Aust. J. Biol. Sci., 1980, **33**, 53–61

Production of Severe Iodine Deficiency in Sheep using a Prepared Low-iodine Diet

B. J. Potter, G. B. Jones, R. A. Buckley, G. B. Belling, G. H. McIntosh and B. S. Hetzel

Division of Human Nutrition, CSIRO, Kintore Avenue, Adelaide, S.A. 5000.

Abstract

Extensive tests on dietary materials suitable for ingestion by sheep have led to the preparation of an appropriate diet which, when fed to the sheep, caused severe iodine deficiency. The deficiency was manifested by daily urinary excretion values which fell to levels of less than 20 μg iodine and by thyroxine (T_4) and triiodothyronine (T_3) concentrations in blood plasma which were reduced from more than 90 and 1·80 nmol/l to the low levels of less than 2·58 and 0·31 nmol/l respectively. The values were attained 5 months after feeding the low-iodine diet. Goitre was present in most of the animals and the reductions in T_4 and T_3 values were accompanied by increased concentrations of plasma thyroid stimulating hormone (TSH) from less than 8·6 to more than 68 ng/ml. Samples of wool removed from selected areas of the sheep showed that the iodine-deficient diet also caused a reduction in the growth of wool.

Extra keywords: thyroid, hypothyroidism.

Introduction

Inadequate intake of dietary iodine is second only to protein-calorie malnutrition as a world health problem and is associated with endemic goitre which has been estimated to affect over 200 million people (Kelly and Snedden 1960). A more severe iodine deficiency in which the iodine intake falls below 20 μg per day may lead to the syndrome of endemic cretinism with symptoms of neurological defects including spasticity, squint and neuromotor incoordination or to hypothyroidism and myxoedema. The importance of iodine in the aetiology of the syndrome has been amply demonstrated by iodine prophylaxis as an intramuscular injection which has led to the disappearance of endemic goitre and cretinism (Buttfield *et al.* 1966; Buttfield and Hetzel 1967; Pharoah *et al.* 1971; Thilly *et al.* 1973). However, the actual role of iodine in the prevention of endemic cretinism is still unknown and controlled experimental studies with animals may help in the elucidation of the role of iodine.

The sheep has attracted attention as an animal model for studying the thyroid gland because of the close resemblance of thyroid hormone metabolism in the foetal sheep to that in the human foetus (Dussault *et al.* 1971, 1972; Erenberg and Fisher 1973; Thorburn and Hopkins 1973; Hollingsworth *et al.* 1975). Because of this close resemblance it would seem reasonable to expect the sheep to behave similarly to the human being in the manner in which iodine also is metabolized in the foetus. It should be possible therefore to study the effects of iodine deficiency on maternal–foetal relationships of iodine and the thyroid gland by feeding a diet containing very low levels of iodine to ewes before and during pregnancy.

Iodine-deficient diets containing less than 20 ng iodine per gram and which have been derived from a diet originally developed by Remington (1937) are available commercially for feeding to rats, but no reports are available of specially prepared iodine-deficient diets for sheep. In an attempt to formulate such a diet, individually selected food components were assayed and, when found to be very low in iodine content, used to prepare a low-iodine diet. The prepared diet was fed to the sheep and observations made on the severity of iodine deficiency caused by exposure to the diet for known periods.

Materials and Methods

Animals

The sheep used in the study were 3-year-old Merino ewes weighing 39–53 kg. They were divided into two groups, one of which, the control group, received the specially prepared iodine-deficient food ration and iodine supplementation and the other received the low-iodine diet only. The iodine for the control group was added twice a week as a subcutaneous injection of 1 ml of a solution containing 1 mg sodium iodide.

Diet

The low-iodine diet was prepared from crushed maize and pea pollard obtained from various areas of Australia but supplied locally. Samples from each area were assayed until a sample of sufficiently low iodine content became available. The selected diet consisted of 70% (w/w) crushed maize and 30% (w/w) pea pollard (pelleted) with an iodine content of 7 and 13 μg/kg respectively; de-ionized water was used for pelleting the pea pollard. Calcium carbonate, also with a low iodine content, was added to the diet to augment the low calcium levels in the maize and pea pollard. Thus 14 g $CaCO_3$, which supplied 6 g Ca^{2+} for each kilogram of diet, contained 0·15 ng iodine.

