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The Goitrogen 3-Hydroxy-4(1*H*)-pyridone, a Ruminal Metabolite from *Leucaena leucocephala*: Effects in Mice and Rats

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Abstract

Mice fed a diet containing 1% (w/w) 3-hydroxy-4(1*H*)-pyridone (DHP) developed goitre even with a diet high in iodine whereas mimosine (0·5% w/w) did not produce goitre even with a low-iodine diet. Thyroid enlargement was apparent (measured morphometrically) by the 7th week and was advanced by the 11th week. Histologically the goitre was hyperplastic in type. No marked histological changes were found in other organs of mice fed DHP or any organs of mice fed mimosine, except for some atrophy of hair follicles.

A single intragastric dose of DHP inhibited the uptake of ^{125}I by the thyroid in the rat but an equivalent dose of mimosine did not. Evidence is presented that the inhibition occurs at the iodine binding step, as with methyl thiouracil, rather than at the iodide trapping step, as with thiocyanate. Chronic treatment of mice with DHP, as with 6-methyl thiouracil, increased the avidity of the thyroid in taking up ^{125}I.

The major conjugated form of DHP in mammals, DHP-3-*O*-glucuronide, was almost as effective a goitrogen as the unconjugated compound when given by mouth but considerably less active than the free form in the blood stream.

It was concluded that DHP is a potent antithyroid compound of the thiouracil type with low general toxicity, since mammals can tolerate a level of intake sufficient to produce goitre in spite of iodine supplementation.
[Other keywords: legume.]

Introduction

Enlarged thyroid glands, low live weight gains and hair loss have been reported in cattle after prolonged grazing of the tropical legume *Leucaena leucocephala* (leucaena) in Queensland, north Western Australia and New Guinea (Holmes 1976; Jones *et al.* 1976, 1978). Goitrous offspring have been produced by sheep (Bindon and Lamond 1966) and cattle (Hamilton *et al.* 1971) fed during pregnancy on diets containing a high proportion of leucaena. Mimosine (β-[*N*-(3-hydroxy-4-oxopyridyl)]-α-aminopropionic acid), a non-protein amino acid which occurs in large amounts in the plant, and which has several unusual physiological properties (for review see Hegarty and Peterson 1973), was suspected as the causative agent. However, Hegarty *et al.* (1976) briefly reported that mimosine was not goitrogenic in rats and mice, but that 3-hydroxy-4(1*H*)-pyridone (DHP), to which a major part of the ingested mimosine is converted in the rumen, was a potent goitrogen.

This paper describes chronic and acute experiments in which mimosine, DHP and DHP-3-*O*-glucuronide (DHPOG) were examined for their actions on the thyroid glands in mice and rats. The mode of action of DHP as an antithyroid substance is

defined. A second paper will deal with the chemistry and biochemistry of DHP relevant to its antithyroid properties and its distribution, metabolism and excretion in some mammals.

Materials and Methods

Animals and Diets

Swiss mice (sex and body weights as shown in plan of experiments) were housed in cages in an air-conditioned mouse room at 24°C. Food and water were available *ad libitum*.

A high and a low-iodine diet were used. The high-iodine diet was a normal rat and mouse diet (IRM pellets, Victorian Wheatgrowers Corporation, Melbourne), which had the following composition (manufacturer's analysis): protein 14%, fat 17%, fibre 8·5%, NaCl 0·5%, calcium 0·8%, phosphorus 0·7%. The diet was found on analysis (Laboratory Services, Mt Waverley, Vic.) to contain 2700 μg I kg^{-1}. The low-iodine diet (low-iodine diet fortified with vitamins, ICN Pharmaceuticals, Life Sciences Group, Cleveland, U.S.A.), contained corn starch, 56%; vitamin-free casein, 27%; hydrogenated vegetable oil, 14%; Hubbell–Mendel–Wakeman salt mix (free of KI), 3%; plus vitamin diet fortification mixture. The iodine content was 80 μg kg^{-1} (Laboratory Services, Mt Waverley, Vic.).

Test substances [mimosine, molecular weight 198, 0·5% (w/w); DHP, molecular weight 111, 1% (w/w); DHPOG dihydrate, molecular weight 323, 3% (w/w)] were incorporated into the ground diets with a mechanical mixer. The amounts of test substances available were insufficient for processing the mixtures in a pelletting machine, and the final diets were prepared in the following way. Distilled water was mixed in to form a dough which was rolled out in shallow trays and dried in a forced-draught oven at 80°C for 1–2 h. The resulting dry biscuit was broken into pieces of about 2 cm^2 for feeding. The mixture of the low-iodine diet with mimosine and DHP (but not with DHPOG) was red from reaction of the ferric phosphate in the salt mixture with the test substances to form the ferric complexes. These complexes are not so readily absorbed from the gastrointestinal tract as the parent substances (Tsai and Ling 1974) and calculations based on the amount of ferric phosphate in the diet indicate that a maximum of 4% of the DHP, and 16% of the mimosine added to the diets could have been complexed in this way. Chromatographic and electrophoretic analysis (Hegarty, Sloots and Court, unpublished data) of the biscuits confirmed that no appreciable decomposition of the test substances took place during processing of the diets.

