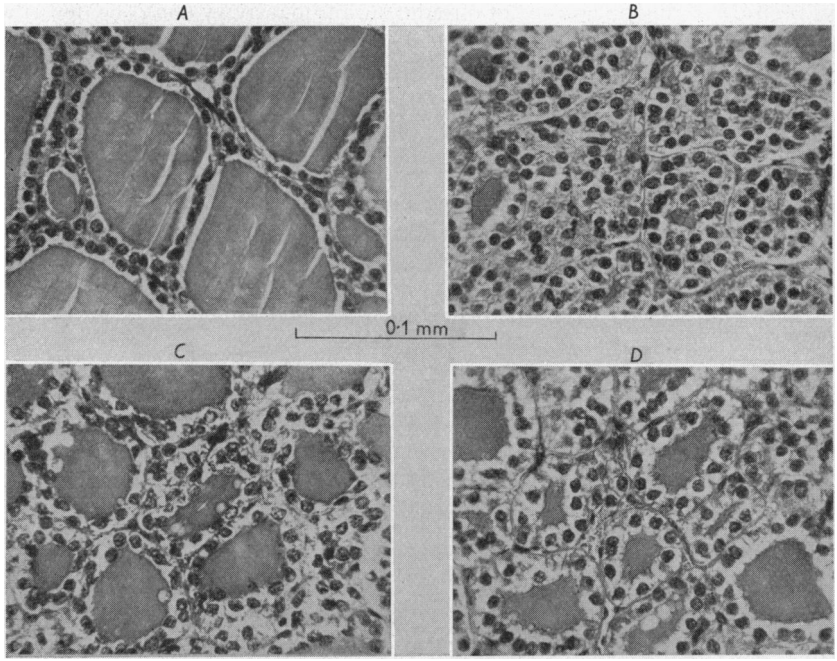

Fig. 3. Photomicrographs of the thyroids: sections stained with haematoxylin and eosin. *A*, sheep 137, unshorn; *B*, sheep 133, shorn; *C*, sheep 136, unshorn, treated with thyrotropin 5 hr before being killed; *D*, sheep 139, unshorn, treated with thyrotropin 7 hr before being killed.

Weights. In Expts. A and B both the shorn and unshorn animals maintained their weights within ± 3 kg throughout the experimental period (Table 3). Statistical differences in weight changes could not be demonstrated. Loss of weight coupled with diminished food intake occurred in all the sheep in Expt. C. Of the shorn sheep in this experiment, one died on the 9th day following the administration of iodide, and a second died 4 days after the end of the experiment, or 16 days after the start of iodide therapy. Weight losses in these animals were 5·5 and 6·4 kg respectively. One of the unshorn animals survived and lost 4·1 kg during the course of the experiment. The second unshorn animal died 3 days after the completion of the experiment and lost 4·4 kg during the period of observation. At autopsy, heavy infestations with gastro-intestinal helminths were present in all three animals. Save for diffuse broncho-pneumonia in one of the shorn animals, no other pathological findings

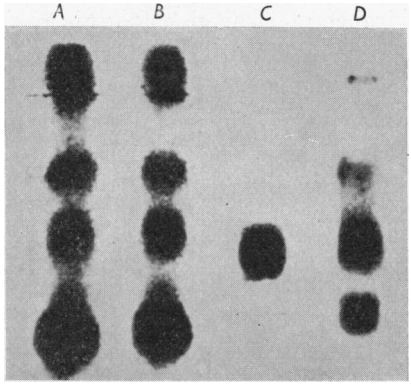

Fig. 4. Paper electrophoresis of plasma from sheep 133 (shorn) and 139 (unshorn), withdrawn 12 hr after the administration of ^{131}I-thyroxine. Paper strips *A* and *B* (sheep 133 and 139 respectively) stained with bromphenol blue to localize the plasma proteins as indicated; *C*, radio-autograph showing the position of radioactivity (sheep 133) principally associated with the sheep *a*-globulin; *D*, radio-autograph showing the displacement of radioactivity from the globulin to the albumin when the plasma (from sheep 139) was supplemented *in vitro* with 5 μg/ml. of unlabelled L-thyroxine.

