Low-iodine diet for the production of severe I deficiency in marmosets (*Callithrix jacchus jacchus*)

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1. A low-iodine diet consisting of maize, peas (*Pisum sativum*), torula yeast, meat meal, maize oil and added vitamins, minerals and amino acids was given to eight pairs of adult, common cotton-eared marmosets (*Callithrix jacchus jacchus*). Eight control pairs were given the same diet to which potassium iodate was added. Both groups also received low-I apple and deionized water.

2. The diet provided adequate nutrition, as confirmed by the maintenance of body-weight and good health.

3. In the I-deficient marmosets the concentration of plasma thyroxine was decreased from 140·1 nmol/l to 22·4 nmol/l and thyroid-stimulating hormone increased significantly from 1·8 ng/ml to 9·0 ng/ml compared with control marmosets, thereby indicating severe I deficiency.

4. Compared with newborn offspring from control marmosets, the thyroid glands from the I-deficient offspring showed (a) an increase in weight, (b) a decrease in I content and (c), on histological examination, hyperplasia, hypertrophy and a total absence of colloid material in the follicles.

Severe iodine deficiency, associated with high rates of endemic goitre, endemic cretinism and other defects, produces a spectrum of conditions now designated 'I-deficiency disorders' (Hetzel, 1983).

There has been a need to develop animal models to investigate the relation of I deficiency to development (particularly fetal brain development) further and determine the mechanisms involved. In the sheep, severe I deficiency has been shown to cause severe hypothyroidism with brain retardation in the fetus (Potter *et al.* 1980, 1982). Less striking effects have been observed in the rat (McIntosh *et al.* 1980).

In the present study, a low-I diet has been produced to assess the effects of severe I deficiency on a non-human primate. The common cotton-eared marmoset (*Callithrix jacchus jacchus*) was chosen because it is phylogenetically similar to man, relatively easy to handle, inexpensive to house and feed (Grist, 1973; Hearn *et al.* 1975; McIntosh & Looker, 1982) and is suitable for teratological research (Poswillo *et al.* 1972).

MATERIALS AND METHODS

Diet

The formulation of the low-I diet (LID) was based on the rat diet used by McIntosh *et al.* (1980), modified for the marmosets by the addition of low-I meat meal, vitamins and amino acids (Table 1). The low-I meat meal was specially produced at this laboratory from sheep which were given a diet of low-I maize and pea pollard (husk which surrounds the pea seed but does not include the pod) 3–5 months before slaughter (Potter *et al.* 1980). Maize and peas (*Pisum sativum*) were used as the major source of carbohydrate, while the meat meal, torula yeast and peas provided the protein.

The dry components of the LID were thoroughly blended in a dough mixer and subsequently pelleted in a moist state (300 g deionised water/kg) using a meat grinder with a plate of 9 mm hole size. The pelleted diet was stored frozen (−20°) rather than dried because of the preference by the marmosets for a moist diet and the fact that the process of drying caused oxidation of vitamin C.

Table 1. *Composition of the low-iodine diet given to marmosets* (Callithrix jacchus jacchus)

Component	Contains (/kg diet)	Source
Maize	600 g	South Queensland, Australia
Peas (*Pisum sativum*) (dried)	150 g	South Island, New Zealand
Torula yeast (dried)	100 g	Sanitarium Health Foods (Australia)
Meat meal (dried)	100 g	From low-I sheep (Potter *et al.* 1980)
Maize oil	15 g	Nuttelex Food Products (Australia)
$CaCO_3$	15 g*	—
NaCl	5 g*	—
$CoCl.6H_2O$	30 mg*	—
$CuSO_4.5H_2O$	39 mg*	—
$MnSO_4.H_2O$	156 mg*	—
$ZnSO_4.7H_2O$	110 mg*	—
$FeSO_4$	300 mg*	—
		Sigma catalogue no.
Choline chloride	1250 mg*	C1879
L-Lysine monohydrochloride	1200 mg	L5626
L-Methionine	850 mg	M9625
Vitamin C (ascorbic acid)	3000 mg	A7506
Vitamin A (retinol palmitate *trans* type IV)	18000 μg†	R3375
Vitamin D_3 (cholecalciferol)	280 μg†	C9756
Vitamin E (D-α-tocopherol acetate type III)	24300 μg†	T3001
Vitamin K_3 (menadione)	3000 μg	M5750
Vitamin B_{12} (cyanocobalamin)	40 μg	V2876
Vitamin B_1 (thiamin hydrochloride)	27000 μg	T4625
Vitamin B_2 (riboflavin)	18000 μg	R4500
Vitamin B_6 (pyridoxine monohydrochloride)	13000 μg	P9755
D-Pantothenic acid	40000 μg	P2250
Folic acid	10000 μg	F7876
D-Biotin	500 μg	B4501
Niacin (nicotinic acid)	78000 μg	N4126

* Dissolved in 200 ml deionized water to moisten diet for pelleting.
† Dissolved in the 15 g maize oil.

