CONGENITAL GOITRE IN SHEEP IN SOUTHERN TASMANIA

by

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Submitted in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy.

UNIVERSITY OF TASMANIA

HOBART

February, 1974.

(degree conferred April 1974)

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge contains no copy or paraphrase of material previously published or written by any other person except where due reference is made in the text of the thesis.

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ACKNOWLEDGEMENTS

I am indebted to my supervisors Dr. A.C. Bray and Dr. J.J. Yates, Senior Lecturers in Animal Production and Agronomy respectively, Faculty of Agricultural Science, for their help and guidance throughout this study.

I express my thanks to Dr. L.P. Jones, Lecturer in Agronomy, Faculty of Agricultural Science, for assistance in experimental design and statistical analysis and to Dr. K.C. Marshall, Reader in Microbiology, Faculty of Agricultural Science, for constructive criticism of the work in this thesis.

I wish to thank Messrs. H.R.C. Edgell and G.B. Edgell of Dennistoun, Bothwell for allowing me use of animals, and part of their property, for experimental work, and assistance throughout the course of the project.

To members of the Technical staff of the Faculty of Agricultural Science, I extend my appreciation for assistance, particularly to Mr. I. Cameron for help in field work and experiments in the Animal House.

Financial support was provided by the Australian Meat Research Committee and to them I am deeply grateful.

CONTENTS

SUMMARY

Iodine deficiency goitre has been quite common in the human population of Tasmania since the turn of the century, and it has also been found in horses and other farm animals. The recent studies of Clements and associates on schoolchildren, led to the conclusion that the condition was not a simple iodine deficiency, and goitrogenic agents were involved.

The first outbreak of congenital goitre in sheep was recorded in 1945. Since then, severe outbreaks have been recorded in 1956, 1964 and 1968. Features of congenital goitre in sheep were that it was sporadic in nature, associated with particular farms, and there were often marked differences in the incidence of the disease between ewe flocks on the one farm.

From a survey conducted amongst Department of Agriculture veterinarians we found that congenital goitre of sheep was mainly confined to the Derwent valley and the Northern midland areas of the state.

The studies reported here cover the results of a grazing trial established on a property near Bothwell where goitre was endemic, and the results of associated animal house and plant growth trials.

The soil type on which pastures were grown was found to be a major factor in the disease, in that ewes grazing pasture grown on a sandy soil had a much higher incidence of goitrous lambs than ewes grazing pastures on clay soil. Analyses of pastures grown on these soil types revealed that the sand soil pastures were lower in iodine than the clay soil pastures. However, neither pasture contained the level of iodine considered by Butler and associates as the minimum level necessary to prevent goitre. An animal house feeding trial using ewes from the grazing trial property indicated that these sheep had similar iodine requirements to those of Butler's.

Observations made by Department of Agriculture personnel and ourselves revealed that outbreaks of the disease were associated with conditions of lush pasture growth during the pregnancy period, i.e. that associated with

higher than average autumn rains. This correlation could be explained if pasture grown under conditions of liberal water availability had lower iodine levels than pasture grown when moisture limited plant growth. However, experiments with the two main pasture species (perennial ryegrass and subterranean clover) grown under conditions of varied water stress, showed that plant iodine levels were not affected by water availability.

Two goitrogenic agents, nitrate and thiocyanate, were shown to be present under field conditions. From animal house experiments and field observations, it was concluded that these substances were not important factors in the etiology of the disease. Similarly, the source of drinking water was found to be unimportant, even though there were marked differences in the iodine content of the two sources available.

It seems that between-year variation in goitre incidence and the effect of soil type can best be explained by a varying iodine intake dependent on soil ingestion which varies with pasture availability. This hypothesis is discussed in light of the results obtained.

INTRODUCTION

GOITRE IN SHEEP

An outbreak of congenital goitre in sheep, which occurred in a Department of Agriculture stocking rate trial at Bothwell in 1968, stimulated the interest of the University of Tasmania. Subsequent investigations revealed that little was known of the nature of this goitre, despite it having been an economic problem for a number of years.

The first report of congenital goitre in sheep in Tasmania was a description of an outbreak at Huonville (see Fig.1:5) by Southcott (1945), who found some 40% of a flock of two-tooth ewes (which themselves grew considerably during the year) gave birth to goitrous lambs, while lambs from older ewes on the same farm were unaffected. Green (1956) reported that since 1945 goitre in lambs had been observed in the Derwent River valley and at several places in the southern midlands. Pillinger (pers. comm. 1971) indicated that goitre in sheep appeared to be of a sporadic nature and had occurred in the Derwent valley or northern midlands in 1956, 1964 and 1968.

The disease appears to be more or less confined to areas in which human goitre occurs, and where animals remain untreated it appears to be increasing in frequency of occurrence.

GOITRE IN HUMANS

Endemic goitre in humans has been recognised as a problem in Tasmania for many years. The first assessment of the incidence and distribution of the disease appears to be the school medical reports published in 1916 and 1918 (Anon, 1916, 1918) and a paper read to the Aust. Medical Association in 1914 (Anon 1914). The 1918 survey showed that in the north of the state 0.99% of the children had goitre, while in Hobart the incidence was 0.78%

(Anon 1918). This latter figure, when compared with the 2.36% goitre incidence found in children in the southern region (Anon 1916), generally indicated that a degree of variability existed in goitre incidence in southern Tasmania. Further evidence for this was given in 1923 when it was noted that 239 of 644 notified cases of goitre in Tasmania (Anon 1923) lived in the New Norfolk area, a southern rural district (Fig.1:5 shows position of this area in relation to Hobart).

