Studies of the Congenitally Goitrous Sheep

THE IODINATED COMPOUNDS OF SERUM, AND CIRCULATING THYROID-STIMULATING HORMONE

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1. A group of normal and congenitally goitrous Merino sheep were investigated to identify the metabolic defect present in the abnormal animals. 2. Protein-bound iodine concentrations of serum from goitrous animals (average $5.7\,\mu$g./100ml.) were higher than normal (average $4.2\,\mu$g./100ml.; P 0.001), but the hormonal iodine measured as butanol-extractable ^{131}I was low in the serum of goitrous (average 40.3% of protein-bound ^{131}I) compared with that of normal (84.2%; P 0.02) sheep. The non-hormonal iodine of the serum of goitrous sheep appeared to include iodotyrosines and iodinated protein. 3. Starch-gel-electrophoretic separations of sera from normal and goitrous sheep after ^{131}I injection (100–500μc) showed no qualitative differences in the radioactivity of protein components. No significant differences in thyroxine-binding *in vitro* by serum proteins of normal and goitrous sheep were observed. 4. The clearance rates of ^{131}I-labelled iodotyrosines ($t_{\frac{1}{2}}$ 1.2–2.9hr.) and iodothyronines ($t_{\frac{1}{2}}$ 33.5–47.4hr.) were similar in normal and goitrous sheep. 5. The concentration of circulating thyroid-stimulating hormone was significantly higher ($P < 0.01$ in three sheep, $P < 0.05$ in one sheep) in goitrous sheep. 6. The congenital goitre appears to be due to compensatory hypertrophy of the gland resulting from an inability to synthesize an adequate supply of thyroid hormone.

The appearance of increasing numbers of South Australian Merino sheep born with enlarged thyroid glands prompted an investigation into the nature of this disorder. Preliminary genetic investigation indicated a congenital defect, unrelated to any environmental or dietary factor (G. Mayo, personal communication). Most lambs having enlarged thyroid glands at birth died within a few weeks, but others survived to maturity with an increasingly large goitre.

Defects in the biosynthesis of thyroid hormones in man resulting in goitre have been described and fall into several distinct categories. The first identified was a defect in the ability of the thyroid to convert accumulated inorganic iodide into organically bound iodine (Stanbury & Hedge, 1950), leading to a deficiency of thyroid hormone. This was expected to have caused an increased secretion of thyroid-stimulating hormone and to result in the large goitres present in recorded cases (Parker & Beierwaltes, 1961).

Another thyroid defect of congenital origin was an inability to deiodinate free iodotyrosines, either in the gland itself or in peripheral tissues (Stanbury, Kassenaar & Meijer, 1956; Choufoer, Kassenaar & Querido, 1960). This led to an accumulation of iodotyrosines in the thyroid and release into the blood. Most of intravenously administered doses of iodotyrosine was recovered in the urine, which demonstrated the lack of deiodination in the body as a whole. Goitre in these cases was assumed to be due to iodide deficiency, caused by the loss of iodotyrosines in the urine.

Defects in the biosynthesis of iodothyronines from iodotyrosines have also been implicated as causes of congenital goitre (Werner, Block, Mandl & Kassenaar, 1957) but the actual nature of these defects is as yet undecided. The presence of abnormal iodinated proteins, which may arise from the thyroid gland, has been described in the blood of some congenitally goitrous patients (Dowling, Ingbar & Freinkel, 1961). It was therefore decided to study the biochemical basis of the congenital goitre in Merino sheep, in the hope that it might offer new evidence of defective thyroid-hormone biosynthesis.

EXPERIMENTAL

Animals. South Australian Merino rams and ewes. The sheep with goitres were provided by Mr C. Mulhearn of the South Australian Department of Agriculture. All animals were kept in partially roofed pens on a constant diet of hay chaff and oats.

General chemicals. All reagents and solvents were of A.R. grade or equivalent unless otherwise specified, except for $Ce(SO_4)_2,4H_2O$ (low in other rare earths) from British Drug Houses Ltd., Poole, Dorset. The starch used for gel electrophoresis was obtained from Connaught Laboratories, Toronto, Ontario, Canada. L-3-Monoiodotyrosine and L-3,5-di-iodotyrosine were obtained from California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A., and L-thyroxine was from the Sigma Chemical Co., St Louis, Mo., U.S.A.