Tissues

Mice were killed by ether overdosage. The larynx and thyroid were removed as a block, fixed in 10% (v/v) formol saline, embedded in paraffin, cut serially at 7 μm and stained with haematoxylin and eosin. Thyroid enlargement was assessed morphometrically, rather than by weight, to avoid damage by manipulation which might adversely affect histological quality. Each value for lobe diameter in Figs 1 and 2 is a mean of 12 measurements on the stained sections, made with an eyepiece micrometer, in two directions at right angles, at three levels near the maximum diameter. The eyepiece micrometer was calibrated by means of a stage ruling. The degree of response of the thyroid gland to the test conditions was assessed histologically and graded according to the convention used by Astwood (1943). Sections were also made from liver, kidney, skin and bone marrow (experiments with mimosine only) as described above.

Test Substances

Mimosine was isolated from the seeds of leucaena by the method of Hegarty *et al.* (1964*b*). DHP was prepared from mimosine by a modification of the method of Hegarty *et al.* (1964*a*). The DHPOG was isolated from urine of cattle grazing leucaena, by means of an anion exchange resin in the acetate form. The DHPOG was eluted from the resin with 4 M acetic acid. Details of the method will be presented elsewhere.

The antithyroid compounds 6-methyl thiouracil, resorcinol and potassium thiocyanate were British Pharmacopoeia preparations. Na ^{125}I was obtained from the Radiochemical Centre, Amersham, England.

Plan of Feeding Experiments

(i) *Feeding experiments with mimosine*

In a preliminary experiment groups of 10 male and 10 female mice (average body weights 25 and 21 g respectively) were maintained for 20 weeks on the high-iodine diet, with or without added mimosine (0·5%). This concentration of mimosine was chosen because Lin and Ling (1961) had shown that mice could be maintained for many weeks on a diet containing 0·5% mimosine, but died within 2 weeks when the concentration was 1%. Mice were killed at intervals of 2 weeks, and the thyroids examined for enlargement and histological changes. As none were detected, a confirmatory experiment was designed using the low-iodine diet with or without 0·5% mimosine, and using female mice only to reduce losses from fighting. Groups of 10 female mice (body weight about 20 g) were maintained for 13 weeks. The groups were weighed weekly, and the mean body weights calculated. At the end of week 5, and every 2 weeks thereafter, two mice from each group were killed, and samples of thyroid, liver, kidney and skin were taken for histological examination.

(ii) *Feeding experiments with DHP*

Experiment 1. Four groups of five young female mice (initial body weight about 15 g) were fed for 12 weeks on low, or high-iodine diets, with or without added DHP (1%). This concentration was used because DHP is less toxic than mimosine (Hegarty *et al.* 1978). One mouse from each of the four treatment groups was killed at the times shown in Fig. 1, except for week 12 when two mice from the high-iodine diet with and without DHP were killed. The tissues were removed and treated as described for the mimosine experiments.

Experiment 2. Two groups of seven adult female mice (initial body weight 24 g) were fed the same low-iodine diets as in experiment 1 for 14 weeks, and a further two groups of four mice were fed the high-iodine diet, with or without DHP, for 16 weeks. One mouse from each group was killed at each of times shown in Fig. 2, except for week 14 when two mice in the DHP-treated group (low-iodine) and three mice in the low-iodine control group were killed.

(iii) *Feeding experiments with DHPOG*

Two groups of 10 young female mice (initial body weight 19·0±0·5 g) were fed for 13 weeks on the low-iodine diet, with or without added DHPOG (3%). Two mice from each group were killed at 5, 11 and 13 weeks, and four mice were killed at 7 weeks. The thyroid, liver, kidney and skin were prepared for histological examination as described above.

Acute Tests for Antithyroid Activity

Female Sprague–Dawley rats weighing about 150 g were stratified by body weight and in each experiment were selected from the same weight groups. The test was carried out using a modification of the method of Arnott and Doniach (1952). The test substances were administered by stomach tube, under shallow ether anaesthesia, as aqueous solutions (1 ml) unless otherwise stated. Control rats received 0·9% NaCl (1 ml). Two hours later Na ^{125}I (37 kBq in 1 ml of 0·9% NaCl) was injected intravenously. One hour later the rats were killed by exsanguination under ether anaesthesia, and the tissue block comprising the thyroid and larynx was removed and dissolved in 2 ml of 1 M NaOH at room temperature overnight. Counts were made on 1 ml of the digest by the toluene-Triton X-100 method (Madsen 1969) except that the scintillation mixture also contained POPOP (0·2 g l^{-1}), as recommended by Cerceo (1974) for ^{125}I. The final sample volume was 15 ml. The sample vials were counted in a Packard model 2425 liquid scintillation spectrometer. The mean uptake (cpm ml^{-1}) of digest, corrected for the quenching effect using the automatic external standard, was determined for each group of rats. The residual variances associated with treatment effects were always heterogeneous. A square root transformation of the radioactive counts is usually the correct transform to equalize the variances. However, a log transformation was necessary for two experiments.