TABLE 3. The live weights of the sheep before and after each experiment, the rectal temperatures before, during and after Expts. B and C, and the PBI concentration in pooled plasma samples of the 3rd, 4th and 5th days of Expt. B. Whether shorn or not can be seen from Table 1. Series 1 of rectal temperatures taken 4 days after the sheep were shorn (i.e. 5 days before start of Expt. B), series 2 taken 4 days after start of Expt. B and series 3 taken on last day of Expt. B

Sheep no.	Weights of sheep (kg)				Rectal temperatures (° F)			PBI (μg/100 ml. plasma)
	Expt. A		Expt. B or C*					
	Before	After	Before	After	1	2	3	
131	33·5	32·9	29·0	29·6	103·6	104·1	104·0	7·6
133	33·3	31·8	25·4	22·9	104·2	103·8	103·2	8·7
135	28·7	27·9	25·7	23·7	103·0†	103·0	103·4	6·2
140	38·5	37·1	33·4	30·3	102·8	103·8	104·2	8·2
125	—	—	31·7	30·1	102·8	103·2	104·4	8·0
136	33·4	32·3	31·1	31·8	104·2	104·3	104·0	5·0
137	32·2	30·6	28·3	26·0	104·4	103·5	104·8	7·3
138	34·9	35·6	30·1	30·7	104·0‡	104·5	104·4	6·6
139	38·1	38·7	35·4	33·1	105·0§	104·5	103·8	7·0
124	—	—	38·1	38·4	104·8	103·4	104·6	6·9
132	33·0	33·3	27·5*	22·0	103·8	104·3*	—	—
141	26·3	26·8	22·9*	16·5	103·2	104·0*	103·8	—
134	31·9	31·4	29·2*	25·1	105·2	104·8*	104·8	—
142	28·5	27·8	26·2*	22·2	104·0	103·9*	104·6	—

* Four sheep used in Expt. C; † 39·5° C; ‡ 40·0° C; § 40·5° C.

could be demonstrated. It is remarkable that these three animals died, and it is not clear what significance must be given to this event.

Rectal temperatures. In general during the experimental periods the rectal temperatures of the shorn and unshorn animals did not differ significantly (Table 3). In Expt. B, however, the rectal temperatures obtained 4 days following shearing (i.e. 5 days before the administration of ^{131}I-thyroxine) were significantly lower in the shorn animals ($P < 0{\cdot}05$).

DISCUSSION

Theoretically, three components may be expected in the disappearance curves of injected ^{131}I-thyroxine (Ingbar & Freinkel, 1955*a*). There is an immediate decline which represents both mixing of the labelled material within its virtual volume of distribution (thyroxine distribution space) and irreversible removal from that compartment by peripheral degradation. When mixing is complete, a slower rate supervenes which is governed exclusively by the rate at which a constant proportion of the extrathyroidal-thyroxine is degraded. During the peripheral catabolism of the injected ^{131}I-thyroxine, inorganic ^{131}I is liberated and is apportioned between the thyroid and urine. Thus, thirdly, after a finite interval, the apparent decline of plasma radioactivity may be further damped by the release of newly synthesized ^{131}I-thyroxine from the thyroid into the circulation. The contribution of such thyroidal re-utilization to disappearance slopes will depend upon the thyroidal avidity for ionic iodide and the size and the rate of turnover of the glandular pool of hormone. Variations in re-utilization may in part at least account for the paradoxical differences in the effects of cold and extreme cold upon the thyroidal release of ^{131}I which have been reported by Brown-Grant (1956).

In the present studies, mixing phenomena were not fully examined and few blood samples were drawn before 24 hr after injection. Under these conditions, the disappearance of circulating radioactivity in the animals of Expts. A and B could be described in terms of two components: (*a*) a rapid exponential decline upon which all values obtained within 24–100 hr after the administration of ^{131}I-thyroxine could be projected and above which all earlier points fell; and (*b*) a slower exponential decline which became manifest at about 120 hr after injection. It is considered that the first slope reflects chiefly peripheral degradation, and that the later component represents the composite of degradation and re-utilization.

