Studies of the Congenitally Goitrous Sheep

COMPOSITION AND METABOLISM OF GOITROUS THYROID TISSUE

BY IAN R. FALCONER

Department of Agricultural Biochemistry, The Waite Agricultural Research Institute, Glen Osmond, South Australia, and University of Nottingham School of Agriculture, Sutton Bonington, near Loughborough, Leicestershire

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1. Normal and congenitally goitrous thyroid tissue was examined to identify the defective mechanism in the goitrous glands. 2. The uptake of [^{131}I]iodide from the blood stream into the goitrous glands (average 74·9%) was significantly greater than normal (average 43·5%; $P < 0.005$), as was the rate of [^{131}I]iodide release (goitrous $t_{\frac{1}{2}}$ average 72·3hr., normal $t_{\frac{1}{2}}$ average 198·7hr.; P 0·025). 3. The L-[^{131}I]-iodotyrosine-deiodinase activity was significantly (P 0·02) greater than normal in goitrous-thyroid slices. 4. The 0·9%-sodium chloride-soluble proteins of [^{131}I]-iodide-labelled thyroid glands were fractionated with ammonium sulphate: 68·7 ± 4·0% of the total radioactivity appeared in the 35–45%-saturation precipitate from normal gland extracts, but less than 20% of the total radioactivity was in in this fraction from goitre extracts. 5. Ultracentrifugal analysis of 0·9%-sodium chloride-soluble proteins of goitrous glands showed no protein of $S_{20,w}$ 19–20s (thyroglobulin) even when the animals had previously received 0·1–2·0mg. of L-thyroxine/day intramuscularly for 40 days. The major proteins of goitrous glands had $S_{20,w}$ 3·2–7·6s. 6. The incorporation in incubated slices of [^{14}C]proline and [^{14}C]leucine into soluble proteins precipitated by 35–42%-saturated ammonium sulphate was markedly lower in goitrous tissue. 7. It was concluded that the goitrous tissue exhibited defective biosynthesis of thyroglobulin.

A congenital defect in Merino sheep that appears as a gross enlargement of the thyroid from birth has been reported. Animals with this defect have a low concentration of circulating thyroid hormone, and abnormal iodinated compounds in the serum. Bioassay of thyroid-stimulating hormone in sera of normal and the congenitally goitrous sheep showed greatly elevated concentrations in the goitrous animals, indicating that the hypertrophy of the thyroid was a compensatory mechanism for an inadequate rate of hormone production (Falconer, 1966).

Biochemical defects of thyroid metabolism in man, leading to goitre, have been attributed to inability of the gland to concentrate iodide, to iodinate tyrosines, to synthesize iodothyronines or to deiodinate iodotyrosines (see Stanbury, 1963). Thyroid tissue obtained from the congenitally goitrous Merino sheep was examined to identify the nature of the defect in thyroid-hormone biosynthesis. Some aspects of this work have already been reported (Falconer, 1965).

EXPERIMENTAL

Materials. The materials employed were identical with those described by Falconer (1966), with the following additions: cycloheximide (Actidione) was obtained from the Upjohn Co., Kalamazoo, Mich., U.S.A.; L-[U-^{14}C]-leucine and L-[U-^{14}C]proline (both 100mc/m-mole) were from Schwartz Bio-Research Inc., New York, N.Y., U.S.A.; L-[U-^{14}C]proline (33mc/m-mole) was from The Radiochemical Centre, Amersham, Bucks. For liquid-scintillation counting Diotol scintillator fluid (Herberg, 1960) was made up from A.R.-grade solvents and naphthalene, 2,5-diphenyloxazole and 1,4-bis-(5-phenyloxazol-2-yl)benzene of A.R. scintillation grade [Nuclear Enterprises (G.B.) Ltd., Edinburgh].

Methods. Measurements of thyroid ^{131}I content were carried out by using a directional scintillation counter (Ekco model N559D) held 20cm. from the centre of the thyroid. All results were corrected for radioactive decay to the time of injection. Half-times ($t_{\frac{1}{2}}$, the time taken for a 50% change in thyroid ^{131}I) were corrected by deduction of the renal clearance rate from the apparent thyroid uptake, and by deduction of the iodide recirculation from the apparent thyroid release rate (see Robertson & Falconer, 1961).