Radioactive materials. Carrier-free $Na^{131}I$ in dilute $Na_2S_2O_3$ was obtained from The Radiochemical Centre, Amersham, Bucks., and L-[^{131}I]thyroxine (39·6 μc/μg.) from Abbott Laboratories, Oakridge, Tenn., U.S.A.; L-[^{131}I]-iodotyrosines were prepared by the method of Lemmon, Tarpey & Scott (1950).

Methods. The analyses of protein-bound iodine were carried out by the method of Acland (1957). Butan-1-ol-extractable ^{131}I was estimated by the method of Man, Kydd & Peters (1951), the procedure being terminated after evaporation of the alkali-washed butan-1-ol extracts to 0·5 ml. Protein-bound ^{131}I was determined as described by Falconer (1963).

Starch-gel electrophoresis of serum was carried out in a vertical apparatus (Graham, 1963) with a discontinuous buffer system [gel buffer: 9 vol. of 3 mM-citric acid–0·16 M-tris (Sigma Chemical Co.) plus 1 vol. of 20 mN-LiOH–76 mM-boric acid, final pH 9·4; electrolyte buffer: 0·1 N-LiOH–0·38 M-boric acid, final pH 8·5].

Assay of thyroxine-binding proteins was done as described by Good, Potter & Hetzel (1965), with 10 μg. of L-[^{131}I]-thyroxine/100 ml. of serum. The paper strips after electrophoretic separation of the serum proteins were cut into 0·5 cm. sections and radioactivity was measured in a well scintillation counter. Radioautographs were prepared from duplicate strips by exposing Kodak Kodirex X-ray film for 48 hr. to the dried paper strip.

The clearance of [^{131}I]thyroxine *in vivo* was measured after the intravenous injection of 25·6 μc (1·65 μg.) of L-[^{131}I]thyroxine in 1 ml. of 0·9% NaCl soln. Blood samples were taken at intervals over 75 hr. and the sera obtained were assayed for protein-bound ^{131}I and butan-1-ol-extractable ^{131}I.

Iodotyrosine clearance from the blood was measured with L-[^{131}I]iodotyrosines (64·2% monoiodotyrosine, 14·2% di-iodotyrosine, 2·8% free iodide) containing 0·57 μc/μg., a dose of 56 μg. in 1 ml. of 0·9% NaCl being injected intravenously into each animal. A mixture of iodotyrosines was used, since both occur in thyroid tissue. Blood samples were taken up to 3 hr. after injection. One sample of each serum obtained was used for the determination of butan-1-ol-extractable ^{131}I and was measured for ^{131}I in a well scintillation counter as whole serum, as acid–butan-1-ol extract and as alkali-washed butan-1-ol extract. The alkaline washing of the butan-1-ol extract removes iodo-tyrosines and any inorganic iodide (Man *et al.* 1951). A further sample was measured for protein-bound ^{131}I

content and the remainder acidified with 1 ml. of N-HCl to 3 ml. of serum, 20 μg. of L-monoiodotyrosine, 20 μg. of L-di-iodotyrosine and 50 μg. of KI were added and the whole was extracted with three equal volumes of 0·1 N-HCl-saturated butan-1-ol. The butan-1-ol extract was evaporated to dryness and the residue taken up in 0·2 ml. of butan-1-ol. Portions of this were spotted on Whatman no. 1 chromatography paper and one-dimensional separation (descending) was carried out with butan-1-ol–acetic acid–water (74:19:51, by vol). After separation the chromatography paper was cut into 1 cm. strips for well scintillation counting of ^{131}I.

For measurement of thyroid-stimulating hormone, blood was obtained by jugular puncture, allowed to clot for 1–2 hr. and then centrifuged at room temperature at about 2000 g for 30 min. in an MSE Minor centrifuge. The supernatant serum was stored at 5° until assay. Assay of thyroid-stimulating hormone was carried out by the method of McKenzie (1958b). In this method five mice/serum sample to be assayed were injected with 5–6 μc of [^{131}I]iodide, followed by 10 μg. of L-thyroxine. Thyroid powder was then added to the diet to continue to suppress the release of endogenous thyroid-stimulating hormone. A 0·5 ml. sample of serum was intravenously injected and measurements of blood radioactivity were made at 3 hr. and 8 hr. after injection. A standard of 2 milliunits of thyroid-stimulating hormone (International Standard; Medical Research Council, Mill Hill, London, N.W. 7) was administered to five similar mice for comparative purposes.