To test this interpretation Expt. C was performed. Here sodium iodide was administered so that dilution with inorganic ^{127}I might minimize the re-utilization of inorganic ^{131}I. From data in euthyroid humans (Ingbar & Freinkel, 1955*a*), it was thought that such doses of inorganic iodide would not significantly alter the fractional rate of thyroxine degradation. In the iodide-treated sheep, all plasma values obtained after 24 hr following the injection

of ^{131}I-thyroxine could be projected on to a single exponential. The constancy of the slopes in this experiment during observations for as long as 240 hr confirms that mixing phenomena were complete within 24 hr. The rate constants for these single slopes in iodide-treated sheep coincided with the values for slope 1 which were observed simultaneously in untreated animals and also agreed with values which had been obtained with the same animals in Expt. A before the administration of sodium iodide. Although the sheep tolerated the iodide poorly, the close parallelism in the values would strongly suggest that (*a*) the monophasic disappearance rates in Expt. C resulted from a damping of the re-utilization of inorganic ^{131}I rather than from inanition or changes in the metabolism of thyroxine immediately before death; and (*b*) the disappearance rates observed in untreated animals within 24–100 hr following the administration of ^{131}I-thyroxine represent at least the minimal values for the rates of thyroxine degradation.

On the basis of these considerations it may be concluded that the fractional rate of thyroxine degradation is augmented in sheep when exposure to cold is induced by shearing. Since shearing was performed 9 days before the injection of radioactive thyroxine, it is doubtful whether any of the observations can be attributed to shearing *per se*. Similarly, although the relatively low specific activity of the labelled thyroxine necessitated the administration of 60–70 μg quantities of thyroxine, the electrophoretic evidence that this had not exceeded the binding capacities of the specific plasma thyroxine-binding protein would tend to rule out 'saturation phenomena' (Gross & Leblond, 1950).

It is tempting to formulate the available data in more quantitative terms (Ingbar & Freinkel, 1955*a*).

Let k = the fractional rate of turnover of extrathyroidal thyroxine expressed as percentage per day,
PBI = protein-bound iodine of serum (μg I/100 ml.),
ETT = extrathyroidal thyroxine (μg thyroxine-I),
TDS = the 'thyroxine distribution space' or virtual volume of distribution of thyroxine, and
D = thyroxine degradation rate (μg thyroxine-I/day):

$$\text{then } \mathrm{ETT} = \mathrm{PBI} \times 10 \times \mathrm{TDS},$$
$$\text{and } \mathrm{D} = \mathrm{ETT} \times k.$$

Field conditions precluded the collection of faeces and urine, and the external neck counting, which are necessary for precise estimations of TDS. An approximate value for TDS may be derived by extrapolating slope 1 to zero time and by dividing the amount injected by the theoretical concentration of ^{131}I-thyroxine in the plasma which would have been obtained if mixing had been instantaneous.

As calculated in this manner, the TDS averaged 6·80 l. or 21·1% of body weight in the unshorn sheep of Expt. B and 6·45 l. or 22·3% of body weight in the shorn animals. At average PBI levels of 6·6 and 7·7 μg/100 ml. plasma and fractional turnover rates of 45 and 69% per day respectively, the unshorn sheep degraded an average of 199 μg of thyroxine-iodine per day as opposed to an average of 343 μg per day in the shorn animals, values within the range found for sheep by Henneman, Reineke & Griffin (1955) using a more indirect method. Ingbar & Freinkel (1955*a*) found the normal rate of degradation of thyroxine to be 53·6 μg thyroxine-iodine per day in humans of 70 kg body weight. It should be stressed that errors inherent in extrapolation techniques (Freinkel, Schreiner & Athens, 1953) would tend towards an overestimation of TDS. At the same time, early re-utilization of inorganic ^{131}I would damp slope 1 and result in an underestimation of k, and an overestimation of TDS. None the less the approximate doubling of hormonal degradation in sheep during exposure to cold is similar to the observations of Dempsey & Astwood (1943) in their studies with rats.

Others have demonstrated that exposure to reduced environmental temperature can lower the PBI in athyroidal animals maintained on constant amounts of exogenous hormone (Stevens *et al.* 1955; Rand *et al.* 1952; Bondy & Hagewood, 1952; Kassenaar *et al.* 1956). These changes could indicate either an alteration in the peripheral degradation or a change in the volume of distribution of thyroxine (Ingbar & Freinkel, 1955*b*). The present studies afford the first direct confirmation of the former interpretation.

Because available methods do not permit differentiation between the disappearance of thyroxine as a result of its action on end organs and breakdown unrelated to hormonal action, the term 'degradation' has been used throughout this report. However, since the elevation of the basal metabolic rate (B.M.R.) in animals exposed to cold is related to thyroidal integrity (Ring, 1939; Stevens *et al.* 1955), it does not seem unlikely that the above differences represent, at least in part, true augmentation of hormone utilization.