Each marmoset was offered 30 g diet/d but consumed about 18 g in addition to 8 g apple. The I content of the diet and apple was 12·1 (SE 1·4) and 11·0 (SE 1·0) μg I/kg (wet weight) respectively, giving an I intake of 0·3 μg/d. Control animals were given the same diet to which potassium iodate was added to give 420 (SE 21) μg I/kg (wet weight) of high-I diet (HID) or an intake of approximately 7·6 μg I/d.

The animals were supplied with deionized water *ad lib.*

Animals

Sixteen pairs of marmosets between the ages of 14 and 24 months were divided into two groups, one being given the LID and the other the HID. The marmosets were kept in pairs of opposite sex in aluminium cages (North Kent Plastics Ltd, Home Gardens, Dartford, Kent, UK) in a room maintained at 26° and 50% humidity and provided with 12 h fluorescent lighting and 45 min u.v. light/d (Poswillo *et al.* 1972; McIntosh & Looker, 1982).

Body-weight was measured weekly and blood samples were withdrawn from the femoral

vein (Hearn *et al.* 1975) into dry heparinized tubes every 2 months to assess the onset and progress of I deficiency by the plasma levels of thyroxine (T_4) and thyroid-stimulating hormone (TSH).

I analysis

I in the diet and thyroid tissue was measured using alkaline ashing (Foss *et al.* 1960) suitably modified, followed by a spectrophotometric determination based on the catalytic effect of I on the oxidation–reduction reaction between Ce(IV) and As(III) (Potter *et al.* 1980; Belling, 1982).

T_4 and TSH determination

Plasma obtained by centrifugation of heparinized blood was used for determination of T_4 (Potter *et al.* 1980) and TSH by a modification of the method of Patel *et al.* (1971) using human TSH antibody and the International Reference Preparation 68/38, National Institute of Medical Research, Mill Hill, London, UK.

Thyroid gland

To allow further investigations to be made on the two groups of marmosets, thyroid assessment was made on their newborn progeny (gestational age 150 d).

The newborn offspring were killed with an overdose of the steroid anaesthetic Saffan obtained from Glaxo Laboratories Ltd, Greenford, Middlesex, UK (Phillips & Grist, 1975) before the thyroid glands were removed and weighed. Half the gland was frozen (−20°) for I determination and half fixed in neutral buffered formaline (100 ml/l) for histology. After embedding in paraffin, the fixed thyroid tissue was cut into 6-μm sections and stained with haemotoxylin and eosin for examination by light microscopy.

Statistics

The Student's *t* test was used to assess significance.

RESULTS

Body-weight and general condition

After eating the diet for 6 months the marmosets were I deficient and yet maintained their condition. In fact at the end of that period their body-weights remained slightly more than that of the controls (Fig. 1). The animals exhibited normal activity with no apparent change in their behaviour.

T_4 and TSH

Plasma T_4 was used to monitor the progress of I deficiency and, as shown in Fig. 2, T_4 levels declined rapidly in the first 9 months to a value of 22·4 (SE 2·1) nmol/l, being significantly lower ($P < 0{\cdot}001$) than 140·1 (SE 7·7) nmol/l for the controls.

Plasma TSH values for I-deficient and control marmosets were 9·0 (SE 1·6) ng/ml and 1·8 (SE 0·2) ng/ml respectively ($P < 0{\cdot}001$) but the values for both treatment groups were lower than might be expected. This is possibly due to a low cross-reaction of marmoset TSH with the human antibody used in the radioimmunoassay.

Thyroid gland

Histology of the thyroid gland from I-deficient newborn offspring revealed obvious hyperplasia, hypertrophy and a complete absence of colloid material in the follicles (Plate 1) in comparison with the offspring of the control group.

Highly-significant effects of I deficiency on thyroid weight and I content of newborn marmosets are shown in Table 2.