The amount of food offered to each sheep was 500 g per day and this represented a daily dietary intake of 4·48 μg iodine. Analyses of the diet showed that it provided adequate amounts of sodium, potassium and magnesium.

A solution of trace minerals containing 28 mg copper, 35 mg zinc and 7 mg cobalt was administered to each sheep *per os* once a week. On a daily basis each ewe then received the following trace minerals: 4 mg copper (15·7 mg $CuSO_4.5H_2O$); 5 mg zinc (22 mg $ZnSO_4.7H_2O$); 1 mg cobalt (4·0 mg $CoCl_2.6H_2O$). The iodine content of this solution was below the level of detection, and iodine concentration was therefore less than 1 ng/g.

The animals received only de-ionized water for drinking purposes.

Measurement of Iodine in Diets and Thyroid Tissue

An alkaline ashing procedure (Foss *et al.* 1960), suitably modified to determine small amounts of iodine in biological material, was used for the estimation of iodine in the diet.

Duplicate samples of 100–300 mg of the diet, previously ground in a stainless-steel knife mill, were placed in 16 by 125 mm Pyrex test tubes. 1·0 ml 1 M KOH was added to each tube and, when the sample was thoroughly wet by mixing, it was dried in an oven at 105°C for at least 24 h. Each tube was then placed in a cold muffle furnace and heated to 150°C for 30 min after which the temperature was raised to 600°C for 2 h. During this period the furnace door was opened for 15 s on three or four occasions to renew air in the furnace. The tubes were cooled, removed from the furnace, and 6 ml water added to each tube. The contents were then heated almost to boiling and, on cooling, centrifuged in the same tubes at 650 *g* for 10 min.

Into each of 25 by 150 mm Pyrex test tubes were pipetted 4·0 ml of the ash solution, after which 5·0 ml of 1·0 mM sodium arsenite were added and the tubes shaken. They were then placed in a water bath at 37°C and heated for 20 min after which 1·0 ml 1·5 mM ceric ammonium sulfate was added to each tube with a micropipette, the tubes shaken and replaced in the water bath. The ceric ammonium sulfate was added to consecutive tubes at regular intervals and at t_1 (30 min after ceric ammonium sulfate addition) a 3-ml aliquot was withdrawn from each tube and the absorbance (A) read at 340 nm, using a quartz cell in which the temperature was maintained at 37°C. The solutions

were withdrawn from the spectrophotometer cell by suction and fresh aliquots read again for absorbance at t_2 (60 min after the addition of the ceric ammonium sulfate).

A calibration curve was drawn from ($\log At_1 - \log At_2$) versus concentrations of iodine in the standard solutions. After subtraction of blank values, calculated by the same method as that for the standards, the iodine values of the unknown samples were obtained. At least four blanks and a standard curve were included with each batch of assays. To test the reliability of the method at low levels of iodine, a sample of maize was analysed at 2-weekly intervals for a period of 3 months. Results obtained were 8·5, 9·0, 10·3, 9·0, 8·7, 10·0 ng/g.

It should be noted that at all times only de-ionized glass-distilled water was used for reagent preparation, and glassware was carefully cleansed with nitric acid and rinsed with the de-ionized water to remove all traces of iodine.

For thyroid tissue the same procedure was followed except that the 1 M KOH, for ashing, was added to a known wet weight of tissue.

Measurement of Iodine in Urine

The sheep were placed in metabolism cages equipped to allow separate collection of urine and faeces. The urine was collected daily into polythene receptacles containing 8 M acetic acid to maintain acidity of the samples. Aliquots were refrigerated at 4°C until assayed.

The method used for assaying urinary iodine was based on the automated method described by Garry *et al.* (1973) suitably modified for use with a Technicon Auto-Analyser II, the main changes being the use of different-sized pump tubing and 1% (v/v) H_2SO_4 containing 1 ml BRIJ 35 per litre for the diluent.