Experimental Details and Results

Feeding Experiments with Mimosine

The groups of mice fed high- and low-iodine diets containing 0·5% mimosine showed diminished weight gains relative to their control groups throughout the

experimental period, but no mice died. The thyroid lobe diameters were comparable to those of the controls at all times, and the histological appearances were indistinguishable from those of the controls. The data are therefore not reported in detail. It was concluded that mimosine is not goitrogenic at a level of 0·5% in the diet.

Feeding Experiments with DHP

Experiment 1. Fig. 1 summarizes the changes in body weight and thyroid size during the course of the experiments using young mice. The presence of 1% DHP in the high-iodine diet increased thyroid size only slightly, and weight gain was almost normal, indicating the low general toxicity of DHP at this concentration. The mice fed the low-iodine diet alone gained weight normally, thus demonstrating the nutritional adequacy of this diet, and their thyroid glands were not enlarged. Fig. 1 also

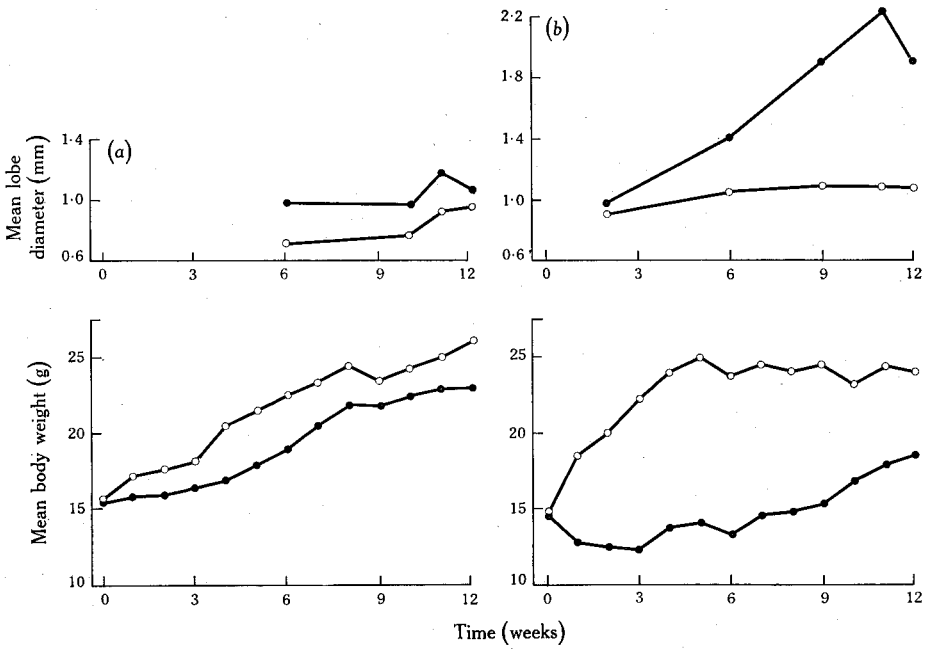


Fig. 1. Mean body weights and mean diameters of thyroid lobes of young mice during 12 weeks' ingestion of (*a*) high-iodine diets and (*b*) low-iodine diets, with 1% DHP (●) or without DHP (○). For experimental details see text (experiment 1).

demonstrates that the mice fed the low-iodine diet plus 1% DHP showed depressed weight gain and progressive increase of thyroid size. The increase in lobe diameter from 0·89 to 2·06 mm indicates more than a 10-fold increase in thyroid volume. No mice died during the experiment.

Experiment 2. Fig. 2 summarizes similar data from the experiment using adult mice. Even with the high-iodine diet the mice ingesting DHP showed consistently larger thyroids than their controls. The mice fed the low-iodine diet maintained their body weight satisfactorily and developed slightly enlarged thyroids. The group fed the low-iodine diet plus 1% DHP lost weight and the thyroid size increased con-

siderably, especially after the 6th week. One DHP-treated mouse died during the experiments. Thyroid size, like body weight, appears to vary slightly from week to week, even in the controls. The graphs show that in spite of this variation the thyroids of the mice fed DHP were always larger than those of the control mice fed the same diets without DHP, killed at the same times.