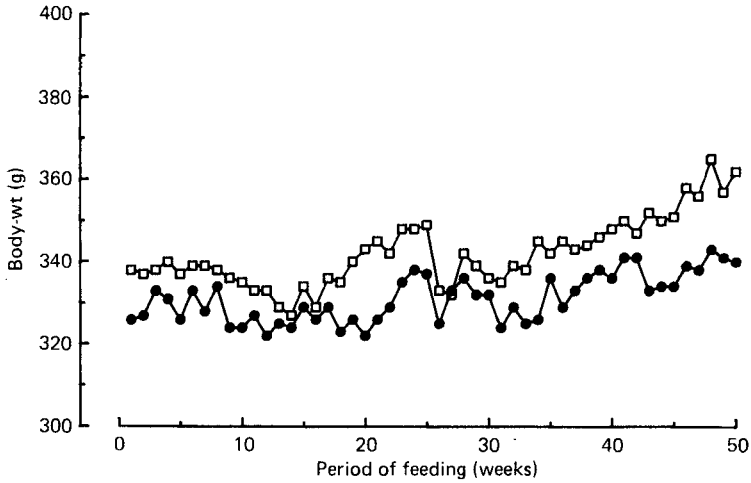

Fig. 1. Body-weights of marmosets (*Callithrix jacchus jacchus*) given an iodine-deficient (□) or control (●) diet for 50 weeks.

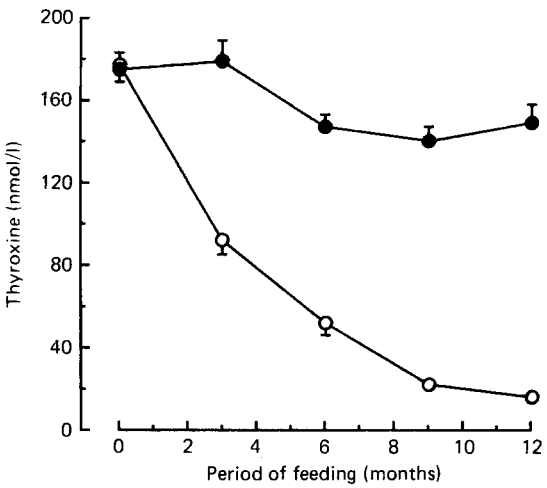

Fig. 2. The decline observed in plasma thyroxine levels (nmol/l) of iodine-deficient (○) compared with control (●) marmosets (*Callithrix jacchus jacchus*) during the first 12 months of diet consumption. Points are means with their standard errors represented by vertical bars.

Table 2. *Weight and iodine content of the thyroid glands from newborn marmosets* (Callithrix jacchus jacchus)

(Mean values with their standard errors)

	No. of observations	Wt (mg)		I content (ng)	
		Mean	SE	Mean	SE
Control	6	9·6	0·6	14780	1920
I-deficient	11	50·6	4·8	57	6
Statistical significance of difference between treatments		$P < 0{\cdot}001$		$P < 0{\cdot}001$	

DISCUSSION

The diet described in the present paper achieved severe I deficiency in the marmoset and yet normal body-weight (Fig. 1) and good health were maintained. 'Wasting marmoset syndrome', believed to be of dietary origin, was not evident at any stage, probably due to a sustained adequate protein intake which was achieved by limiting the amount of soft foods such as banana and apple which the marmosets would consume in preference to the more nutritious diet pellets (Shimwell *et al.* 1979).

The success of the diet was achieved largely by enhancing the palatability with the addition of low-I meat meal prepared from sheep given a LID (Potter *et al.* 1980) although any source of low-I meat protein should be acceptable provided it remains palatable to the marmoset. The advantages of meat meal are emphasized by the observations of McIntosh & Looker (1982) that marmosets prefer a diet with a high meat content and the fact that they require a high-protein intake (King, 1978; Shimwell *et al.* 1979). In addition, feeding the diet as a soft moist pellet (300 g water/kg) was more acceptable to the marmosets than a hard, dry pellet. This agrees with the observations of Wirth & Buselmaier (1982) that consumption was improved when the diet was softened with water.

The degree of I deficiency achieved with this diet is clearly demonstrated by the significant reduction in plasma T_4 and increased plasma TSH. Further evidence of severe I deficiency can be seen from the significant effects on the thyroid gland of newborn offspring, as shown by an increase in weight, a decrease in I content (Table 2) and histologically by a total absence of colloid material in the follicles (Plate 1).

In conclusion, our results show that with this diet formulation it is feasible to study the effects of I deficiency and, in particular, the nutritional stress it imposes on the fetus, in a non-human primate. This kind of study can be related to man, in whom some investigations of I deficiency are unacceptable for ethical reasons, and dietary regimens difficult to control.

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EXPLANATION OF PLATE

Thyroid tissue of newborn marmosets (*Callithrix jacchus jacchus*) showing a total absence of colloid material (col) in the follicles of (*a*) the iodine-deficient gland compared with (*b*) the control gland.