Determination of Plasma Thyroxine (T_4), Triiodothyronine (T_3), Thyroid Stimulating Hormone (TSH) and Reverse Triiodothyronine (rT_3)

T_4, T_3, TSH and rT_3 were determined in plasma obtained by centrifugation of heparinized blood and immediately frozen. They were determined by radioimmunoassay on unextracted plasma using 8-anilino-1-naphthalene sulfonic acid (ANS) to inhibit binding to plasma proteins. The method for T_4 was based on that described by Chopra (1972) and modified by using polyethylene glycol to separate free and antibody-bound T_4 (Cheung and Slaunwhite 1976) A standard curve, usable to a concentration of 258 nmol/l, was obtained with a sensitivity of 1·3 nmol/l. T_3 determinations were based on the methods of Gharib *et al.* (1971) and Fang and Refetoff (1974) with dextran-coated charcoal being used to remove free T_3, instead of the double antibody technique which is used for the separation of bound T_3. A sensitivity of 0·08 nmol/l was obtained with a limit of 7·68 nmol/l. The double antibody radioimmunoassay was used for measuring both TSH and rT_3; sensitivities were 0·2 ng/ml and 0·09 nmol/l, and the upper limits of the standard curves 5 ng/ml and 5·12 nmol/l for the TSH and rT_3 assays respectively.

Thyroid Gland Tissues

Thyroid gland tissues, obtained from the sheep by biopsy under general anaesthesia, were fixed in 10% (v/v) neutral buffered formalin. After embedding in paraffin, sections were made at 6 μm and stained with haematoxylin and eosin for histological examination.

Hair and Wool Growth

Wool growth of the sheep was assessed by weighing a sample of raw wool clipped from an identical and clean area of the shoulder (75 by 50 mm) at 4-weekly (monthly) intervals. In this way the influence of the deficient diet on wool growth could be measured and compared in the iodine-deficient and iodine-replete sheep.

Results

Health of the Sheep Eating the Iodine-deficient Diet

Eight ewes matched in weight and age were confined to metabolism cages and fed the iodine-deficient diet which was introduced gradually over a period of 2 weeks.

Thereafter the sheep mostly ate all the food offered them and no differences in health and well-being could be detected between the four ewes which became iodine-deficient and the four which had their iodine status maintained by sodium iodide injections. Both groups behaved similarly to other ewes maintained on a normal mixed diet of chaffed wheaten hay and lucerne hay.

Body Weights

The sheep maintained their weights while eating the low-iodine diet: the iodine-deficient sheep weighed 46·4±2·1 kg and the replete sheep 43·3±2·1 kg 5 months after first receiving the diet.

Wool Growth

The selected wool samples from the sheep (Fig. 1) showed that lack of iodine reduced the rate of growth of wool. Cleansing the wool of fat and suint failed to alter the differences shown in the values for the raw wool samples.

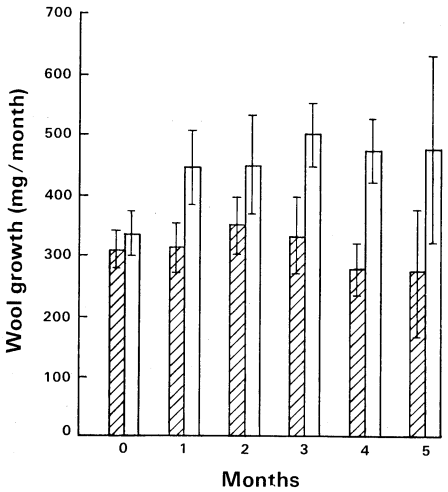

Fig. 1. Growth of wool taken at 4-weekly intervals from a selected area of skin of sheep receiving the iodine-deficient diet and the diet supplemented with sodium iodide. Values given are means±standard error of the mean (s.e.m.). Open histograms, iodine-replete sheep; shaded histograms, iodine-deficient sheep.

Urinary Excretion of Iodine

The mean daily loss of iodine by the sheep is shown in Fig. 2 where it may be seen that the excretion of iodine in the iodine-deficient sheep gradually fell from an initial mean daily value of about 25 μg to a constant value of 14 μg 5 months later, while the weekly administration of 1 mg and later 2 mg sodium iodide to the control sheep increased the mean daily loss of iodine to values which remained about 40 μg.