The next survey was not carried out until 1949, when it was found that goitre was widespread throughout the state, the incidence varying from a low level along the North West Coast to high levels in the south. On the basis of this survey it was recommended that school children be given 10mg KI as a weekly tablet (Clements and Wishart 1956).

A further survey 5 years later carried out by the same authors, showed an increased incidence of goitre in some areas. There was a marked change in the incidence of this disease between age groups. Young children (5-8 yr) were found to be the most affected, while the degree of the disease in older girls declined. The falling incidence of this condition in older girls was thought to be a result of the iodine medication in schools initiated in 1950, on the recommendations of the 1949 survey. The increase in goitre in young children was thought to be due to the presence of a goitrogen in milk (Clements and Wishart 1956) since a free milk scheme was introduced in Tasmanian schools in 1950, and at the same time farmers increased the amount of brassica crops being grown as dairy cattle feed. Clements and Wishart (1956) considered the increase in goitre was due to antithyroid agents present in the brassicae being passed into milk of the cows after ingestion. An outbreak of goitre in sheep in 1956 led Clements (1957) to include weeds as a possible source of goitrogenic agents. Clements (1957) stated;

"...Goitre appeared to be associated with a large consumption by the ewes in the last four to six weeks of the pregnancy of a cruciferous

> weed known locally as "carrot weed". It was subsequently shown that two species of weed were involved, the common crowsfoot *(Erodium cicutarium)* and long storkbill *(Erodium botrys)*."

He continued

> "Throughout southern Tasmania there was an extensive growth of cruciferous weeds in 1956. Although it was not possible to make a survey of farms and grazing lands in the Snug and Margate districts to determine the possible consumption of cruciferous weeds by milking cows, this seems a possible explanation for the abrupt rise in the incidence of goitre."

No cruciferous weeds other than *Erodium* are named in this paper and the inference given is that these are the same ones being considered in the latter quote. However, *Erodium* is not a cruciferous weed as it belongs to the family Geraniaceae, (Curtis 1956) a quite unrelated family, which has not been found to contain goitrogens. There is therefore no evidence to support the statements made by Clements.

A biochemical investigation was undertaken by Clements and Wishart (1956) to investigate the goitrogenic effects of milk upon both humans and rats. They found a depression of radioiodine uptake in human volunteers who were fed milk from cows grazing on chou-mollier *(Brassica oleracea)*, but the number of patients used was very low and the differences in radioiodine uptake were not large. An ethanol extract of the milk injected into rats caused a marked depression in radioiodine uptake which supported the hypothesis that milk from chou-mollier fed cows contained a goitrogenic agent.

Virtanen (1964a) and Greene, *et al.* (1958) repeating this work, but using larger test groups, found no differences in radioiodine uptake between control groups and those fed milk from cows grazing on chou-mollier. Virtanen (1964a) also showed that the results obtained were affected by fluctuations in the subjects' previous iodine intake. This author (Virtanen,

1964b) also discovered that the effects of injections of ethanol extracts of milk on radioiodine uptake in rats was mainly due to the milk salts extracted by the alcohol. Pure mixtures of these salts when injected produced a similar depression of iodine uptake to that of the alcoholic extract. He also showed that the amounts of the antithyroid compounds thio-cyanate and L-5 vinyl-2-thio-oxazolidone, which were found in cows milk after the animals had been fed known amounts of the purified compounds, were too low to cause thyroid disfunction in humans. Arstila *et al.* (1969) and Krusius and Peltola (1966) however, dispute this, as they report levels of L-5-vinyl-2-thio-oxazolidone in milk, from cows grazing cruciferous weeds, in sufficient concentrations to cause goitre in rats, and possibly also cause goitre in humans.

To increase the general iodine intake of the Tasmanian population, mass medication was undertaken in 1966 by adding potassium iodate to bread, to give a final concentration of 2-4 ppm (D.W.) iodine. Surveys of bread consumption have shown that this provides a mean iodine intake ranging from 80 to 270 µg/day, depending upon age and sex of the individual. This scheme has reduced the incidence of goitre in school children to that of a non-goitrous area (Clements, Gibson and Howler-Coy 1970). The introduction of a new form of dairy sterilant which contains up to 2% iodine has resulted in a significant increase in the iodine content of milk (Connolly 1971), with consequent increases in such milk products as cheese, chocolate and ice-cream. These would also tend to reduce the level of goitre in the population, particularly in children.

The evidence for dietary goitrogens acting on the human population is tenuous, but cannot be entirely discounted as unexplained rises in goitre incidence were found in the surveys of school children (Clements, Gibson and Howler-Coy 1968), particularly in northern Tasmania between 1949 and 1954. It seems that if dietary goitrogens are involved they are of the thiocyanate type, since mass medication has reduced the incidence of the

disease to that of a non-goitrous population.

SHEEP IN TASMANIA

Sheep were first introduced into Tasmania in the early 1800's and the numbers expanded rapidly to reach 1.8 million in 1864. Population growth declined in the latter part of the century and it was not until 1929 that the 2 million mark was passed (Cumming 1969). The population increased until 1970 when there were 4.6 million sheep, but with a recent fall in wool prices this number had declined to 4.2 million by 1972 (Comm. Bureau Census and Statistics 1972).

The major breeds and their approximate proportion of the flock are Polworth (40%), Corriedale (18%), Crossbred (18%), Comeback (10%), Merino (8%) and British Breeds (6%).

The sheep carrying area of the state (Fig.1:4) can be divided into two, the predominantly wool area and the fat lamb area.