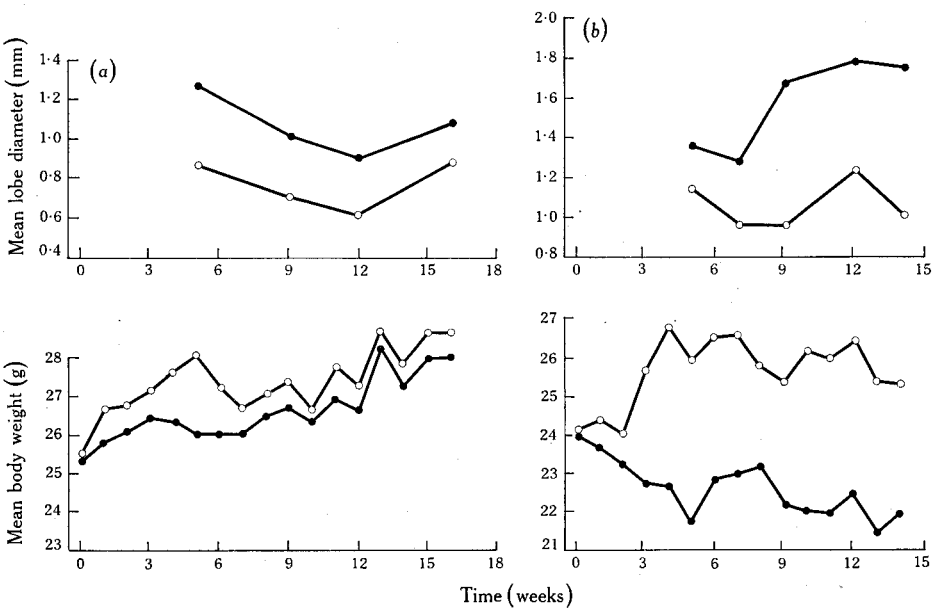


Fig. 2. Mean body weights and mean diameters of thyroid lobes of adult mice during (*a*) 16 weeks' ingestion of high-iodine diets, and (*b*) 14 weeks' ingestion of low-iodine diets, with 1% DHP (●) or without DHP (○). For further experimental details see text (experiment 2).

Histological Observations on Thyroid Glands

The main histological changes in the thyroids are graded with respect to their times of onset and degree of development in Table 1, and are illustrated in Figs 3–5 and 7–9. Fig. 6 shows the macroscopic appearance of a goitrous thyroid from a young mouse resulting from ingestion of the low-iodine diet plus 1% DHP for 11 weeks. Figs 5 and 9 demonstrate that the goitres were of the hyperplastic type histologically, and resembled those observed in cattle grazing leucaena in field experiments (Fig. 10). Mild but definite epithelial hyperplasia developed in the mice fed the high-iodine diet plus 1% DHP, but did not progress beyond this stage.

Feeding Experiment with DHPOG

The control mice in this experiment were fed the low-iodine diet, and showed weight gains and thyroid lobe diameters similar to those of the comparable group in experiment 1 (Fig. 1). The test group received the low-iodine diet plus 3% DHPOG. Throughout the experiment the mean body weight of the test group was a little below that of the control group, and the thyroid lobe diameter was moderately

Table 1. Histological changes in the thyroids of mice fed low-iodine diets with or without 1% DHP

Three major histological criteria of thyroid activity are graded according to the convention of Astwood (1943) as follows: − no change; + distinct change; ++ moderate change; +++ marked change; ++++ maximum change. Experimental details are given in Materials and Methods and in Figs 1 and 2. The histological changes are illustrated in Figs 3–9

Diet	Histological change	Time of killing (weeks)									
		Young mice					Adult mice				
		2	6	9	11	12	5	7	9	12	14
Low iodine	Colloid loss	−	−	−	−	−	−	−	−	−	+
	Follicle enlargement	−	−	−	−	−	−	−	−	−	±
	Cell enlargement	−	−	±	±	±	−	±	±	+	+
Low iodine plus 1% DHP	Colloid loss	+	−	+	+++	+	++++	+++	+++	++++	+++
	Follicle enlargement	−	++	++	+++	+++	++	++	+	+++	++
	Cell enlargement	++	++	++++	++++	++	++++	++++	+++	++++	++++

increased (up to 1·3 mm). Histologically, the cells lining the follicles were moderately enlarged. It was concluded that DHPOG is goitrogenic when given by mouth.

Histological Observations on Other Organs

The skin sections from the mice fed diets containing mimosine showed inactivity or atrophy of some hair follicles, as described by Hegarty *et al.* (1964*b*) in sheep. Similar but milder changes were found in mice fed DHP or DHPOG. There were focal infiltrates of lymphocytes in the portal tracts of the livers of the mice fed DHPOG, but no abnormalities were found in any of the other organs examined including the bone marrow.

Experiments to Determine Mode of Action of DHP

(i) *Effect of DHP on radioiodide uptake*

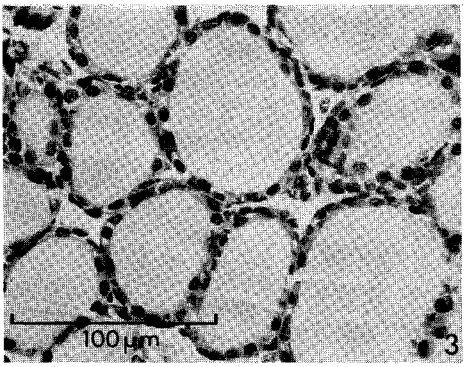
It is well established that antithyroid agents inhibit the uptake of radioiodide by the thyroid gland (Arnott and Doniach 1952). It was therefore necessary to determine whether DHP also showed this effect, and if so, to compare its activity with that of a typical phenolic antithyroid compound such as resorcinol. In these experiments each rat received 0·25 mmol of one of the test substances in aqueous solution by stomach tube in 1 ml (for DHP and resorcinol) or 2·5 ml (for mimosine Na salt). The control rat received 1·0 ml of 0·9% NaCl solution only. The results are shown in Table 2. Mimosine induced a slight inhibition of iodide uptake, but this was not significant. The inhibitory activity of DHP was greater than that of resorcinol at the same dose level.