Thyroid Iodine and Goitre

Goitre became apparent and the thyroids palpable after ingestion of the low-iodine diet for 5 months. The iodine concentration in a wedge-shaped sample of the gland, obtained at biopsy, fell in the iodine-deficient group from a mean initial level of 850±124 μg/g wet tissue to 27·5±7·0 μg/g. Meanwhile in the control group, which received iodine supplementation, mean thyroid gland iodine concentration actually increased from 962±201 μg/g wet tissue to 2217±461 μg/g over the same period.

Plasma Thyroid Hormones

Thyroxine (T_4) and triiodothyronine (T_3) concentrations in the sheep declined steadily and the values shown in Table 1 for T_4 were reached after ingestion of the diet for 3–4 months, while those for T_3 and rT_3 were observed about 1 month later.

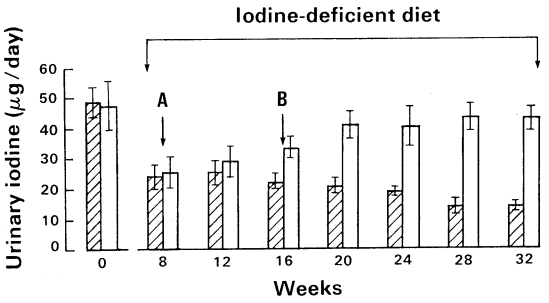

Fig. 2. Daily urinary excretion of iodine in sheep receiving the iodine-deficient diet and in sheep receiving the diet supplemented with sodium iodide. Values given are means±s.e.m. *A*, supplementation with 1 mg sodium iodide per week commenced (controls); *B*, supplementation with 2 mg sodium iodide per week commenced (controls).

The changes in TSH values tended to follow the same time pattern as that of the T_4 changes, rather than the longer period required for T_3 and rT_3 reduction. The concentrations of thyroid hormones in sheep plasma are shown in Table 1.

Table 1. Concentrations of thyroid hormones in sheep plasma before and after receiving iodine-deficient diet for 5 months

Sheep	Group[A]	T_4 (nmol/l)		T_3 (nmol/l)		TSH (ng/ml)		rT_3 (nmol/l)	
		Before	After	Before	After	Before	After	Before	After
107	I−	126·4	<2·58	2·58	<0·31	4·8	68	1·24	0·39
177	I−	130·3	<2·58	2·75	0·38	7·2	116	1·03	0·29
154	I−	91·6	<2·58	2·57	0·34	6·1	174	0·61	0·46
122	I−	94·2	<2·58	1·87	<0·31	8·6	98	1·04	0·28
188	I+	89·0	103·2	1·63	1·95	6·1	3·4	0·75	0·39
109	I+	131·6	152·2	3·01	1·77	2·9	4·2	1·10	1·23
146	I+	121·3	194·8	1·86	2·00	3·0	5·1	0·98	1·66
119	I+	81·3	107·1	0·91	1·51	6·0	5·9	1·06	0·85

[A] I−, iodine-deficient diet; I+, sheep received 2 mg sodium iodide each week.

Thyroid Gland Histology

The histological appearance of the goitrous glands (Fig. 3) indicated the intense hyperplasia and hypertrophy characteristic of iodine deficiency. The follicles were replaced by narrow spaces with barely detectable amounts of colloidal material and the epithelium lining the follicular spaces consisted of columnar cells instead of the normal cuboidal cells.