The wool growing area is in general the area of low rainfall and high evaporation, and in fact occupies very closely the area in which evaporation is greater than precipitation (Fig.1:3). Fat lambs are produced mainly in the high rainfall areas in the north and to some extent in the south east.

TASMANIA : GEOGRAPHY AND CLIMATE

Tasmania is an island of 67,897 sq. km , latitude 40-44^{o}S. Physically it is a part of the Eastern Australian Highlands and this is reflected in the north-south direction of the major mountain ranges (Fig.1:1). It has a temperate marine climate which tends to produce abnormally mild winters and cool summers for the latitude.

The mean January temperature varies from about 10^{o}C in the central highlands to 18^{o}C around the coast and in the midlands. The mean July

temperatures vary from 10°C along the coast to 1°C in the highlands (Langford 1965). Above 600 m the winter months are too cold to allow growth of agricultural crops.

Annual evaporation (measured from a free water surface in a sunken tank) is fairly uniform in the low level country of the central north, east and south east, being between 78 and 86 cm. In the west and south west the evaporation drops to less than 50 cm (Langford 1965).

Rainfall is distributed throughout the year, but there are relatively dry periods in late summer and late winter and relatively wet periods in late autumn and late spring. As the Westerly winds are the dominant feature of air movement over the State the rainfall pattern is characterized by concentration of rainfall over the western highlands and to a lesser extent over the north-eastern highlands (Langford 1965). This results in a rain shadow in the central part of the state, (Fig.1:2) and there is quite a large area of agricultural land in which annual evaporation is greater than annual precipitation (Fig.1:3) (Buckney 1971). The rain falling on the highlands is rapidly returned to the sea by extensive river systems draining both to the south and west.

The soils of Tasmania are generally infertile by world standards. Most of the soils are moderately to strongly leached and acidic, at least in the surface horizons. The most extensive soils are podzolics, then kraznozems on basalt and moor peats in highland areas with restricted drainage (Nicolls and Dimmock 1965).

Glacial and periglacial activity in Tasmania only occurred in the highland areas and had little or no effect in the regions below 450 m (Derbyshire *et al.* 1965).

The low rainfall and acid, leached soil in the lowland areas, and recent glaciation in the western highlands, are all features which are considered (Chilean Iodine Education Bureau 1956) to give rise to soils of low iodine content.

Fig. 1:1

RELIEF

(Davies 1965)

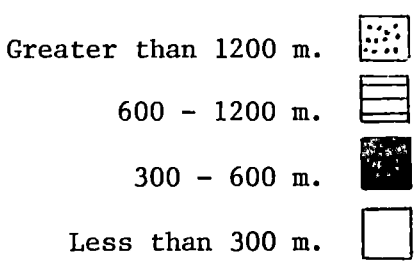
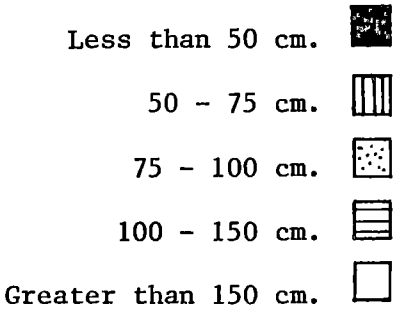


Fig. 1:2

ANNUAL RAINFALL

(Buckney 1971)