Measurements of radioactivity were made as follows. Scintillation counting of ^{131}I was carried out in an Ekco type N 664A detector fitted with a 2$\frac{1}{4}$ in. NaI (Th) well-type scintillation crystal, the counts being recorded on an Ekco type N 610A scaler. The efficiency of this system was 44% with 1 ml. samples at 1250 v (extra high tension) and 30 v discriminator bias. For the measurement of whole-blood ^{131}I in the assay of thyroid-stimulating hormone, samples were counted on a Nuclear–Chicago Corp. D 47 windowless gas-flow counter with automatic sample changer, of efficiency 60% at 1200 v (extra high tension) and 15 v discriminator bias.

RESULTS AND DISCUSSION

Iodinated components of serum. A group of three sheep having large goitres, and four normal sheep, were injected intramuscularly with 50 μc of [^{131}I]-iodide in 0·9% sodium chloride solution. At 50 or 96 hr. after injection blood samples were drawn by jugular puncture, allowed to clot and the serum was separated. In further experiments a goitrous sheep was injected with 500 μc of [^{131}I]iodide and blood samples were taken at 96 and 168 hr. An additional goitrous sheep was given L-thyroxine intramuscularly for 20 days at 1 mg./day before injection of 50 μc of [^{131}I]iodide and blood was withdrawn at 50 hr. after injection. The sera obtained were analysed for protein-bound iodine, protein-bound ^{131}I and butan-1-ol-extractable ^{131}I. The results of these experiments are given in Table 1.

The concentrations of hormone ^{131}I in the sera

Table 1. *Iodinated components of serum in normal and goitrous sheep*

Analyses for protein-bound iodine and protein-bound ^{131}I of the serum were carried out as described in the text. Measurements of the radioactivity of the acid–butan-1-ol-extractable ^{131}I were done before and after alkaline washing of the butan-1-ol extract. The results were corrected for radioactive decay to the time of blood sampling. In this and subsequent Tables the numbers of normal sheep are prefixed 'N' and those of goitrous sheep 'G'.

Serum from sheep no.	Time-interval from [^{131}I]iodide injection to blood sampling (hr.)	Protein-bound iodine (μg./100 ml. of serum)	Protein-bound ^{131}I (% of total radioactivity)	Hormone ^{131}I butanol-extracted, not removed by alkaline washing (% of protein-bound ^{131}I)	^{131}I removed from butanol extract by alkaline washing (% of total radioactivity)	Free serum ^{131}I (% of total radioactivity)	Serum [^{131}I]-iodoprotein, after butan-1-ol extraction (% of total radioactivity)
N (mean ± S.E.M. of four sheep)	96	4·2±0·42	65·2±5·3	84·2±8·0	15·9±6·7	35·4±5·7	8·8±4·3
GR 201	50	5·3	96·7	29·0	36·1	3·3	0
GBl 50	50	6·1	89·5	30·5	19·0	10·5	22·3
GR 77	50	5·8	81·4	38·5	30·2	18·6	7·7
GY 190	96	5·6	92·6	50·8	28·9	7·4	15·7
GY 190	168	5·8	90·0	52·9	30·3	10·0	16·9
GY 87*	50	17·7	58·7	15·2	17·5	41·3	35·2

* L-Thyroxine was administered intramuscularly (1 mg./day) for 20 days before blood sampling.

of normal sheep (Table 1) show that almost all the protein-bound ^{131}I is hormone; however, the sera of goitrous sheep show a much lower proportion (29–53 %) of hormone in the protein-bound ^{131}I, as measured by the results for the butan-1-ol-extractable ^{131}I (Man *et al.* 1951). The low concentrations of hormone iodine, calculated from these results (1·5–3·0 μg./100 ml.), in the sera of goitrous sheep demonstrates the cause of decreased viability at birth and in some cases the poor growth of the few surviving animals.

The total amount of iodinated material bound to serum proteins in the goitrous sheep is 1·3–1·4 times the normal. In a group of congenitally goitrous cattle the protein-bound iodine concentrations were shown to be approx. 10 times the normal (Van Zyl, Schulz, Wilson & Pansegrouw, 1965). The radioactivity of acid–butanol-precipitated proteins (Table 1, last column) demonstrates that circulating iodoprotein constitutes an additional component of the protein-bound ^{131}I in goitrous sheep.