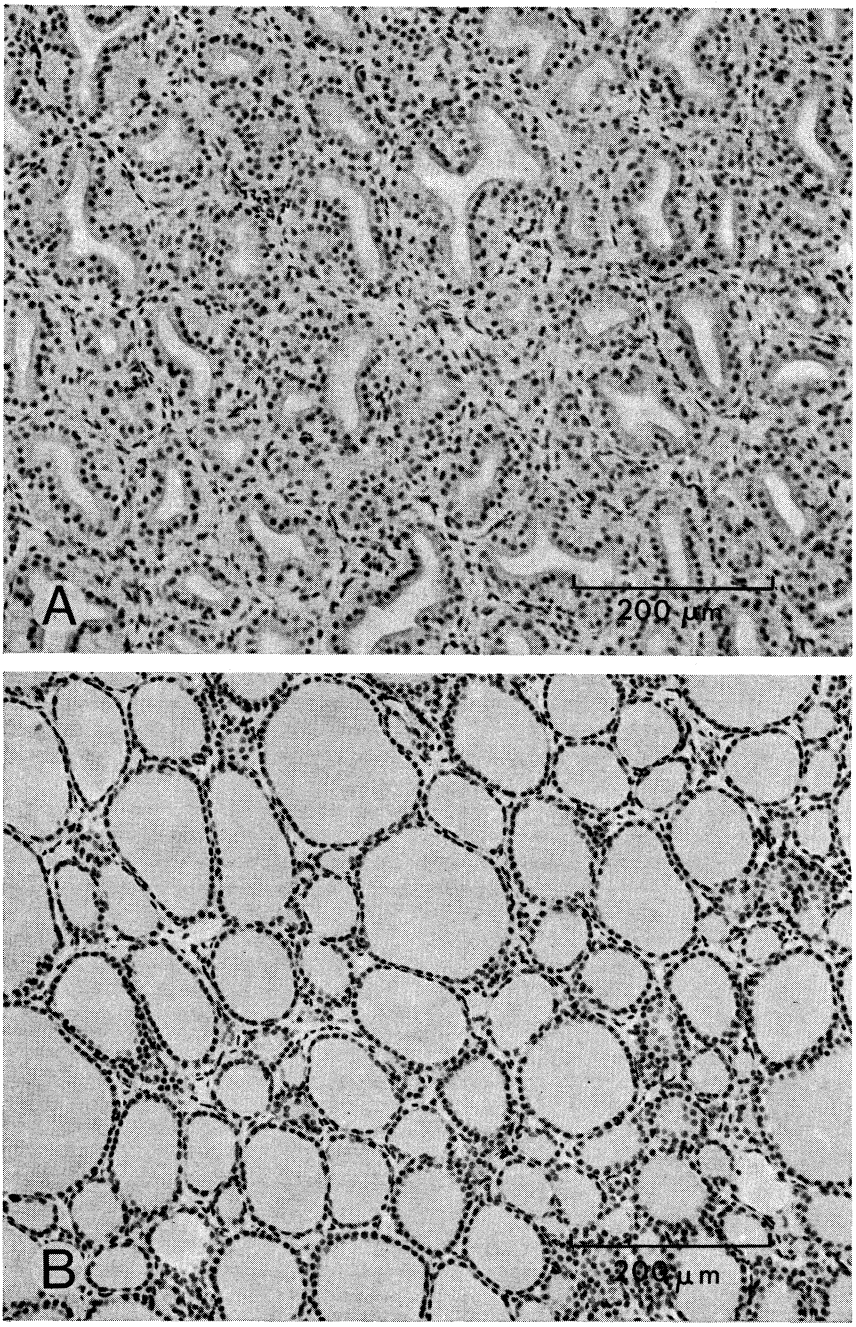

Fig. 3. The thyroid tissue of a sheep (*A*) which received the iodine-deficient diet for 5 months and another sheep (*B*) which received the diet and subcutaneous injections of sodium iodide for the same period. Haematoxylin and eosin stain.

Discussion

The ability to produce iodine deficiency in experimental animals is essential in the quest for new knowledge on the role of iodine in preventing endemic goitre, hypothyroidism and cretinism.

The sheep has received considerable attention experimentally in relation to thyroid hormone metabolism but the response of the thyroid to iodine deficiency in sheep has been studied only in association with ingested goitrogens and pasture from low-iodine tracts of land (Wright and Sinclair 1959; Statham and Bray 1975; Mason 1976; Wallace *et al.* 1978).

The iodine-deficient diet described in this paper has been prepared for sheep to enable iodine deficiency of a severe nature to be studied in these animals. The sheep required about 2 weeks of gradual substitution before they accepted the new diet. Body weights appeared to be unaffected by the lack of iodine in the diet and, although no visible changes were apparent in wool growth, measurements of wool weight from a selected area of the sheep clearly indicated that iodine is necessary to maintain wool growth. This would undoubtedly be the result of interference with hormonogenesis by the thyroid gland since lowering of T_4 values as a result of thyroidectomy was observed by Simpson (1924) to lead to retarded growth and fleece weights. Later Ferguson *et al.* (1956) reported that thyroxine requirements for normal wool growth and secondary follicle maturation surpass the requirements for general body growth. This might explain why the body weights remained unaffected by the iodine lack and yet wool growth was reduced.