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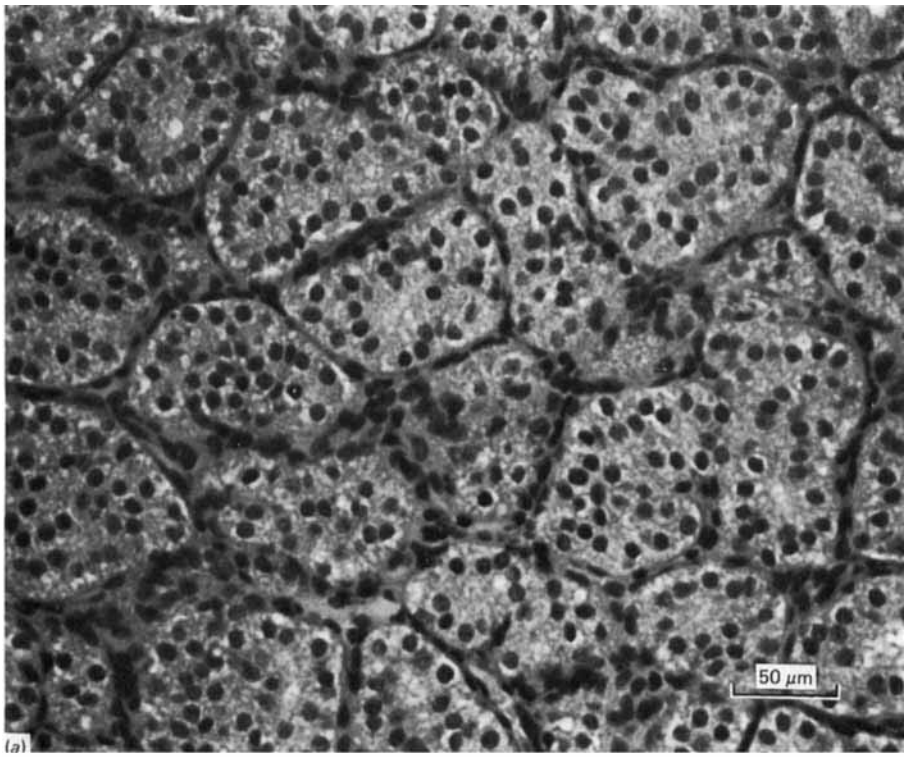

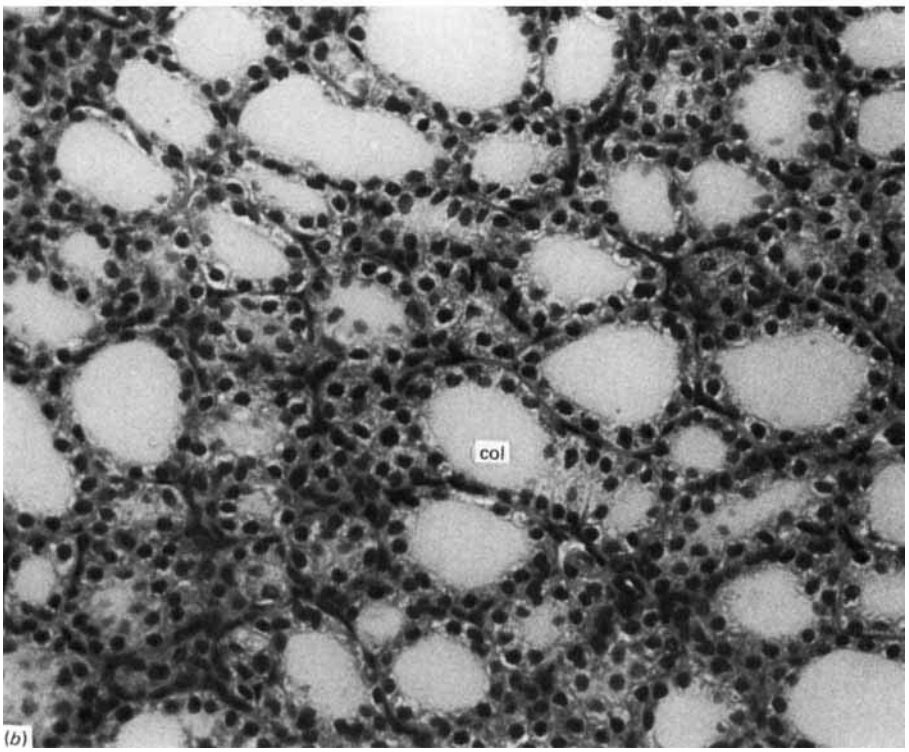

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