Several studies of congenital goitre in man (Wiener & Lindeboom, 1963; Fraser, 1964) and in cattle (Van Zyl *et al.* 1965) have shown abnormal serum iodoproteins and iodopeptides. The varying concentrations of iodoprotein in the sera of the goitrous sheep are comparable with the amounts found in human goitrous patients (Dowling *et al.* 1961).

Separation of serum proteins from a goitrous sheep injected earlier with [^{131}I]iodide, by the method of Cohn *et al.* (1946), showed that the ^{131}I-labelled protein occurred in the albumin fraction. This fraction contained 12·4 % of the total serum radioactivity compared with 15·7 % determined as acid–butan-1-ol-insoluble ^{131}I.

Alkali washing of the butan-1-ol extract removes iodotyrosines and any free iodide present in the butan-1-ol. The presence of 19–36 % of the serum radioactivity in this fraction in the goitrous sheep, with free serum [^{131}I]iodide concentrations of only 3·3–18·6 % in goitrous animals not given thyroxine therapy, strongly indicates that iodotyrosines are present in the serum.

In confirmation of this, iodotyrosines were observed after chromatographic separation of extracts of serum from goitrous sheep. Iodotyrosines have also been demonstrated in the serum of patients with congenitally defective mechanisms for deiodinating these compounds, which resulted in goitre (Stanbury, Kassenaar, Meijer & Terpstra, 1955).

Results obtained for the sheep given 1 mg. of L-thyroxine/day show a markedly low level of endogenous thyroid-hormone production as measured by butan-1-ol-extractable ^{131}I, with a very high concentration of circulating thyroid hormone. This indicates the normal suppression of thyroid activity by high concentrations of exogenous

hormone, which is in agreement with the elevated free [131I]iodide concentration of the serum.

To identify the serum proteins transporting the iodinated compounds in the blood of normal and of goitrous sheep, a series of starch-gel-electrophoretic separations were carried out on sera from sheep injected with 100 μc (normal) or 500 μc (goitrous sheep) of [131I]iodide 96 hr. previously. The serum proteins were separated in a discontinuous buffer system, described above, at 5 v/cm. for 18 hr. The gel after separation was sliced into two layers, the upper being stained for protein (Graham, 1963) and the lower cut up into 1 cm. strips for radioactivity measurement on a well scintillation counter. The results of a representative separation are presented in Fig. 1. No qualitative differences were observed between serum-protein radioactivities of normal and goitrous sheep. This indicates that no unique iodoprotein occurred in the serum of goitrous animals, unless it was similar in electrophoretic mobility to α-globulin or albumin, in which case it would not be observed.
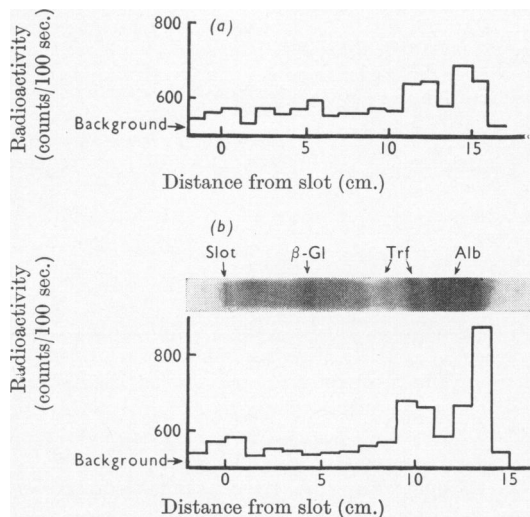

Fig. 1. Radioactivity associated with serum proteins from normal (b) and goitrous (a) sheep, after the intramuscular injection of [131I]iodide (100 μc for normal sheep, 500 μc for goitrous sheep) in 1 ml. of 0·9% NaCl soln. 96 hr. previously. Proteins were separated by starch-gel electrophoresis in a discontinuous buffer system at 5 v/cm. for 18 hr. One half of the gel was stained for protein, the location of characteristic bands being illustrated for normal serum (β-Gl, β-globulin; Trf, transferrin; Alb, albumin); goitrous-sheep serum gave a similar distribution. The other half of the gel was cut into 1 cm. strips for measurement of 131I radioactivity by well scintillation counting: 10000 counts were recorded per strip, all counts being corrected for radioactive decay to the time of blood sampling.

In both groups of animals clear peaks of 131I radioactivity occurred close to the transferrins (α-globulins) and superimposed on the front edge of albumin. This distribution of radioactivity is similar to that described by Dowling et al. (1961) in goitrous human adults.