The activity of iodotyrosine-deiodinating enzymes was determined as described by Querido, Stanbury, Kassenaar & Meijer (1956) in 0·5mm.-thick slices of normal and goitrous thyroid gland cut on a mechanical chopper (Mickle, Gomshall, Surrey). L-[^{131}I]Iodotyrosines (71·8% monoiodotyrosine, 27·1% di-iodotyrosine) were prepared (Falconer, 1966), the product containing 0·15 μc/μg. A 0·015 μc sample was taken, together with 25 μg. of monoiodotyrosine plus 25 μg. of di-iodotyrosine, and incubated with 0·5g. of

slices in Krebs–Ringer phosphate buffer, pH 7·4 (Krebs & Henseleit, 1932), for 4 hr. A mixture of iodotyrosines was used, since both occur in the thyroid gland. The reaction was terminated by the addition of N-HCl, and the iodinated amino acids were extracted into butan-1-ol saturated with 0·1 N-HCl. The butan-1-ol was then evaporated to dryness and the residue redissolved in butan-1-ol. Descending chromatography in butan-1-ol–acetic acid–water (74:19:51, by vol.) was used to separate the iodotyrosines, as described by Roche, Michel, Michel & Lissitzky (1952). Measurement of radioactivity was carried out in a well scintillation counter (see Falconer, 1966).

The fractionation of thyroid proteins was carried out after homogenizing 0·5–1·0 g. of fresh thyroid tissue in 10 ml. of 0·9% NaCl soln./g. in an MSE top-drive homogenizer (Measuring and Scientific Equipment Ltd., Birmingham) cooled in ice. The disrupted material was then centrifuged at 105000*g* for 30 min. in a Spinco model L ultracentrifuge (no. 40 rotor) at 4°. The supernatant was brought to 35% saturation with $(NH_4)_2SO_4$ and the precipitate collected after 15 min. at 3000*g* at room temperature in an MSE bench angle-head centrifuge. The supernatant was then adjusted to 45% saturation with $(NH_4)_2SO_4$ and the centrifugation repeated. The final supernatant was brought to 5% (w/v) with 20% (w/v) trichloroacetic acid and the precipitate collected as before. All $(NH_4)_2SO_4$-fractionation precipitates were redissolved in water and reprecipitated by the addition of trichloroacetic acid solution to 5% (w/v). The radioactivity of the precipitate was measured in a well scintillation counter (Falconer, 1966) and nitrogen determination was carried out by a micro-Kjeldahl method (McKenzie & Wallace, 1954).

Ultracentrifugal analysis of the 0·9%-NaCl-soluble proteins of normal and goitrous thyroid tissue was carried out in a Spinco model E analytical ultracentrifuge (rotor An E) by standard procedures (Shulman, Rose & Witebsky, 1955). For the study of goitrous thyroid tissue before thyroxine treatment, portions of the goitre were excised by aseptic surgery under anaesthesia, without harm to the animal. Thyroid-protein solutions were adjusted to less than 1% protein concentration before centrifugation in a double-sector cell, with the diffusate after 24 hr. dialysis against 0·9% NaCl of the protein solution in the reference sector.

Starch-gel separation of 0·9%-NaCl-soluble thyroid proteins was carried out in a discontinuous buffer system (Falconer, 1966). Samples of goitrous tissue were collected by surgical excision 7 days after the intramuscular administration of 500 μc of [^{131}I]iodide, perfused with chilled 0·9% NaCl soln., homogenized (1 g./3 ml. of 0·9% NaCl) and the homogenate was dialysed against 0·9% NaCl for a minimum of 18 hr. A suitable dilution of the 20000*g* supernatant of this material was placed on the gel and the proteins were separated at 15 v/cm. for 18 hr. Half of the gel sliced through its thickness was laid on radioautographic film protected by a thin polythene sheet for 7 days before development. The other half was stained for protein and subsequently sliced into 1 cm. strips for measurement of ^{131}I in a well scintillation counter.