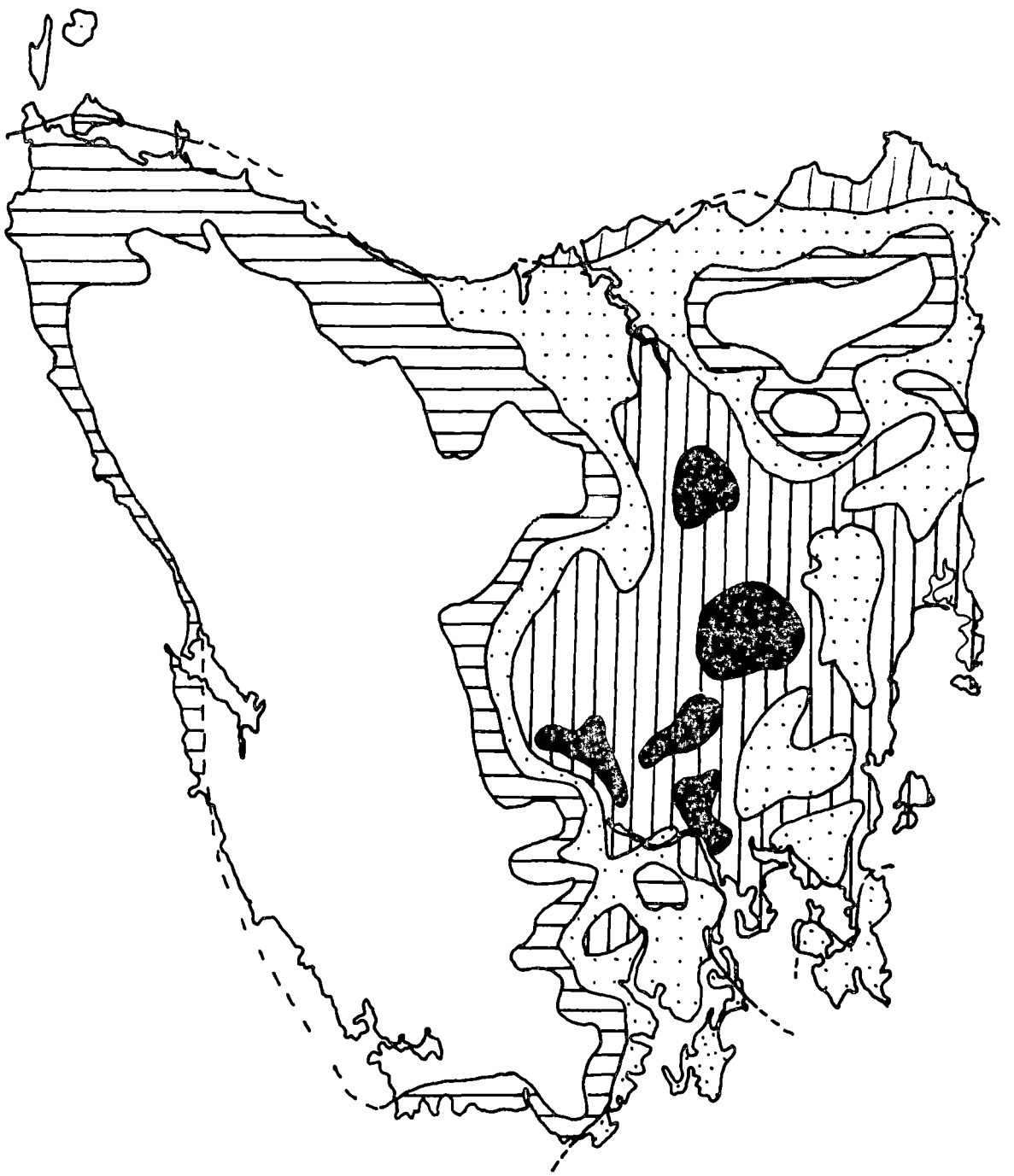


Fig. 1:3

Approximate minimum area for which annual evaporation is greater than or equal to annual precipitation. (Based on mean July temperature)

(Buckney 1971)

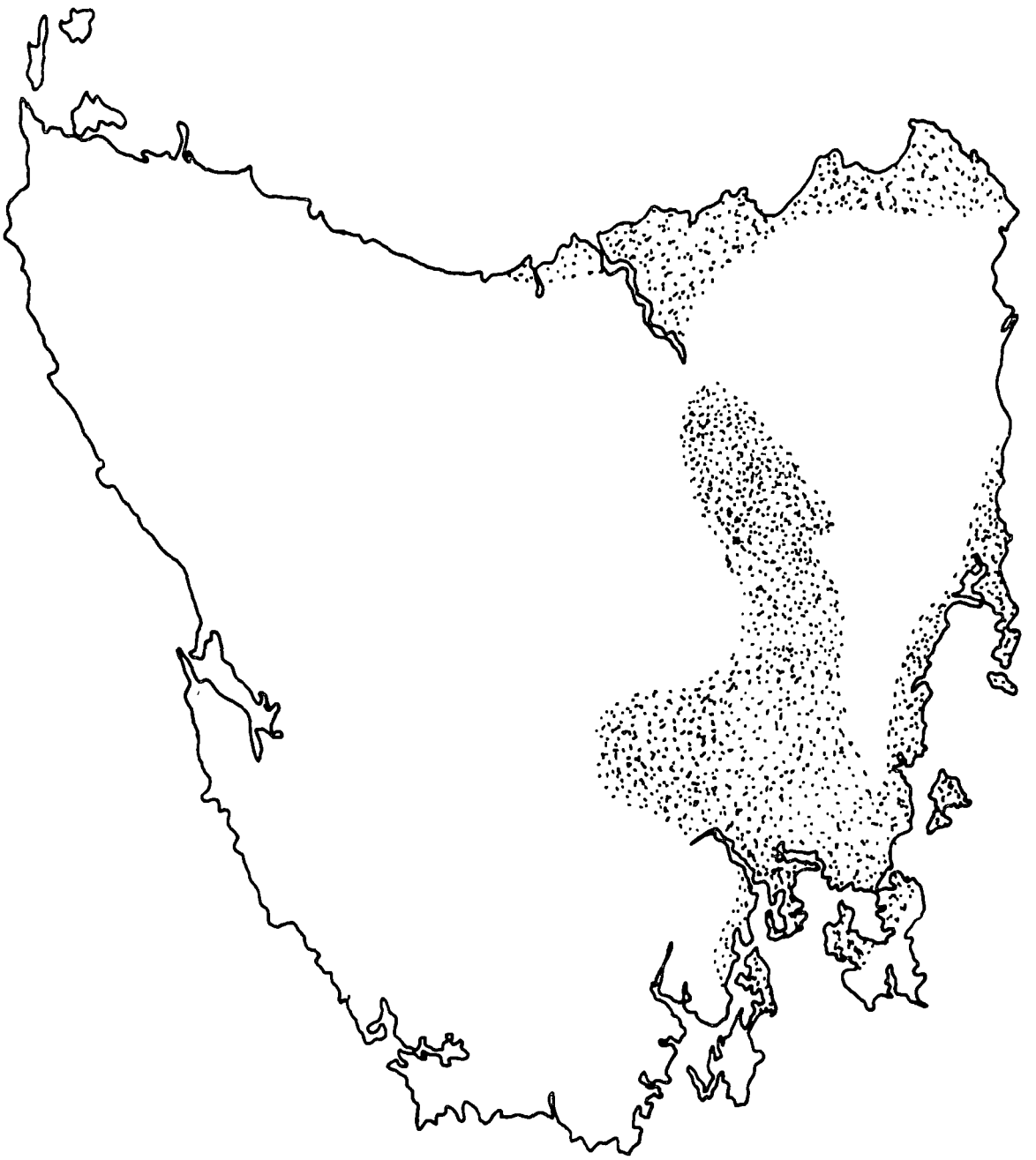
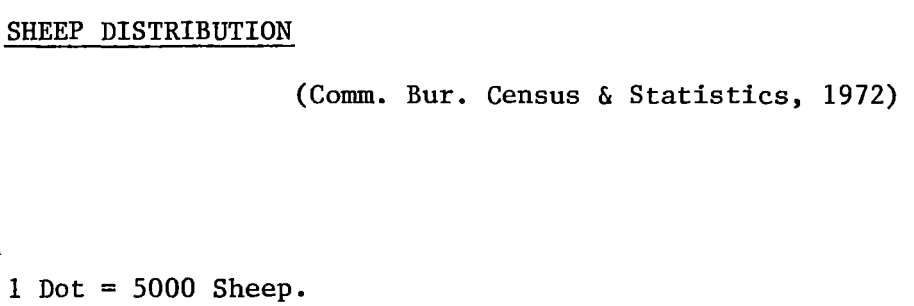