(ii) *Effect of DHP on organic binding of iodine*

Some antithyroid agents such as KSCN inhibit the uptake and concentration of iodide by the thyroid gland and can cause discharge from the gland of any iodine which is not organically bound (Vanderlaan and Vanderlaan 1947). In contrast, antithyroid drugs such as resorcinol and thiouracil inhibit the organic binding of iodine but not the trapping mechanism. To determine to which group DHP belongs, the following experiment, based on that of Bachelard *et al.* (1963), was carried out. This experiment determines if the test substance behaves like KSCN in causing a discharge of trapped iodide from the thiouracil-inhibited gland, or like thiouracil in that its maximum inhibitory effect does not prevent KSCN causing a discharge of trapped iodide.

The several substances (*A*, Table 3) were administered in 1 ml of 0·9% NaCl, by subcutaneous injection of 0·25 ml at four different sites, in the following doses: DHP, 0·25 mmol; 6-methyl thiouracil, 0·03 mmol; KSCN, 0·05 mmol. One hour later all rats received ^{125}I (37 kBq in 1 ml) intravenously. After a further 1 h the rats were injected with the appropriate solutions (*B*, Table 3), and 1 h later (3 h from the start of the experiment) the rats were killed and the thyroids removed, processed and counted as previously described.

As the test substances were administered at dose levels which gave maximum effects on the thyroid gland, the results (Table 3) show that thiocyanate discharged as many counts from the DHP-inhibited gland as from the thiouracil-inhibited gland, indicating in each case that the iodine taken up by that gland was not organi-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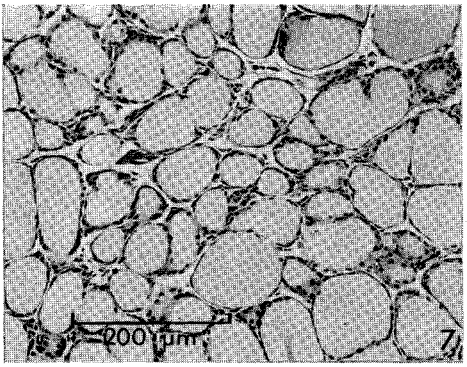
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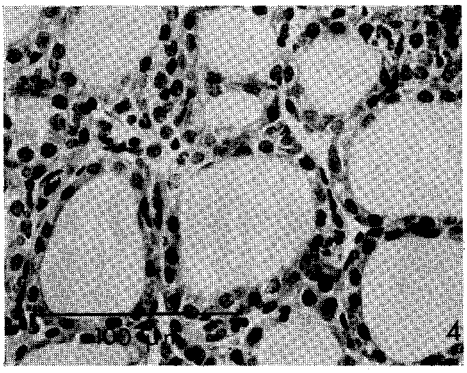
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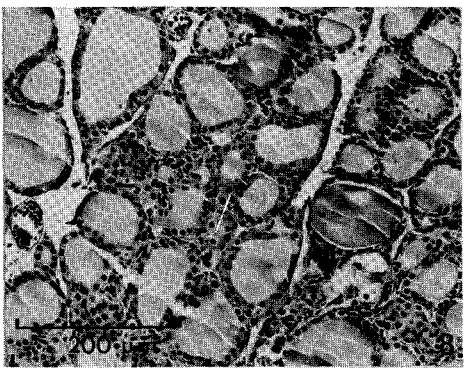
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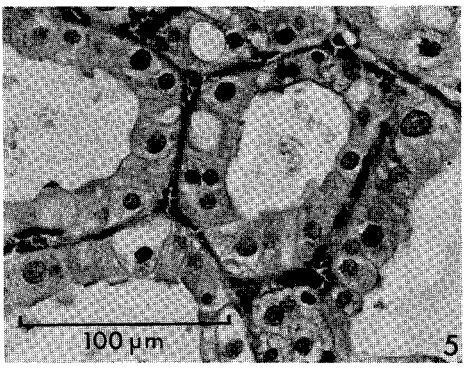
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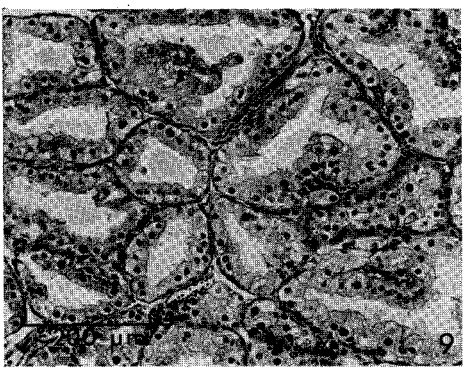
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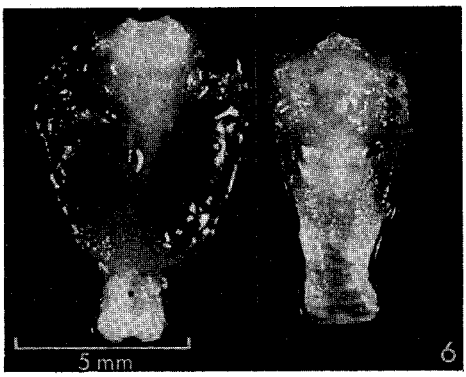
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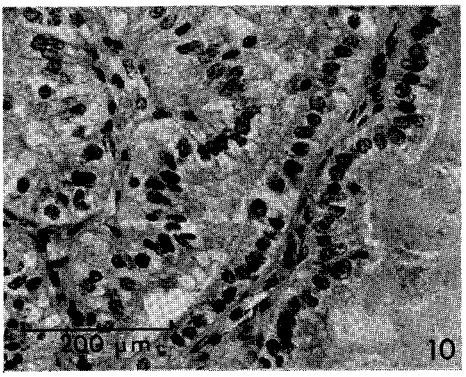
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cally bound. Unlike KSCN, DHP did not discharge a significant number of counts from the methyl thiouracil-inhibited gland. It was therefore concluded that the antithyroid action of DHP was similar to that of thiouracil and other thioureylene type drugs, but its activity was of a lower order than that of methyl thiouracil under our experimental conditions.