To measure quantitatively any differences in thyroxine-binding between the serum proteins of normal and goitrous sheep, fresh serum samples were incubated for 3 hr. at 37° with L-[131I]thyroxine (10 μg. of thyroxine/100 ml. of serum) and the serum proteins separated by reverse-flow paper electrophoresis (Elzinga, Carr & Beierwaltes, 1961). This quantity of exogenous thyroxine more than doubled the normal concentration of thyroxine in sheep serum, and therefore differences in the amount of L-[131I]thyroxine bound to serum proteins would indicate differences in affinity of binding. Duplicate electrophoretic separations were carried out on each serum sample, one paper strip being cut into 0·5 cm. sections for radioactivity measurement and the other used to prepare a radioautograph. The second strip was finally stained for protein with bromophenol blue (Durrum, 1950). Radioactivity was present only in two protein bands, corresponding to albumin and the α-globulin region. The proportions of labelled thyroxine in each zone are shown in Table 2. These results indicate a close similarity in the proportion of thyroxine bound by albumin and α-globulin in sera from normal and goitrous sheep. No other zones of radioactivity were observed. It is therefore apparent that no major defect in thyroxine-binding by serum proteins exists in the goitrous sheep.

Metabolism of thyroxine and iodotyrosines. L-[131I]Thyroxine (25 μc/1·6 μg.) in 0·9% sodium chloride solution was injected intravenously into normal and goitrous sheep. At intervals up to 75 hr. after injection blood samples were withdrawn and the sera measured for total radioactivity, protein-bound 131I and butan-1-ol-extractable 131I. The results of the analyses for protein-bound 131I are given in Fig. 2. Calculations of the half-life of L-[131I]thyroxine in normal and goitrous sheep are given in Table 3. Annison & Lewis (1959) used this technique on normal non-pregnant sheep, and showed an average half-life for circulating thyroxine of 37·1 hr. (range 33–44 hr.). This is in close agreement with the results given in Table 3, indicating that no extra thyroidal defect in thyroxine metabolism occurs in the goitrous sheep.

The clearance of iodotyrosines from the blood was measured after the intravenous injection of L-[131I]iodotyrosines (32 μc/56 μg. in 1 ml. of 0·9% sodium chloride solution) into normal and goitrous sheep. Blood samples were withdrawn at intervals up to 3 hr. after injection. Serum from these

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Table 2. *Thyroxine-binding proteins in serum from normal and goitrous sheep*

L-[131I]Thyroxine (10 μg./100 ml. of serum) was incubated *in vitro* and the serum proteins were separated by reverse-flow paper electrophoresis. Radioactivity measurements are expressed as counts/sec. after deduction of background radiation and as a percentage of total protein-bound radioactivity, all results being corrected for radioactive decay to the time of addition of L-[131I]thyroxine.

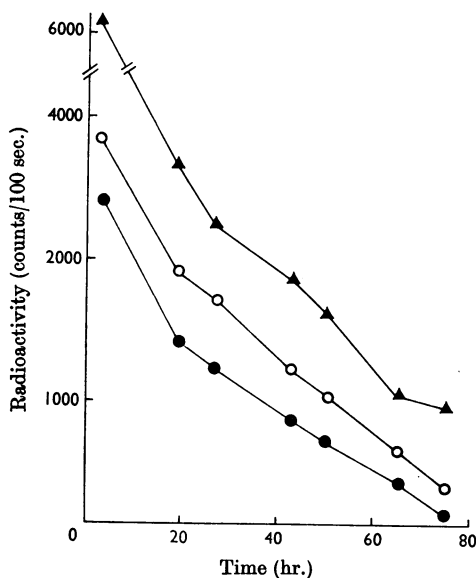| Serum from sheep no.	Radioactivity of albumin		Radioactivity of α-globulin	
	(counts/ sec.)	(% of protein-bound)	(counts/ sec.)	(% of protein-bound)
N 250	44·4	59·3	30·5	40·7
GY 135	40·2	58·1	29·0	41·9
GY 190	44·0	59·7	29·7	40·3
GY 87	33·0	47·3	36·8	52·7

Fig. 2. Protein-bound 131I in the serum of normal and goitrous sheep after the intravenous administration of 25 μc (1·6 μg.) of L-[131I]thyroxine in 1 ml. of 0·9% NaCl soln. All counts were corrected for radioactive decay to the time of injection. ▲, Normal sheep NC1; ○, goitrous sheep GY 190; ●, goitrous sheep G 87. Further details are given in the text.