The incorporation of [^{14}C]proline and [^{14}C]leucine into the proteins of 0·5 mm.-thick thyroid slices was carried out as described by Falconer (1965). Additional incubations of thyroid slices under identical conditions but with the addition of 20 μg. of Actidione/ml. were performed. Actidione is an inhibitor of protein biosynthesis, and has been used in these experiments to indicate the magnitude of the non-biosynthetic incorporation of ^{14}C-labelled amino acids into proteins (Bennett, Ward & Brockman, 1965). Measurements of ^{14}C radioactivity in 0·5 ml. of aqueous protein suspension was carried out by the liquid-scintillator method, with 10 ml. of Diotol scintillator fluid (Herberg, 1960) with a suitable detector and scaler (Ekco Electronics Ltd., Southend; models N 664A and N 610A). The protein suspension was dispersed thoroughly in the scintillator fluid by a Vibromix (A.G. für Chemie-Apparatebau, Zurich, Switzerland) driving a small stainless-steel paddle. The dispersed samples were stored at 24 hr. in the dark before the measurement of radioactivity. Measurements of the efficiency of this system showed that 42% of the disintegrations of a standard [^{14}C]cholesterol sample were recorded, at 1450 v (extra high tension) and 15 v discriminator bias. Additional samples of protein suspension were analysed for nitrogen by a micro-Kjeldahl method (McKenzie & Wallace, 1954).

RESULTS AND DISCUSSION

Uptake of [^{131}I]*iodide into normal and goitrous thyroid glands of sheep.* Four goitrous Merino sheep, of which one had received 1 mg. of L-thyroxine/day intramuscularly for 20 days, were compared with three normal Merinos. The results of measurement of the half-times ($t_{\frac{1}{2}}$) for [^{131}I]iodide uptake into and release from the thyroid, and the maximum uptake, are shown in Table 1. The apparent rate of release of ^{131}I from the goitrous glands was considerably lower ($t_{\frac{1}{2}}$ 202–295 hr.) than the true rate given in Table 1, owing to the high proportion of ^{131}I recirculated after peripheral deiodination of iodinated amino acids.

An elevated thyroid uptake after the injection of [^{131}I]iodide has been shown to be a feature of most types of congenital goitre in man (Stanbury, Ohela & Pitt-Rivers, 1955). The goitrous sheep showed a markedly more rapid thyroid [^{131}I]iodide uptake than the normal animals (Table 1; $P < 0{\cdot}005$), except for the thyroxine-treated animals, in which the [^{131}I]iodide transport closely resembled the normal. The true rate of release of ^{131}I from the goitrous glands was higher than from the normal glands (P 0·025), indicating that iodine-containing compounds were being secreted by the glands at an elevated rate. The circulating concentration of thyroid hormones was shown to be lower than normal in the goitrous animals (Falconer, 1966), so that the high rate of release of ^{131}I from the goitre confirmed the release of the abnormal iodinated compounds previously described in the blood.

Deiodination of iodotyrosines by thyroid slices. Slices of fresh thyroid glands were incubated with L-[^{131}I]iodotyrosines for 4 hr. at 37·5°, the reaction was stopped with hydrochloric acid, the iodinated amino acids were separated and their radioactivity was measured. To identify any effects due to the biosynthesis of new iodotyrosines from [^{131}I]-

Table 1. *Thyroid [^{131}I]iodide uptake and release in normal and goitrous sheep*

A 50μc dose of [^{131}I]iodide in 1ml. of 0·9% NaCl soln. was injected intramuscularly into goitrous sheep, and 100μc of [^{131}I]iodide into normal sheep. Further details are given in the text. Dimensions of normal thyroid glands are quoted for adult sheep *post mortem* and goitres were measured *in situ* by palpation. *P* is the significance of difference between normal and untreated goitrous sheep in $t_{\frac{1}{2}}$ (by the *t* test). In this and subsequent Tables the numbers of normal sheep are prefixed 'N', and those of goitrous sheep 'G'.