Fig. 1:4


SHEEP DISTRIBUTION
(Comm. Bur. Census & Statistics, 1972)
1 Dot = 5000 Sheep.

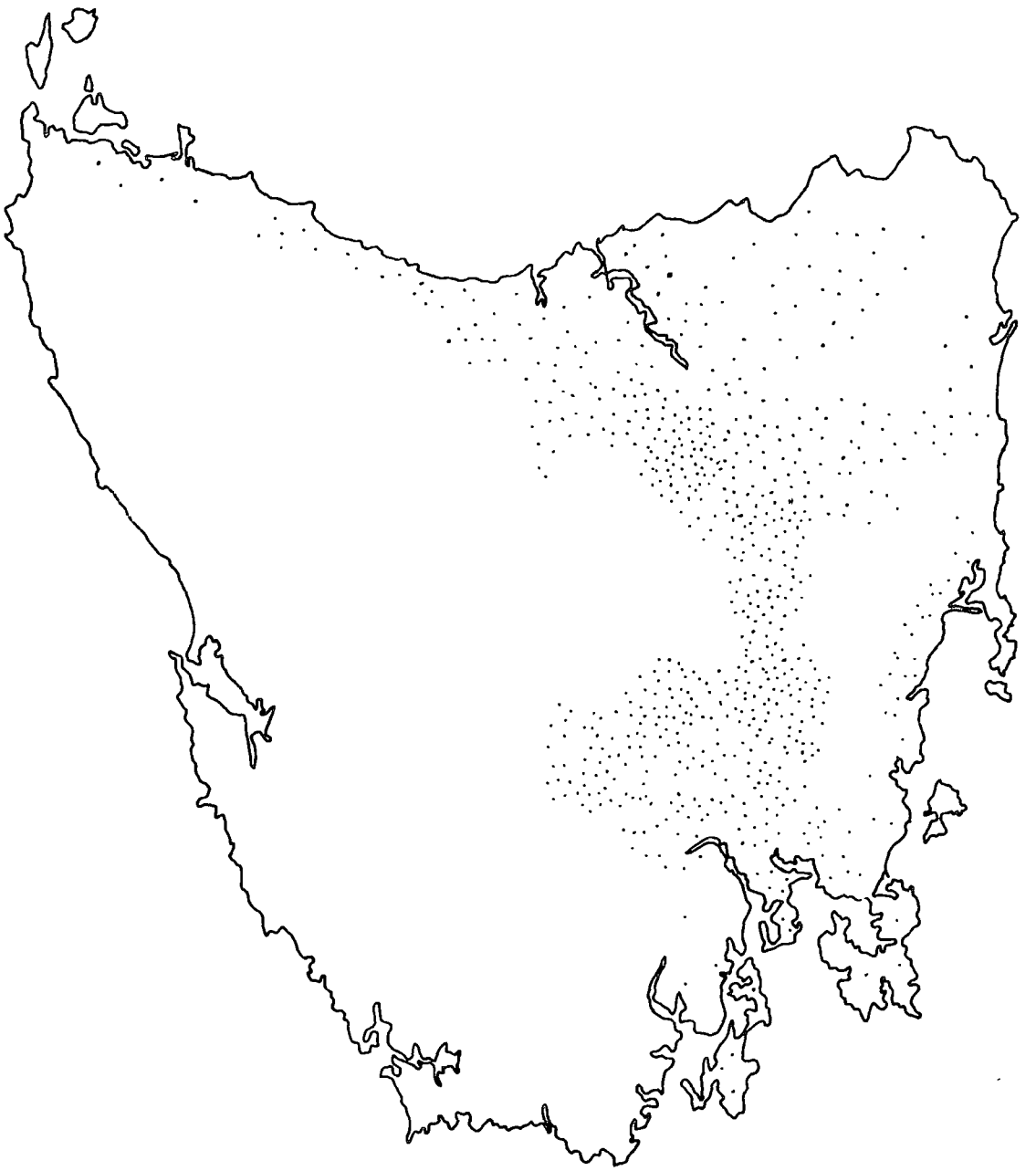


Fig. 1:5

TASMANIA GEOGRAPHY


Smithton
Burnie
Devonport
Scottsdale
Launceston
Westbury
Longford
Fingal
Campbell Town
Ross
Queenstown
Oatlands
Bothwell
Ouse
Hamilton
Derwent R.
Sorell
New Norfolk
HOBART
Huonville
Huon R.
0
50
Km

LITERATURE REVIEW

INTRODUCTION

In 1921 Marine and Kimball (1921) wrote:

"Simple or endemic goitre most commonly develops during (1) foetal life, (2) around the age of puberty and (3) during pregnancy, and we believe that any plan which provides for its control during these three periods of life will practically eliminate endemic goitre."