The success of the diet in producing iodine deficiency in sheep was amply demonstrated by the reduction in daily urinary iodine excretion and by decreased T_4 values and increased TSH concentrations. It was noticeable that plasma concentrations of T_3 and rT_3 also were reduced. This is contrary to most reports that a depression in T_3 levels is inconsistent with dietary iodine deficiency (Abrams and Larsen 1973). However, it is more likely, as suggested by Riesco *et al.* (1977) and Thilly *et al.* (1978), that when iodine deficiency is sufficiently prolonged or severe, any shift of synthesis from T_4 to T_3 may no longer be sufficient to maintain adequate circulating levels of T_3 and rT_3.

The severity of the deficiency is substantiated also by evidence of visible goitre and by the histological picture showing follicular cell hyperplasia.

It is evident therefore that a dietary regimen suitable for producing severe iodine deficiency in sheep has been developed. The diet causes hypothyroidism and, from preliminary reports (Potter *et al.* 1977, 1978), concurrent changes in the developing foetal brain, and should enable a more complete study of hypothyroidism and cretinism to be made.

Acknowledgments

The bovine TSH used for the preparation of ^{125}I-labelled TSH was kindly donated by Professor J. G. Pierce, University of California School of Medicine, Los Angeles, California, and the NIH-S8 ovine TSH used for reference standards was obtained from the National Pituitary Agency through the generosity of the National Institute of Arthritis, Metabolism and Digestive Diseases. TSH antibody was kindly supplied by Mr A. L. C. Wallace, Division of Animal Production, CSIRO, Prospect, N.S.W.

The authors gratefully acknowledge the technical assistance of Deborah M. Martin,

R. C. Watson, P. F. Rogers and M. T. Mano and the advice and assistance given by Dr M. L. Wellby and Janet Marshall of the Queen Elizabeth Hospital, Woodville, South Australia.

References

Abrams, G. M., and Larsen, P. R. (1973). Triiodothyronine and thyroxine in the serum and thyroid glands of iodine-deficient rats. *J. Clin. Invest.* **52**, 2522–31.

Buttfield, I. H., Black, M. L., Hoffman, M. J., Mason, E. K., Wellby, M. L., Good, B. F., and Hetzel, B. S. (1966). Studies of the control of thyroid function in endemic goitre. *J. Clin. Endocrinol. Metab.* **26**, 1201–7.

Buttfield, I. H., and Hetzel, B. S. (1967). Endemic goitre in eastern New Guinea with special reference to the use of iodized oil in prophylaxis and treatment. *Bull. WHO* **36**, 243–62.

Cheung, M. C., and Slaunwhite, W. R. (1976). Use of polyethylene glycol in separating bound from unbound ligand in radioimmunoassay of thyroxine. *Clin. Chem.* **22**, 299–304.

Chopra, I. J. (1972). A radioimmunoassay for measurement of thyroxine in unextracted serum. *J. Clin. Endocrinol. Metab.* **34**, 938–47.

Dussault, J. H., Hobel, C. J., and Fisher, D. A. (1971). Maternal and fetal thyroxine secretion during pregnancy in the sheep. *Endocrinology* **88**, 47–51.

Dussault, J. H., Hobel, C. J., Di Stefano III, J. J., Erenberg, A., and Fisher, D. A. (1972). Triiodothyronine turnover in maternal and fetal sheep. *Endocrinology* **90**, 1301–8.

Erenberg, A., and Fisher, D. A. (1973). Thyroid hormone metabolism in the foetus. In 'Foetal and Neonatal Physiology'. [Barcroft Centenary Symposium.] pp. 508–26. (Cambridge University Press.)

Fang, V. S., and Refetoff, S. (1974). Radioimmunoassay for serum triiodothyronine: evaluation of simple techniques to control interference from binding proteins. *Clin. Chem.* **20**, 1150–4.