Sheep no.	Thyroid size (cm.)	Thyroid [^{131}I]iodide uptake $t_{\frac{1}{2}}$ (hr.)	Max. uptake at $t = 0$ (% of dose)	Thyroid [^{131}I]iodide release $t_{\frac{1}{2}}$ (hr.)
N246	Two lobes of approx. equal size, 4·5 × 1·8 × 0·8, in each case	41·7	47·7	157
N247		39·8	51·3	173
N248		63·0	31·6	266
GR77	One fused goitre, 12·5 × 11·5 × 10·0	0·64	76·1	89
GR201	Two lobes, 7·5 × 2·5 × 2·5 and 5·0 × 2·5 × 2·0	0·87	75·0	74
GB150	Two lobes, 5·0 × 3·5 × 2·5 and 6·0 × 3·0 × 2·5	1·20	73·5	54
GY87*	Two lobes, 7·5 × 2·5 × 2·0 and 4·0 × 2·0 × 1·5	33·0	32·9	145
P value		0·005	0·005	0·025

* L-Thyroxine was administered intramuscularly (1 mg./day) from 20 days before [^{131}I]iodide administration to the end of the experiment.

Table 2. L-[^{131}I]*Iodotyrosine-deiodinase activity in thyroid slices obtained from normal and goitrous sheep*

A 0·5g. portion of 0·5mm.-thick slices was incubated in 4ml. of Krebs–Ringer phosphate buffer, pH7·4, with 0·015μc (0·1μg.) of L-[^{131}I]iodotyrosines. After incubation of both fresh and heated slices for 4hr. iodotyrosines were extracted into acidified butanol and separated by paper chromatography. Further details are given in the text. The results are expressed as the counts/100sec. above background for iodotyrosines, and are given as means ± S.E.M. with the numbers of determinations in parentheses.

Thyroid sample	Radioactivity (counts/100sec.) No additional iodide	Radioactivity (counts/100sec.) 100μg. of KI added to incubation mixture
Normal	8580 ± 325 (4)	8969 ± 767 (4)
Goitrous	6848 ± 592 (4)	7650 ± 574 (3)*
Heated normal	20229 ± 1065 (4)	

* No significant effect due to idoide; goitrous slices showed significantly less residual [^{131}I]iodotyrosines than normal slices; *P* 0·02 by analysis of variance.

iodide released by deiodination of the added L-[^{131}I]-iodotyrosines, one group of each of normal and goitrous slices had 100μg. of potassium iodide added to each incubation. A further group of slices were heated at 100° for 5min., which gives a measure of the total radioactivity added. The results of the deiodinase estimation are given in Table 2, which shows that considerable deiodination has occurred in both normal and goitrous-thyroid slices. The radioactivity recovered as iodotyrosines was higher in both groups of samples incubated with 100μg. of potassium iodide, but the increase was not significant. It is therefore clear that the iodotyrosine-deiodinase defect that has been demonstrated in some cases of human congenital goitre (Querido *et al.* 1956) is not present in the goitrous sheep. Examination of the iodotyrosine content of the sheep goitres showed only 10% of the normal concentration of iodotyrosines/g. net weight (Falconer, 1965); in serum, however, iodotyrosines occur only in goitrous sheep (Falconer, 1966).

Determination of thyroid proteins in normal and goitrous glands. Thyroid tissue was removed by aseptic surgery from a normal sheep, a goitrous sheep and a goitrous sheep receiving 1mg. of L-thyroxine/day, 2 weeks after the administration of 50μc of [^{131}I]iodide to each animal. The tissue was homogenized, centrifuged and fractionated by ammonium sulphate precipitation as described above. The weight and [^{131}I]iodide content of the precipitates are given in Table 3, which shows that the distribution of radioactivity between the endogenously labelled iodoprotein fractions in the goitrous glands was approximately uniform, which contrasted with about 70% of the radioactivity in the thyroglobulin fraction (35–45%-saturation precipitate) in the normal glands.

The properties of iodoproteins obtained from cases of congenital goitre have been used as guides to the nature of the defect (Roche, Michel & Tubiana,

Table 3. *Ammonium sulphate fractionation of thyroid tissue from normal and goitrous sheep*

Tissue samples were collected 2 weeks after the intramuscular administration of 50 μc of [^{131}I]iodide in 1 ml. of 0·9% NaCl to sheep. Samples (0·5 g.) of fresh gland were homogenized in 5 ml. of 0·9% NaCl, centrifuged at 105000*g* (Spinco model L centrifuge, no. 40 rotor) and the supernatant proteins fractionated with $(NH_4)_2SO_4$ as described in the Experimental section.