(iii) *Effect of DHPOG on radioiodide uptake*

In view of the reported 10-fold reduction of the activity of propyl thiouracil as a result of conjugation (Lindsay *et al.* 1974), an attempt was made to evaluate the antithyroid activity of DHPOG, the major metabolite of DHP, relative to the free compound. Experiments were carried out as described above (0·25 mmol of the conjugate intragastrically, 37 kBq ^{125}I intravenously 2 h later, rats killed at 3 h). The mean uptake of ^{125}I for the thyroids of the 12 treated rats (4068 ± 1738 cpm) was significantly lower ($P < 0{\cdot}001$) than the uptake of the 16 control rats (7532 ± 1440 cpm), indicating a 49% inhibition. In other experiments the conjugate and the ^{125}I were injected intravenously at the same time, and the rats were killed 1 h later. The time was decreased to limit possible hydrolysis of the conjugate in the blood or tissues. Under these conditions the mean iodide uptake by the thyroids of the four treated rats was less than half (47%) that of the four controls. A direct comparison with unconjugated DHP under the same conditions was not possible because 0·25 mmol of DHP injected intravenously caused convulsions and death within 10 min. The results suggest that the conjugate has some activity in the systemic circulation.

(iv) *Radioiodide uptake after chronic administration of DHPOG*

Although a single dose of an effective goitrogen inhibits iodide uptake by the thyroid of a normal animal, the hyperplastic thyroid after persistent administration

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Fig. 3. Thyroid from young mouse fed the high-iodine diet and killed 11 weeks after the start of the experiment. The gland is shown in Fig. 6. The appearances are normal. Stained with haematoxylin and eosin.

Fig. 4. Thyroid from young mouse fed the low-iodine diet for 11 weeks, showing slight increase in height of the epithelial cells, but preservation of the normal size and colloid content of the follicles. Stained with haematoxylin and eosin.

Fig. 5. Thyroid from young mouse fed the low-iodine diet plus 1% DHP for 11 weeks, showing hyperplastic epithelium and colloid deficiency. The gland is shown in Fig. 6. Stained with haematoxylin and eosin.

Fig. 6. Anterior view of larynx and thyroid of mouse of Fig. 3 (right), and of Fig. 5 (left).

Fig. 7. Thyroid from adult mouse fed the high-iodine diet, and killed 9 weeks after the start of the experiment. The appearances are normal. Stained with haematoxylin and eosin.

Fig. 8. Thyroid from adult mouse fed the low-iodine diet for 12 weeks, showing a slight increase in the height of the epithelium, but the follicles are normal in size and colloid content. Stained with haematoxylin and eosin.

Fig. 9. Thyroid from adult mouse fed the low-iodine diet plus 1% DHP for 12 weeks, showing hyperplastic epithelium and colloid deficiency. Stained with haematoxylin and eosin.

Fig. 10. Thyroid from heifer which grazed leucaena for 24 weeks, showing extreme hyperplasia of the epithelium and deficiency of colloid. Stained with haematoxylin and eosin.

of the goitrogen takes up iodide more avidly than the normal gland (Vanderlaan and Vanderlaan 1947; Bull and Fraser 1950). This method was applied to obtain further evidence for the antithyroid activity of DHP. Four groups of five adult mice were fed for 4 weeks either on the high-iodine diet, the low-iodine diet, the low-iodine diet plus 3% DHPOG, or the low-iodine diet plus 0·05% (w/v) 6-methyl thiouracil in the drinking water. After 4 weeks each mouse received 37 kBq of ^{125}I in 0·2 ml of isotonic saline intraperitoneally. One hour later the mice were killed, and the thyroid glands removed and prepared for counting. Table 4 shows that the groups receiving DHPOG and methyl thiouracil showed higher counts than the control groups.