Table 3. *Half-lives of intravenously injected* L-[131I]-*thyroxine and* L-[131I]*iodotyrosines in normal and goitrous sheep*

L-[131I]Thyroxine (25 μc/1·6 μg.) was injected in 1 ml. of 0·9% NaCl soln. Blood samples were withdrawn by jugular puncture 3–75 hr. after injection. The half-life of L-[131I]thyroxine was calculated from the protein-bound 131I concentration of serum, 20–75 hr. after injection. L-[131I]Iodotyrosines (32 μc/56 μg.) were injected in 1 ml. of 0·9% NaCl soln. Blood samples were withdrawn 5 min.–3 hr. after injection. The half-life of L-[131I]iodotyrosines was calculated from the 131I radioactivity removed from the acid–butan-1-ol extract of serum by alkaline washing. Further details are given in the text.

Serum from sheep no.	Half-life of L-[131I]thyroxine (hr.)	Half-life of L-[131I]iodotyrosines (hr.)
NC 1	33·5	1·2
NC 2	—	2·9
GY 87	35·6	1·9
GY 111	47·4	1·6

samples was analysed for butan-1-ol-extractable 131I by measuring the radioactivity of both the total acid–butan-1-ol-extractable 131I and also that fraction retained in the butan-1-ol phase after alkaline (4N-sodium hydroxide containing 50 g. of sodium carbonate/l.) washing. The remainder of the serum was extracted with acid butan-1-ol and the iodinated amino acids separated by paper chromatography as described above. A marker strip was run with authentic samples of L-iodo-tyrosines and L-thyroxine and was sprayed with ceric sulphate–arsenate reagent to locate the position of the iodine-containing amino acids. The radioactivity data derived from the chromatograms indicated a relatively constant proportion of L-[131I]iodotyrosines to total serum radioactivity from 0·5–3 hr. after injection in the normal [71·2 ± 4·3% (6)] and goitrous [65·5% ± 6·1% (6)] sheep. The results of calculation of the half-life of L-[131I]iodotyrosines in the serum between 1 and 3 hr. after intravenous injection are given in Table 3.

In cases of human congenital goitre in which iodotyrosine deiodinase is lacking, marked increases in the measured half-life of iodotyrosine in the serum are observed (Stanbury, Kassenaar, Meijer & Terpstra, 1955, 1956). It is clear from Table 3 that there are no major differences in the half-life of L-[131I]iodotyrosines between normal and goitrous sheep. As the observed half-life is short,

Table 4. *Estimation of the concentration of thyroid-stimulating hormone in the serum of normal and goitrous sheep*

Thyroid-stimulating hormone was assayed by the method of McKenzie (1958a,b). The results are expressed as percentage increases in blood ^{131}I radioactivity of mice injected 4–6 days earlier with 5–6 μc of [^{131}I]iodide. There were five mice/group, and the results are expressed as means ± S.E.M. of the percentage increase.

Serum sample (0·5 ml.) from sheep no.	Increase in radioactivity 3 hr. after serum injection (%)	Increase in radioactivity 8 hr. after serum injection (%)	Probability of the increase resembling that of the normal serum (P)
NC 1	170 ± 26	174 ± 26	—
GR 77	440 ± 111	393 ± 53	0·01
GY 87	589 ± 64	542 ± 89	0·01
GWh 132	609 ± 65	528 ± 117	0·01
GS 638	378 ± 120	291 ± 64	0·05
Standard thyroid-stimulating hormone (0·20 milliunit)	446 ± 26	420 ± 57	0·01

the presence of iodotyrosines in the blood of these goitrous animals indicates a substantial continuous release of iodotyrosines from the thyroid.