	Thyroid sample	Dry wt. of fraction (mg.)	Radioactivity (% of total ppt. radioactivity)	Radioactivity (% of total ppt. activity/mg. of protein)
0–35%-saturated-$(NH_4)_2SO_4$ ppt.	N (mean ± S.E.M.)	8·8 ± 0·5	8·1 ± 1·6	0·92
	GB150	51·0	24·4	0·48
	GY87*	59·0	23·4	0·25
35–45%-saturated-$(NH_4)_2SO_4$ ppt.	N (mean ± S.E.M.)	61·4 ± 8·5	68·7 ± 4·0	1·12
	GB150	50·9	19·4	0·38
	GY87*	22·5	11·5	0·51
45%-Trichloroacetic acid ppt.	N (mean ± S.E.M.)	27·7 ± 5·5	19·9 ± 5·5	0·72
	GB150	98·6	22·2	0·23
	GY87*	94·1	30·4	0·32
105000*g* ppt.	N (mean ± S.E.M.)	17·9 ± 1·6	3·2 ± 0·4	0·18
	GB150	70·0	33·0	0·47
	GY87*	224·4	34·8	0·16

* L-Thyroxine was administered intramuscularly (1 mg./day) for 40 days before biopsy.

Table 4. *Ultracentrifugal analysis of 0·9%-sodium chloride-soluble proteins of normal and goitrous thyroid glands*

Proteins were separated in the Spinco model E ultracentrifuge (rotor An E). Peak areas were corrected for radial dilution, and the sedimentation coefficient was corrected to 20° in water. A double-sector cell was used, the reference sector containing diffusate after 18 hr. dialysis of protein solution. The bar angle was 60°.

Sheep no.	Treatment	Protein in sample (%)	$S_{20,w}$ of peaks (s)	Area (% of total)	Time at full speed before measurement of peak area (min.)	Rev./min.
N200	None	0·99	3·3	15·4	28	36960
			19·1	72·7	28	36960
			27·9	9·1	28	36960
			32·2	2·8	28	36960
GY190	None	0·52	4·6	100	28	39890
GY190	0·1 mg. of L-thyroxine/day	0·67	7·6	100	28	39900
GY135	None	0·73	2·7	97·4	32	37000
			10·0	2·6	32	37000
GB12	None	0·39	4·0	100	33	37000
GY135	None	0·33	3·2	100	37	36020
GY87	1·0 mg. of L-thyroxine/day	0·96	7·6	93·3	29	35200
			50·4	6·7	29	35200
GWh133	None	0·40	4·8	100	28	37270
GWh133	2·0 mg. of L-thyroxine/day	0·55	7·0	100	28	36990
GB150	None	0·84	6·4	99	28	38950
			55·9	1	28	38950
GB150	0·50%-saturated-$(NH_4)_2SO_4$ ppt. dissolved in 0·9% NaCl	0·80	5·5	87·1	28	36960
			32·9	12·9	28	36960
GB150	35–45%-saturated-$(NH_4)_2SO_4$ ppt. dissolved in 0·9% NaCl	2·4	7·2	100	28	37010

1959; De Groot & Carvalho, 1960), and therefore additional methods were applied to the study of proteins from these sheep goitres.

Ultracentrifugal analysis. Samples of 20000*g* supernatant from whole homogenates of normal and goitrous thyroid tissue were examined in an analytical ultracentrifuge, as were similar preparations from goitrous sheep receiving 0·1, 1·0 and 2·0mg. of L-thyroxine/day intramuscularly. Fractions precipitated by 0–50%- and 35–45%-saturated ammonium sulphate from similar soluble protein preparations of goitrous thyroids were also investigated. The results of these investigations are given in Table 4. The major 0·9%-sodium chloride-soluble protein of normal thyroid glands was thyroglobulin ($S_{20,w}$ 19–20s), which made up approx. 70% of the total. In the extracts of goitrous glands the major components had $S_{20,w}$ values 2·7–6·4s. No evidence of a protein of $S_{20,w}$ 19–20s was seen in extracts from goitrous glands.