The present data also afford some information about thyroid-pituitary interrelationships during the adaptation of large mammals to cold. In the shorn and unshorn animals in which re-utilization of inorganic ^{131}I had not been inhibited, the divergent rates of disappearance of circulating radioactivity became similar after 120 hr. The late parallelism of slopes could only have resulted from an increased turnover of intra-thyroidal hormone in the shorn population. The histological finding of increased cell heights corroborates the isotopic evidence for enhanced thyroidal function. It may be inferred that this hyperactivity was produced at least in part by an increased elaboration of endogenous thyrotropin since similar, though less significant, histological changes were effected by the acute administration of exogenous thyrotropin.

The available data, however, do not aid in the differentiation of primary and secondary events in these sequences. Thus, it cannot be said whether exposure to cold augments peripheral degradation directly and secondarily activates the thyroid-pituitary system, or whether the reverse is true. In intact animals, PBI levels at reduced environmental temperatures have been variously described as unchanged, slightly elevated, or slightly depressed (Stevens *et al.* 1955; Rand *et al.* 1952; Bondy & Hagewood, 1952; Erschoff & Golub, 1951); in the present study, the small elevation of PBI in some of the shorn animals was not statistically significant. Such minor variations in PBI values could be consistent with either thesis, especially if one postulates evanescent phases of lag or overshoot in the adjustments of the secondary component to the new equilibrium. It may even be that central and peripheral mechanisms are concurrently activated by the thermal, vascular or dietary adjustments which occur during exposure to cold. In any event, the fact that both central and peripheral hyperactivity coexist in the face of near normal values for PBI would suggest that the perpetuation of these patterns is conditioned by intracellular events rather than extracellular levels of circulating hormone.

SUMMARY

1. Thyroxine (^{131}I-labelled) was administered during the winter months to grazing shorn and unshorn sheep in order to assess the effects of reduced environmental temperature on the peripheral degradation of thyroid hormone.

2. Half-times for the turnover of extrathyroidal thyroxine averaged 25 hr in the shorn and 38 hr in the unshorn animals.

3. The enhanced rate of thyroxine degradation in shorn sheep was accompanied by significant functional and histological evidence of thyroidal hyperactivity.

4. No significant differences in the circulating levels of protein-bound iodine were demonstrated between the shorn and unshorn groups.

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REFERENCES

Barker, S. B., Humphrey, M. J. & Soley, M. H. (1951). The clinical determination of protein-bound iodine. *J. clin. Invest.* **30**, 55–62.

Bondy, P. K. & Hagewood, M. A. (1952). Effect of stress and cortisone on plasma protein-bound iodine and thyroxine metabolism in rats. *Proc. Soc. exp. Biol., N.Y.*, **81**, 328–331.

Brolin, S. E. (1946). A study of the structural and hormonal reactions of the pituitary body of rats exposed to cold, illustrating the regulatory influence of the anterior lobe on the thyroid gland. *Acta anat.* **2**, Suppl. 3.

Brown-Grant, K. (1956). Changes in the thyroid activity of rats exposed to cold. *J. Physiol.* **131**, 52–57.

CRAMER, W. (1916). On the thyroid-adrenal apparatus and its function in the heat regulation of the body. *J. Physiol.* **50**, xxxviii–xxxix.

CRITCHLOW, A. & GOLDFINCH, M. K. (1954). The preparation of thyroxine labelled with ^{131}I and its use as an analytical tool. In *Radioisotope Conference*, 1954, 1st ed. pp. 271–280. London: Butterworths.

DEMPSEY, E. W. & ASTWOOD, E. B. (1943). Determination of the rate of thyroid hormone secretion at various environmental temperatures. *Endocrinology*, **32**, 509–518.

ERSCHOFF, B. H. & GOLUB, D. J. (1951). Effects of prolonged exposure to cold on the serum protein-bound iodine of the rat. *Arch. Biochem. Biophys.* **30**, 202–206.

FISHER, R. A. (1948). *Statistical Methods for Research Workers.* 10th ed. London: Oliver and Boyd.

FREINKEL, N., DOWLING, J. T. & INGBAR, S. H. (1955). The interaction of thyroxine with plasma proteins: localization of thyroxine-binding protein in Cohn fractions of plasma. *J. clin. Invest.* **34**, 1698–1709.