However, some 50 years later endemic goitre is still a problem in some areas.

It is generally considered that endemic goitre may be caused by insufficient dietary iodine or by an agent which interferes with some stage of the iodine cycle within the body. This agent may be a chemical or an inherited metabolic defect. Iodine may be supplied to an animal in either food or water, and at least in grazing animals such as sheep, soil must also be considered as a direct source of microelement supply, although it is generally considered that grazing animals usually obtain the major proportion of their iodine requirements from plant material.

The minimum iodine requirement for sheep has been estimated by various authors, Albritton (1954) suggested that for a safe margin mature pregnant sheep required 20 μg/kg body wt./day, while the Agric. Research Council (1965) recommended 800 μg/kg dry matter in the feed. One of the few estimates based on experimental evidence was that of Butler and Johnson (1957) who found that congenital goitre in lambs was associated with pasture iodine levels of less than 300 μg/kg dry wt.

PLANT IODINE

The iodine content of some species of plants appears to be less dependent on soil iodine status than on plant strain or varietal differences (Butler and Johnson 1957). Averages of iodine content of plants over the whole year for two areas show that in New Zealand ryegrass *(Lolium perenne* L.) and Cocksfoot (*Dactylis glomerata* L.) had iodine contents of 1450 and 220 µg I/kg respectively (Butler *et al* 1956), while in Wales the same two species averaged 165 and 320 µg I/kg (Alderman and Jones 1967). It would be expected that differences due to climate or soil variation would be reflected by similar variation in the two species, and it is unlikely that the differences actually found could be from these sources. It is more likely that the variation, particularly in ryegrass, was due to different strains of the same species. Variation within species has been recorded several times, Alderman and Jones (1967) found variation within ryegrass and clover seedlings to be 100-280 µg I/kg and 140-440 µg I/kg respectively, while Butler and Glenday (1962) reported ryegrass plants varying from 250 to 2470 µg I/kg. Using these plants in a diallel cross technique Butler and Glenday (1962) showed that iodine content of perennial ryegrass is a strongly inherited character with an apparent maternal effect on the inheritance.

As well as variation between plants of the same species there appears to be variation within plants depending upon the season. Simpson (1930), Butler, *et al* (1956) and Alderman and Jones (1967) all have reported higher plant iodine levels in autumn and winter than in spring and summer. These fluctuations may be related to a dilution effect on total I content by the increased growth in spring, as a dilution of plant iodine concentration has been found when growth has been increased with nitrogen fertilizer (Alderman and Jones 1967).

Some experimental evidence suggests that plants can absorb iodine

from the atmosphere. Collins and Moore (1965) enclosed untreated bean plants in plastic bags with ones which had had I^{131} added to the rooting zone. After four hours, traces of I^{131} were found in the foliar tissue of the untreated plants. Hungate *et al.* (1963), using geranium found I_2 gas sorption by leaves to be proportional to the concentration used, but only a small proportion of the sorbed iodine was translocated away from the leaves within three days. When plants were exposed to the gas at night the sorption was greatly reduced, possibly because leaf permeability was reduced through stomatal closure during this period. A much greater degree of translocation of iodine was found by Dziljanov and Aleksiev (1962) in strawberries. When 0.01% KI was used as a foliar spray, the iodine content of leaves and fruit was approximately doubled. The chemical form of the applied iodine may be important in determining the degree of translocation if these two results are to be compatible.

To test whether plants could obtain iodine directly from the air, Shacklette and Cuthbert (1967) sampled Spanish moss (*Tillandsia usneoides*), a common flowering epiphite in the southern U.S.A. The mature plant derives all of its nutritive elements, and water, from the air. The range in iodine concentration found in this species was 4,000-7,000 μg I/kg compared with 4,300-7,100 μg I/kg in grasses in the same area. Three sources for this iodine seem possible, iodine vapour, rainwater or dust particles.

Although it appears that some iodine is absorbed through the foliage, the greatest bulk of research on iodine nutrition of plants has centered around uptake by the roots.

The form in which iodine is supplied, either by soil or nutrient solution, determines the rate of uptake into the plant. In barley, Umlay and Poel (1971) found that the uptake of iodine by plants was greatest with potassium iodide, followed by iodoacetic acid, potassium iodate and then potassium periodate (Table 1:1), when all were added to the nutrient sol-

TABLE 1:1

(From Umlay and Poel 1971)

Oxidation State and Molecular Wt. of I Compounds

Compound	Oxidation state of I	M.W.
Potassium iodide	-1	166.01
Iodoacetic acid	+1	185.96
Potassium iodate	+5	214.01
Potassium periodate	+7	230.01

ution to the same iodine concentration. It was suggested that either the compounds of lower oxidation state were absorbed more readily, or the heavier, higher valency forms may have had a time lag in reduction to iodide before being absorbed. Borst Pauwels (1962) obtained similar results when using potassium iodide and potassium iodate with oats, but he found a negative, quantitative relationship between iodine content of the oat roots and root growth rate with higher levels of iodine (2.25 ppm KI and 6.75 ppm KIO_3 in the nutrient solution), the effect of both forms of iodine being similar. This was thought to indicate that iodide and iodate, after being absorbed by the plant had the same effects on growth.

A different theory has been proposed by Boszormenyi and Cseh (1960) who found, that after three days in nutrient solution containing $KI^{131}O_3$, the iodine present in wheat roots was almost completely in the form of I^-. They suggested that there was a change in oxidation state of the iodine either before, or very soon after it entered the plant root. In lettuce plants, Cowan and Esfahni (1967) reported that I^{125} taken up from a nutrient solution was present as inorganic unbound iodide, which appeared to be in the cell sap since it was readily extracted.