Since the absence of thyroglobulin could be due to excessive hydrolysis within the goitre, allowing no accumulation of the protein, three sheep were given L-thyroxine intramuscularly at 0·1, 1·0 and 2·0mg./day respectively. The higher two doses were sufficient to elevate the serum protein-bound iodine concentration from approx. 6·0 to 17·7 and 15·2μg./100ml., and to depress [^{131}I]iodide uptake into the goitre to normal levels. After 40 days of L-thyroxine treatment, goitre samples were obtained and the 0·9%-sodium chloride-soluble proteins subjected to ultracentrifugal analysis. As shown in Table 4 there was no evidence of a protein at $S_{20,w}$ 19–20s but the major component of the sedimenting proteins now had $S_{20,w}$ 7·0–7·6s.

As the fractional precipitation of soluble proteins from goitrous tissue indicated some material precipitated by 35–45%-saturated ammonium sulphate, the proteins from this fraction and also that precipitated by 0–50%-saturated ammonium sulphate were, after being dissolved in 0·9% sodium chloride, separated on the ultracentrifuge. As shown in Table 4, the protein precipitated by 35–45%-saturated ammonium sulphate sedimented at $S_{20,w}$ 7·2s as a single peak. In neither sample was there any protein sedimenting at $S_{20,w}$ 19–20s.

Starch-gel-electrophoretic separations of the 0·9%-sodium chloride-soluble proteins of normal and goitrous thyroid tissue were carried out. The results of the densitometric scanning of the radioautograph of a starch-gel-electrophoretic separation of goitrous-thyroid protein and the location of known protein bands are shown in Fig. 1.

The major radioactive peak is not visible on the stained gel, and forms a diffuse zone extending 5–7cm. In this system thyroglobulin from normal sheep migrates as a well-defined band, in the position marked on Fig. 1.

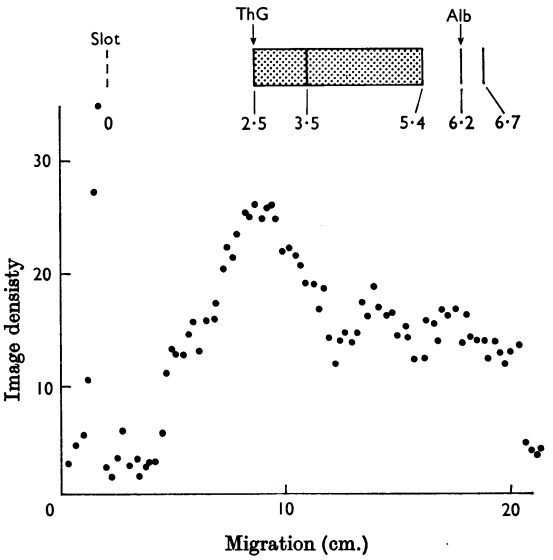

Fig. 1. Separation by starch-gel electrophoresis in a discontinuous buffer system (pH 9·4/8·5) of 0·9%-NaCl-soluble proteins of a congenital goitre. The upper diagram indicates the location of protein bands visible after staining with Nigrosine and Naphthalene Black solution. The lower graph shows the densitometric scan of a radioautograph taken from the gel, the goitrous sheep having been injected with 500μc of [^{131}I]iodide in 2ml. of 0·9% NaCl soln. 7 days before surgical excision of the gland. The two arrows indicate the location of normal thyroglobulin (ThG) and serum albumin (Alb) run in this system.

Incorporation of ^{14}C-labelled amino acids into thyroid protein. Slices of normal and goitrous thyroid tissue were incubated at 37° in Tyrode solution, pH 7·4, for 3hr. with ^{14}C-labelled amino acid. After incubation the tissue was homogenized and centrifuged at 20000*g* for 30min. at 4°. The particulate fraction was separated and the soluble proteins were treated with solid ammonium sulphate to yield material precipitated at 35% saturation, soluble at 35% but precipitated at 42% saturation, and soluble at 42% saturation but insoluble in 5% (w/v) trichloroacetic acid.

The results of the incorporation studies are shown in Table 5. The radioactivity measured in the protein precipitate after incubation in the presence of Actidione records the magnitude of association of ^{14}C-labelled amino acids and protein, unrelated to protein biosynthesis. It is approximately proportional, in any particular fraction, to the weight of protein precipitated. The wide range of incorporation of ^{14}C-labelled amino acids into protein by normal slices is due to the different ages, and hence thyroid activities, of the animals from which the tissue was obtained.