Thyroid-stimulating hormone. In animals or patients having large goitres and a low concentration of circulating thyroid hormone it is likely that the output of thyroid-stimulating hormone from the pituitary will be much higher than normal. It was therefore decided to estimate the concentration of thyroid-stimulating hormone in the blood of normal and goitrous sheep. Samples of blood were withdrawn from normal and goitrous sheep, and the sera separated. Estimation of the content of thyroid-stimulating hormone was carried out by the method of McKenzie (1958b), as described above. The results are expressed as percentage increase in ^{131}I concentration in the blood of mice receiving injections of the sample serum. Each serum was administered to five mice, and five other mice received 0·2 milliunit of thyroid-stimulating hormone for comparative purposes. The results are presented in Table 4, which shows that 3 hr. after the injection of 0·5 ml. of goitrous-sheep serum into mice the increase in mouse-blood radioactivity is significantly greater ($P < 0·01$) than after the injection of normal sheep serum. The increase in blood radioactivity after 0·2 milliunit of thyroid-stimulating hormone is lower than the average for the goitrous serum, indicating that the goitrous serum contains about 0·4 milliunit/ml. The decreased blood radioactivity after 8 hr. shown in Table 4 is an effective demonstration that the stimulating hormone in the goitrous-sheep serum does not have long-acting thyroid-stimulator activity (Adams, 1958; McKenzie, 1958a,b).

It is therefore concluded that, although the iodinated compounds of the serum are abnormal, no defect in the transport or peripheral metabolism of thyroxine or iodotyrosines exists in these

goitrous sheep. The low concentration of circulating thyroid hormones, and elevated concentration of thyroid-stimulating hormone, indicate that the goitre is a result of compensatory enlargement of the gland. The congenital defect may therefore be expected to be in the biosynthesis of hormone in the goitrous gland, and the abnormal consequences described in this paper arise from that defect.

REFERENCES

Acland, J. D. (1957). *Biochem. J.* **66**, 177.

Adams, D. D. (1958). *J. clin. Endocrin. Metab.* **18**, 699.

Annison, E. F. & Lewis, D. (1959). *J. agric. Sci.* **52**, 79.

Choufoer, J. C., Kassenaar, A. A. H. & Querido, A. (1960). *J. clin. Endocrin. Metab.* **20**, 983.

Cohn, E. J., Strong, L. E., Hughes, W. L., Mulford, D. J., Ashworth, J. M., Melin, M. & Taylor, H. L. (1946). *J. Amer. chem. Soc.* **68**, 459.

Dowling, J. T., Ingbar, S. H. & Freinkel, N. (1961). *J. clin. Endocrin. Metab.* **21**, 1390.

Durrum, E. L. (1950). *J. Amer. chem. Soc.* **72**, 2943.

Elzinga, K. E., Carr, E. A. & Beierwaltes, W. H. (1961). *Amer. J. clin. Path.* **36**, 125.

Falconer, I. R. (1963). *J. Endocrin.* **26**, 241.

Fraser, G. R. (1964). In *The Thyroid Gland*, vol. 2, p. 277. Ed. by Pitt-Rivers, R. & Trotter, W. R. London: Butterworths Scientific Publications.

Good, B. F., Potter, H. A. & Hetzel, B. S. (1965). *Aust. J. exp. Biol. med. Sci.* **43**, 291.

Graham, J. S. D. (1963). *Aust. J. biol. Sci.* **16**, 342.

Lemmon, R. M., Tarpey, W. & Scott, K. G. (1950). *J. Amer. chem. Soc.* **72**, 758.

McKenzie, J. M. (1958a). *Endocrinology*, **62**, 865.

McKenzie, J. M. (1958b). *Endocrinology*, **63**, 372.

Man, E. B., Kydd, D. M. & Peters, J. P. (1951). *J. clin. Invest.* **30**, 531.

Parker, R. H. & Beierwaltes, W. H. (1961). *J. clin. Endocrin. Metab.* **21**, 21.

Stanbury, J. B. & Hedge, A. N. (1950). *J. clin. Endocrin. Metab.* **10**, 1471.

Stanbury, J. B., Kassenaar, A. A. H. & Meijer, J. W. A. (1956). *J. clin. Endocrin. Metab.* **16**, 848.

Stanbury, J. B., Kassenaar, A. A. H., Meijer, J. W. A. & Terpstra, J. (1955). *J. clin. Endocrin. Metab.* **15**, 1216.

Stanbury, J. B., Kassenaar, A. A. H., Meijer, J. W. A. & Terpstra, J. (1956). *J. clin. Endocrin. Metab.* **16**, 735.

Van Zyl, A., Schulz, K., Wilson, B. & Pansegrouw, D. (1965). *Endocrinology*, **76**, 353.

Werner, S. C., Block, R. J., Mandl, R. H. & Kassenaar, A. A. H. (1957). *J. clin. Endocrin. Metab.* **17**, 817.

Wiener, J. D. & Lindeboom, G. A. (1963). *Acta endocr., Copenhagen*, **42**, 412.