Ferguson, K. A., Schinckel, P. G., Carter, H. B., and Clarke, W. H. (1956). The influence of the thyroid on wool follicle development in the lamb. *Aust. J. Biol. Sci.* **9**, 575–85.

Foss, O. P., Hankes, L. V., and Slyke, D. D. van (1960). A study of the alkaline ashing method for determination of protein-bound iodine in serum. *Clin. Chem. Acta* **5**, 301–26.

Garry, P. J., Lashley D. W., and Owen, G. M. (1973). Automated measurement of urinary iodine. *Clin. Chem.* **19**, 950–3.

Gharib, H., Ryan, R. J., Mayberry, W. E., and Hockert, T. (1971). Radioimmunoassay for triiodothyronine (T_3). I. Affinity and specificity of the antibody for T_3. *J. Clin. Endocrinol. Metab.* **33**, 509–16.

Hollingsworth, D. R., Belin, R. P., Parker Jr., J. C., Moser, R. J., and McKean, H. (1975). Experimental cretinism in lambs: an intrauterine model with thyroid evaluation in surviving lambs. *Johns Hopkins Med. J.* **137**, 116–22.

Kelly, F. C., and Snedden, W. W. (1960). Prevalence and geographical distribution of endemic goitre. In 'Endemic Goitre'. [Monograph Series 44.] pp. 27. (World Health Organization: Geneva.)

Mason, R. W. (1976). Aetiological studies of ovine congenital goitre in Tasmania with particular reference to the Cressy-Longford Area. M.V.Sc. Thesis, University of Melbourne.

Pharoah, P. O. D., Buttfield, I. H., and Hetzel, B. S. (1971). Neurological damage to the fetus resulting from severe iodine deficiency during pregnancy. *Lancet* **1**, 308–10.

Potter, B. J., Jones, G. B., McIntosh, G. H., and Hetzel, B. S. (1977). A new model of severe iodine deficiency in sheep. *Proc. Endocrinol. Soc. Aust.* **20**, 37.

Potter, B. J., McIntosh, G. H., and Hetzel, B. S. (1978). Changes in foetal brain development caused by severe iodine deficiency in sheep. *Proc. Endocrinol. Soc. Aust.* **21**, 30.

Remington, R. E. (1937). Improved growth in rats on iodine deficient diets. *J. Nutr.* **13**, 223–33.

Riesco, G., Taurog, A., Larsen, P. R., and Krulich, L. (1977). Acute and chronic responses to iodine deficiency in rats. *Endocrinology* **100**, 303–13.

Simpson, S. (1924). Effects of thyroidectomy on the cutaneous system in the sheep and goat. *Q. J. Exp. Physiol.* **14**, 185–97.

Statham, M., and Bray, A. C. (1975). Congenital goitre in sheep in southern Tasmania. *Aust. J. Agric. Res.* **26**, 751–68.

Thilly, C. H., Delange, F., Goldstein-Golaire, J., and Ermans, A. M. (1973). Endemic goitre prevention by iodized oil: a reassessment. *J. Clin. Endocrinol. Metab.* **36**, 1196–204.

Thilly, C. H., Delange, F., Lagasse, R., Bourdoux, P., Ramioul, L., Berquist, H., and Ermans, A. M. (1978). Fetal hypothyroidism and maternal thyroid status in severe endemic goitre. *J. Clin. Endocrinol. Metab.* **47**, 354–60.

Thorburn, G. D., and Hopkins, P. S. (1973). Thyroid function in the foetal lamb. In 'Foetal and Neonatal Physiology'. [Barcroft Centenary Symposium.] pp. 488–507. (Cambridge University Press.)

Wallace, A. L. C., Gleeson, A. R., Hopkins, P. S., Mason, R. W., and White, R. R. (1978). Plasma thyroxine concentrations in grazing sheep in several areas of Australia. *Aust. J. Biol. Sci.* **31**, 39–47.

Wright, E., and Sinclair, D. P. (1959). The concentration of radioiodine by the foetal thyroid gland and its relation to congenital goitre in sheep. *N.Z. J. Agric. Res.* **2**, 933–7.

Manuscript received 23 August 1979, accepted 7 January 1980