Table 5. *Incorporation of* [^{14}C]*proline or* [^{14}C]*leucine into* 0·2*g. of* 0·5*mm.-thick slices of normal and goitrous thyroid tissue*

Slices were incubated at 37° in Tyrode solution, pH 7·4, for 3 hr. with ^{14}C-labelled amino acid and treated as described in the Results and Discussion section. Identical samples of all fractions were incubated with 20 μg. of Actidione/ml. The results are expressed as counts/sec.

Thyroid sample from sheep no.	Particulate fraction		0–35%-saturated-$(NH_4)_2SO_4$ ppt.		35–42%-saturated-$(NH_4)_2SO_4$ ppt.		42%-saturated-$(NH_4)_2SO_4$ 5%-trichloroacetic acid ppt.	
	No Actidione	Actidione	No Actidione	Actidione	No Actidione	Actidione	No Actidione	Actidione
Expt. 1: [^{14}C]proline (0·20 μc)								
N	96·9	17·3	24·6	7·1	31·6	6·2	18·1	4·9
GWh132	130·8	22·3	59·1	8·6	12·8	2·0	39·0	7·9
GWh133	38·7	16·3	34·6	2·1	1·8	0·0	29·3	5·2
Expts. 2 and 3: [^{14}C]proline (0·25 μc)								
N	—	—	36·6	5·2	274·0	47·3	114·8	32·8
GY87*	—	—	37·1	1·7	26·3	11·9	114·2	33·2
N	97·6	26·3	134·4	31·3	161·2	38·1	—	—
GBl50	242·0	15·9	172·3	28·1	60·2	23·0	—	—
Expt. 4: [^{14}C]leucine (0·25 μc)								
N	74·6	11·7	98·3	10·8	77·5	9·4	78·6	7·3
GWh132	173·6	11·4	118·0	5·6	10·8	2·2	136·2	8·8

* L-Thyroxine was administered intramuscularly (1 mg./day) for 40 days before biopsy.

The results in Table 5 show that the incorporation of ^{14}C-labelled amino acids into soluble proteins other than those precipitated by 35–42%-saturated ammonium sulphate is higher in the goitrous than in the normal thyroid slices. In three of four experiments, the ^{14}C incorporated into particulate material in the goitrous-thyroid slices was also much higher than in the normal. However, in the fraction containing thyroglobulin, if present (35–42% saturation with ammonium sulphate), the radioactivity was markedly lower in the extracts from goitrous-thyroid slices. Recent work has indicated that ^{14}C-labelled amino acids can be demonstrated in 'thyroglobulin' of $S_{20,w}$ 12s and 19s after 1·5 hr. in normal tissue. By 3 hr. almost all the ^{14}C in 'thyroglobulin' was in the $S_{20,w}$ 19s fraction. Since all the suspected precursors of the final $S_{20,w}$ 19s molecule were included in the 35–42%-saturated ammonium sulphate precipitate, the lower activity of this fraction in the extracts of goitrous glands represents decreased thyroglobulin synthesis (Lissitzky, Roques, Torresani, Simon & Bouchilloux, 1964). It thus seems likely that any biosynthesis of the $S_{20,w}$ 12s or 19s molecules in the slices of goitre would be detected, and that the incorporation of ^{14}C-labelled amino acids would be greater than normal in goitrous tissue, as a consequence of excessive stimulation of the gland by thyroid-stimulating hormone (Falconer, 1966). Since the actual incorporation of radioactivity in this fraction was markedly lower than normal, and the fraction by ultracentrifugal analysis showed no material at $S_{20,w}$ 12s or 19–20s, it is concluded that thyroglobulin biosynthesis is defective in the goitrous tissue.

It has been suspected that several human congenital goitres result from defects giving abnormal thyroglobulin (Roche *et al.* 1959). In the animals of the present experiments no accumulation of an obvious abnormal iodinated protein occurred, though the biochemical data resembled closely cases of the postulated 'coupling defect' (Stanbury *et al.* 1955; Mosier, Blizzard & Wilkins, 1958). It appears likely that a proportion of cases of 'coupling defect' in man are therefore due to defective thyroglobulin synthesis, and not due to a deficiency of the proposed 'coupling enzyme'.

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