The role of iodine in plant metabolism is still confused, it is not considered to be an essential element for plant growth, although at low levels it may have stimulatory effects on some plants. Some of the stimulatory effects reported are, greater height and weight of barley (Umlay and Poel 1971), accelerated stem growth and fruit yield of tomatoes (Lehr, Wybenga and Rosanow 1958), advanced earing of oats and wheat, and dry weight increases in tomato, fodder beet, white clover and ryegrass (Borst Pauwels 1961).

Symptoms of iodine toxicity in barley and tomato are general chlorosis, yellow interveinal patches and brown necrotic spots which tend to form streaks with later death of some leaves (Umlay and Poel 1970). In rice, the symptoms are generally similar and the disorder known as Kaiden-Akagare

disease has been identified as iodine toxicity (Tensho and Yeh 1970).

The levels of iodine which produce stimulatory or toxic levels in plants are dependent on the plant species, form of iodine and rooting medium. The Chilean Iodine Education Bureau (1950) from an extensive review of the earlier literature has concluded that:-

a) In plant water cultures

50 ppm appeared to be generally toxic

0.125 - 50 ppm depressed growth

0.1 - 0.125 ppm improved growth

b) In soil (applied iodine)

10 ppm harmful

5 - 10 ppm may be harmful

0.1 - 5 ppm was beneficial or had no effect

The use of soil as a root medium makes evaluation of iodine nutrition difficult, since the amount of iodine naturally available in the soil is rarely measured and availability would vary with the soil type.

SOIL IODINE

It is generally considered that most iodine accumulated in plants is derived from the soil. The origin of soil iodine, however, is less well defined. Some authors (Benson and Carter 1927; Remington, Culp and von Kolnitz 1929) consider that the geological origin of the soil is of major importance in determining the amount of iodine which a soil contains, while others (Shacklette and Cuthbert 1967; Goldschmidt 1954) believe that rain, containing iodine from the sea, is a much more important factor in soil iodine accumulation than nature of the bedrock.

The only geological action which has been generally agreed upon as having a marked effect on soil iodine is glaciation. Glacial activity may completely strip the soil strata, and soil formed after glaciation is often iodine deficient.

As the iodine content of soil is generally higher than that of the parent rock (Table 1:2) it appears unlikely that soil iodine would accumulate to such an extent as an insoluble residual component of weathered primary rock (Goldschmidt 1954).

Goldschmidt (1954) reported an iodine content of rain of 1-3 µg/litre in Switzerland, whilst Miyake and Tsunogai(1963) measured it as 1.7 µg/litre in Japan. The Chilean Iodine Education Bureau (1956) considers that, at 1 µg I/litre, the amount of iodine added to an area annually by rain would be insignificant. However, on the basis of 1 µg I/litre of rain, the amount of iodine derived from rain in some of the Tasmanian goitrous areas where rainfall is only 460 mm/yr would be approximately 4.6 g/ha/yr. In these areas an average yield of 4900 kg/ha of clover-grass hay can be expected (Anon.1973), and if the crop absorbed all of the iodine from the rain, it would have an iodine content of 940 µg/kg, which is approximately 3 times the iodine content necessary to prevent goitre in sheep (Butler and Johnson 1957). This example serves to demonstrate that, under conditions of iodine deficiency, the amount supplied by rain could be quite important in the balance of soil and plant iodine.

Miyake and Tsunogai (1963) working on irradiation of sea water and measurement of gases produced, estimated that the total escape of iodine from the sea was 4×10^{11} g/yr. Iodide ions were shown to be oxidised to free iodine in sea water under the influence of light up to a wavelength of 560 nm. Free iodine released is thought to be attracted to dust particles which later form nuclei for rain drops (Goldschmidt 1954).

Factors other than rain and bedrock which are important in altering soil iodine, either by accumulation or depletion are vegetation, soil

TABLE 1:2

(From Chilean Iodine Education Bureau 1956)

Iodine Content (µg/kg) of bedrock and derived soil
(mean of all available published date to 1955)

Parent material		Derived soil
Igneous rock	521	9338
Sedimentary rock	1545	3850
Metamorphic rock	1612	5312

texture, soil pH and depth.

Vegetation may assist in increasing soil iodine by absorbing gaseous iodine or iodine from rainwater and returning it to the soil when the plant dies. Humus formed from dead plant tissue is considered to be the most important soil factor affecting iodine retention. Jee and De (1967) added humic acid to a range of soils in proportions varying between 5% and 40%. The soils were held in a moist condition for three months, then iodide was added to them and after two hours the unabsorbed iodide was measured. In all cases the soil alone had negative adsorption, with some iodine being released while the soil was in a moist state. With the addition of humic acid, some absorption occurred, reaching a maximum at 20-40% humic acid depending on the soil.