Table 2. Effect of mimosine, DHP and resorcinol on uptake of ^{125}I by the thyroid gland in rats

Experimental details are given in Materials and Methods. Thyroid uptake was measured at 3 h after each rat had received the test compound at zero time and ^{125}I at 2 h. The cpm values were transformed to the square root scale because variances were heterogeneous. The pooled standard deviation for the transformed scale was 14·7. Two means which do not have a letter in common were significantly different at $P < 0·01$ on the transformed scale. Body weights of the rats did not influence the results

No. of rats	Test compound	Thyroid uptake of ^{125}I (cpm ± s.d.)	Mean cpm (square root scale)
5	Nil (control)	11 164 ± 2 534	104·9[a]
4	Mimosine, 0·25 mmol	10 072 ± 3 057	98·6[a,b]
5	Resorcinol, 0·25 mmol	5 934 ± 1 365	76·2[b]
5	DHP, 0·25 mmol	2 293 ± 929	47·0[c]

Table 3. Mode of action of DHP on the thyroid gland in rats

Solutions *A* and *B* were injected subcutaneously at 0 and 2 h respectively and contained 30 μmol methyl thiouracil (MT), or 50 μmol KSCN, or 250 μmol DHP in 1 ml 0·9% NaCl. NaCl indicates 1 ml of 0·9% solution only. ^{125}I (37 kBq in 1 ml of 0·9% NaCl) was injected intravenously at 1 h. Rats were killed at 3 h. The cpm values were transformed to a log scale because the variances were heterogeneous. The pooled standard deviation for the transformed scale was 0·12. Any two means which do not have a letter in common were different at $P < 0·001$ on the transformed scale. The body weights did not influence the results

No. of rats	Body wt (g) ± s.d.	Test solutions *A*	Test solutions *B*	Whole thyroid cpm ± s.d.	Mean cpm (log scale)
4	161 ± 10	NaCl	NaCl	19 594 ± 8 254	4·24[a]
4	154 ± 5	MT	NaCl	2 283 ± 625	3·34[b]
4	151 ± 7	DHP	NaCl	3 073 ± 972	3·46[b]
4	153 ± 5	MT	KSCN	401 ± 258	2·59[c]
4	156 ± 9	DHP	KSCN	1 105 ± 364	3·02[d]
4	156 ± 10	MT	DHP	2 162 ± 1 054	3·27[b,d]

Toxicity of DHP

Mice were maintained for a prolonged period on a diet containing 1% DHP and showed only reduced weight gain and goitre whereas 1% mimosine kills mice within 2 weeks (Lin and Ling 1961). In a limited experiment to test the acute toxicity of DHP, a dose of 4 g kg^{-1} body weight (36·0 mmol kg^{-1}) of DHP was given intra-

gastrically to four mice, and a dose level of 2·4 g kg^{-1} (21·6 mmol kg^{-1}) to two mice. All mice died within 12 h. At a dose level of 1·6 g kg^{-1} (14·4 mmol kg^{-1}) one of two mice died within 12 h while the other was alive although weak at 24 h. No histological lesions were found in the heart, kidney, skin, thyroid or lung in these mice. Some enlargement of hepatocyte nuclei was observed, but there was no other abnormality in the liver. A rat weighing 200 g died within 24 h after a dose of 2·5 g kg^{-1} (22·5 mmol kg^{-1}) of DHP intragastrically, but another rat given 1·5 g kg^{-1} (13·5 mmol kg^{-1}) recovered, and showed no long-term ill effects.

The dose level of DHP required for inhibition of iodide uptake in the acute experiments in rats was well below the level of general toxicity. The standard dose for a rat weighing 150 g was 0·25 mmol (1·67 mmol kg^{-1}). This dose was well tolerated when given intragastrically but killed the rats when given intravenously. The rats survived the same dose of DHP given as DHPOG intravenously.

Table 4. Effect of chronic administration of DHPOG and 6-methyl thiouracil (MT) on the uptake of ^{125}I by the thyroid glands in female Swiss mice

After 4 weeks on the diet the thyroid uptake of ^{125}I of the mice was measured 1 h after administration of ^{125}I intraperitoneally. The cpm values were transformed to a log scale to equalize the variances. The pooled standard deviation on the log scale was 0·13. Any two means which do not have a letter in common were different at $P < 0·001$ on the transformed scale

No. of mice	Diets	Thyroid uptake of ^{125}I (cpm ± s.d.)	Mean cpm (log scale)
5	High iodine diet	15 820 ± 3 186	4·19[a]
5	Low iodine diet (LID)	20 947 ± 8 780	4·29[a]
5	LID + 3% DHPOG	62 273 ± 20 708	4·77[b]
5	LID + 0·05% MT	73 792 ± 11 772	4·86[b]

Discussion

Some of the toxic effects shown by cattle and sheep grazing leucaena, notably hair loss, poor weight gain, low fertility and abortion, have now been experimentally reproduced by administration of mimosine, which is present in large quantities in the seed and foliage of the plant. However, the development of goitre in animals grazing leucaena has not been reproduced in the laboratory. The initial experiments reported in this paper were designed to determine whether or not an antithyroid action of mimosine could be demonstrated. In the experiments in which mimosine was fed to mice, one of the diets was very low in iodine, in order to maximize any goitrogenic action, but goitre was not produced, and there was no histological evidence of hyperplasia of the thyroid. Furthermore, mimosine did not significantly affect the uptake of ^{125}I by the thyroid (Table 2). We therefore concluded that mimosine itself cannot be responsible for the development of goitre in the cattle and sheep grazing leucaena.