FREINKEL, N., SCHREINER, G. E. & ATHENS, J. W. (1953). Simultaneous distribution of T-1824 and ^{131}I-labelled human serum albumin in man. *J. clin. Invest.* **32**, 138–148.

GROSS, J. & LEBLOND, C. P. (1950). Metabolism of the thyroid hormone in the rat as shown by physiological doses of labelled thyroxine. *J. biol. Chem.* **184**, 489–500.

HENNEMAN, H. A., REINEKE, E. P. & GRIFFIN, S. A. (1955). The thyroid secretion rate of sheep as affected by season, age, breed, pregnancy and lactation. *J. anim. Sci.* **14**, 419–434.

INGBAR, S. H. & FREINKEL, N. (1955*a*). Simultaneous estimation of rates of thyroxine degradation and thyroid hormone synthesis. *J. clin. Invest.* **34**, 808–819.

INGBAR, S. H. & FREINKEL, N. (1955*b*). The influence of ACTH, cortisone and hydrocortisone on the distribution and peripheral metabolism of thyroxine. *J. clin. Invest.* **34**, 1375–1379.

INGBAR, S. H., KLEEMAN, C. R., QUINN, M. & BASS, D. E. (1954). The effect of prolonged exposure to cold on thyroidal function in man. *Clin. Res. Proc.* **2**, 85.

KASSENAAR, A. A. H., LAMEYER, L. D. F. & QUERIDO, A. (1956). The effect of environmental temperature on the blood protein-bound iodine content of thyroxine maintained thyroidectomized rats. *Acta endocr., Copenhagen*, **21**, 37–40.

KENDALL, E. C. & SIMONSEN, D. G. (1928). Seasonal variations in the iodine and thyroxine content of the thyroid gland. *J. biol. Chem.* **80**, 357–377.

LEBLOND, C. P., GROSS, J., PEACOCK, W. & EVANS, R. D. (1944). Metabolism of radio-iodine in the thyroids of rats exposed to high or low temperatures. *Amer. J. Physiol.* **140**, 671–676.

MILLS, C. A. (1918). Effects of external temperature, morphine, quinine and strychnine on thyroid activity. *Amer. J. Physiol.* **46**, 329–339.

RAND, C. G., RIGGS, D. S. & TALBOT, N. B. (1952). The influence of environmental temperature on the metabolism of the thyroid hormone in the rat. *Endocrinology*, **51**, 562–569.

RING, G. C. (1939). Thyroid stimulation by cold: including the effect of changes in the body temperature upon basal metabolism. *Amer. J. Physiol.* **125**, 244–250.

ROCHE, J., LISSITZKY, S. & MICHEL, R. (1954). *Methods of Biochemical Analysis*, ed. D. Glick, 1st ed. pp. 243–264. New York: Interscience.

SEIDELL, A. & FENGER, F. (1912). Seasonal variation in the iodine content of the thyroid gland. *J. biol. Chem.* **13**, 517–526.

STARR, P. & RAWSON, R. W. (1937). A graphic representation of thyroid response to stimulation by thyrotropic hormone. *Proc. Soc. exp. Biol., N.Y.*, **35**, 603–604.

STARR, P. & ROSKELLEY, R. (1940). A comparison of the effects of cold and thyrotropic hormone on the thyroid gland. *Amer. J. Physiol.* **130**, 549–556.

STEVENS, C. E., D'ANGELO, S. A., PASCHKIS, K. E., CANTAROW, A. & SUNDERMAN, F. W. (1955). The response of the pituitary-thyroid system of the guinea-pig to low environmental temperature. *Endocrinology*, **56**, 143–155.

TAUROG, A., WHEAT, J. D. & CHAIKOFF, I. L. (1956). Nature of the ^{131}I compounds appearing in the thyroid vein after injection of ^{131}I-iodide. *Endocrinology*, **58**, 121–131.

UOTILA, U. U. (1939). On the role of the pituitary stalk in the regulation of the anterior pituitary, with special reference to the thyrotropic hormone. *Endocrinology*, **25**, 605–614.

WOLF, O. & GREEP, R. (1937). Histological study of thyroid gland of hypophysectomized rats exposed to cold. *Proc. Soc. exp. Biol., N.Y.*, **36**, 856–860.