Detailed evidence to be presented in a subsequent paper indicates that most of the mimosine ingested by cattle grazing leucaena is broken down by ruminal flora to DHP. Only traces of mimosine are present in the blood, but the blood levels of DHP and of DHPOG are high. It was therefore of importance to study the effects of the DHP on the mammal, both ruminant and non-ruminant.

The mouse feeding experiments with DHP showed that this compound is goitrogenic, and that its activity is such that even a high iodine intake cannot protect the animal if a sufficiently high dose of the goitrogen is administered. This effect is observed with other antithyroid agents which affect organic binding of iodine provided that their general toxicity allows an effective dose to be given, as with therapeutically useful antithyroid drugs (Purves 1974). The findings in the mouse experiments are in agreement with those of Holmes (1976) who found that goitre in cattle grazing leucaena could not be prevented by iodine supplementation. The control mice fed the low-iodine diet showed minimal changes in thyroid size and histological appearance. This is in agreement with the results of Riesco *et al.* (1976) who fed the same low-iodine diet to rats and attributed the suppressed thyroid response of the animals to the high casein content of the diet.

The tracer experiments showed that a single dose of DHP significantly depressed uptake of ^{125}I by the normal thyroid (Table 2) and that persistent administration increased the avidity of uptake of iodide by the thyroid (Table 4) as has been shown by other workers with resorcinol and methyl thiouracil. The antithyroid activity of DHP as determined in our experiments was intermediate between that of resorcinol and that of methyl thiouracil. The action of DHP within the thyroid is on the binding function, as with phenolic and thioureylene antithyroid agents. Further experiments are in progress to determine whether DHP, like the previously mentioned antithyroid agents, exerts its inhibitory effect on the thyroperoxidase enzyme system (Taurog 1970; Lindsay *et al.* 1974). As DHP has not previously been known to have antithyroid properties, the possible activity of other hydroxy pyridines is of interest, and is being investigated.

In the ruminant grazing leucaena or in the non-ruminant fed DHP, 30–70% of the DHP in the blood is conjugated as the glucuronide. As conjugation often results in decreased biological activity (Lindsay *et al.* 1974), we tested the glucuronide for antithyroid activity. The chronic feeding experiments in mice showed that the goitrogenic activity of the conjugate was of the same order as that of the same molar quantity of the free compound. Thus the glucuronide is goitrogenic when ingested, but this does not necessarily indicate that it is absorbed from the alimentary canal without decomposition or that it has antithyroid activity in the blood. The acute experiments in the rat suggest that the glucuronide retains some antithyroid activity in the blood. Confirmation is being sought by the use of the thyroid peroxidase enzyme system of Lindsay *et al.* (1974).

The present experiments indicate that the general toxicity of DHP is low. This is in agreement with the low cytotoxicity shown by this compound in a mouse bone marrow cell culture system (Hegarty *et al.* 1978), although there is evidence that epidermoid cells may be more susceptible than mesenchymal cells *in vitro* (Ward and Harris 1976).

Hyperactivity has been reported in heifers (Falvey 1976) and sheep (Little and Hamilton 1971) fed leucaena and in the calves of cows fed the plant throughout pregnancy (Hamilton *et al.* 1968). Hyperactivity is the opposite of what would be expected from the action of an antithyroid agent. Borchardt (1973) showed that DHP irreversibly inhibited the enzyme catechol methyltransferase (CMT) *in vitro*. CMT participates in the inactivation of epinephrine and norepinephrine and its inhibition might be expected to potentiate the action of these amines (Guldberg and Marsden 1975). It is a soluble extracellular enzyme and could therefore be accessible

to circulating DHP. The inhibition of CMT by DHP may be associated with the Mg^{2+} dependence of the enzyme since DHP is a strong chelating agent (Tsai and Ling 1973). Although we did not observe hyperactivity in mice ingesting 1% DHP it is possible that CMT inhibition might produce hyperactivity or excitability in some animals when the intake of DHP is sufficiently high and prolonged. Further work is required to determine if DHP is responsible for hyperactivity in cattle.

DHP does not appear to be broken down within the body. Protection of the animal from its pharmacological actions would therefore appear to be best achieved by decreasing the amount absorbed. One approach would be to breed a strain of leucaena low in mimosine but without losing the desirable properties of the plant; this is being done at the Division of Tropical Crops and Pastures, CSIRO. A second possibility would be to modify the ruminal flora to break down the DHP. Although several strains of bacteria can synthesize DHP, only one is known which can degrade it (Watson *et al.* 1974) and this is an aerobe, and would probably not grow in the rumen. Recent studies on the catabolism of phenolic compounds in anaerobic environments (Evans 1977) suggest that while it might be possible to develop a rumen microbial community which could degrade DHP, e.g. by transfer of metabolic plasmids, it would require a major research effort.

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