Raja and Babcock (1961) found that the effect of organic matter was a direct reaction, and microbial activity did not appear to be involved. Several authors (e.g. Remington, Culp and von Kolnitz 1929; Itano and Tuji 1934) have reported that clay soil contains more iodine than sand, and a survey of published figures by the Chilean Iodine Education Bureau (1956) shows that soil iodine levels are correlated with the proportion of clay in a soil (Table 1:3). The difference is due only in a small part to the clay itself, as Raja and Babcock (1961) discovered that less than 10% of added radioiodine was fixed by clay. From a soil chemistry point of view such a result would be expected because the general negative charge on the clay would tend to repel negatively charged iodide and iodate ions. At low pH iodide is bound to oxides of iron and aluminium which are sorbed to clay particles (Whitehead 1973), probably through hydroxyl groups originating from broken bonds in these oxides, as other anions have been shown to be bound in this manner (Bear 1964). These anions are readily exchangeable at pH values found in cultivated soils and are easily lost by leaching. Anions have also been found to bind to clay particles by replacement of hydroxyl groups in the clay lattice. Under these conditions the ion is

TABLE 1:3

Iodine level of some soils (from Chilean Iodine Education Bureau (1956))

Soil Type	Mean Iodine Content (µg/kg)
Clay	7319
Clay loam	5904
Loam	3802
Sand loam	3574
Sand	2113

fixed into the clay lattice and is therefore unavailable for release or exchange with other ions (Bear 1964). If iodine were to react with clay in this manner it would not be an important soil reservoir of available iodine.

Other factors which would enhance the iodine content of clay soils are the generally greater organic matter content and impeded drainage through the clay which would allow more time for fixation of any iodine contained in the soil water and reduce the effect of leaching.

The effect of leaching on iodine content of river terraces was investigated by Hercus, Benson and Carter (1925). Those terraces which were low lying and often flooded contained much less soluble iodine than higher ones not subjected to flooding, although both were composed of river gravels and silt.

Soil pH has been shown to have a marked effect on soil iodine levels. In Japanese soils Itano and Tuji (1934) found a mean iodine level of 7.33 ppm at pH greater than 6.5, but a mean of only 4.35 ppm at pH less than 6.5. The difference is possibly due to acid soils releasing iodine more readily than alkaline soils (Hopkirk *et al.* 1930) with subsequent leaching. This variation could be related to the method of binding of iodine, as organic matter is the main iodine binding agent under alkaline conditions, and as the soil becomes more acid, oxides of iron and aluminium become more important as iodine binding sites (Whitehead 1973).

As plants are the chief concentrators of iodine in the soil, it is logical that the iodine content should decrease down the soil profile. The Chilean Iodine Education Bureau (1956) has surveyed published figures in which the depth of soil sample is specified and the summarized data is shown in Table 1:4.

The iodine content of a soil may have a direct effect on the iodine intake of grazing animals under certain conditions due to direct ingestion of soil.

TABLE 1:4

Iodine content of soil (µg/kg) at increasing depth
(source Chilean Iodine Education Bureau 1956)

Depth	Clay	Loam	Sand
Less than 45 cm	7463	3880	2158
45 - 90 cm	6724	2008	1518
Over 90 cm	5088	1695	1173

Healy (1967) reported that soil structure was important in determining the amount of soil ingested, and generally the weaker the soil structure the greater was the intake of soil (Table 1:5).

Soil ingestion is also related to the stocking rate and season of the year. Arnold, McManus and Bush (1966) found a maximum faecal silica content of 100 g/day with a stocking rate of 5 sheep/ha but at 22.5 sheep/ha the faecal silica rose to 380 g/day. Field (1964), in England, and Healy (1967) showed that the maximum soil intake occurred during winter when feed availability was lowest, and Healy suggested that a sheep could ingest up to 400 g of soil per day. This amount of ingested soil could obviously be important in supplying the animals requirements for microelements, and it was suspected that soil intake was a major factor in the development of congenital goitre in a field experiment in New Zealand in 1971 (Healy *et al.* 1972). It was discovered that soil intake by ewes at a stocking rate of 22 ewes/ha was much greater than that of ewes stocked at 15 ewes/ha, this being reflected in higher faecal soil levels (69% of D.M. at the high stocking rate and 4% of D.M. at the lower stocking rate). As the pasture herbage samples were similar in iodine content throughout the experimental area (less than 0.1 ppm) it was thought that the levels of goitre which occurred in the high stocking rate (4%) compared to the lower (69%) was due to the extra soil iodine ingested at the higher stocking rate.

IODINE IN WATER

The amount of ingested iodine derived from drinking water is not considered to be singificant under most circumstances. It may however be

TABLE 1:5

Soil intake by sheep in relation to structure
(source Healy 1967)

Soil	Structure	% Soil in Faeces
Yellow brown earth	Mod. strong	25 - 35
Podzol	Weak	40
Recent soil from alluvium	Weak	65

a useful indicator of the degree of iodine deficiency of an area (Hetzel 1970). In a British survey conducted by Young, Crabtree and Mason (1936) the incidence of goitre among children in Somerset was found to be 56% and in Suffolk 3%. The levels of water iodine were respectively 2.9 µg/litre in Somerset and 8.2 µg/litre in Suffolk. Mahadeva and Shamnuganathan (1967) in a goitre survey in Ceylon found goitre to be associated with drinking water iodine levels of less than 10 µg/litre. This is not a figure which could be applied to other areas, as factors such as imported food high in iodine would tend to mask marginally goitrogenic areas in more advanced communities. The goitre area of Ceylon was shown to be coincident with heavy rainfall areas with a high annual water flow from river basins into the ocean, a process which should lead to rapid leaching of soluble minerals.

It has been suggested that bacteria may induce goitre if they are present in large enough numbers, as Macchia, Bates and Pastan (1967) have isolated a thyroid stimulating factor from *Clostridium perfringens*. This compound, which was thought to be a protein with a molecular weight of about 30,000, acted in a manner similar to Thyroid Stimulating Hormone when incubated with thyroid slices, and when injected into chickens it depleted the thyroid of radioiodine.

Other bacteria which commonly inhabit the human intestinal tract (particularly *Paracolobacterium*) have been shown to exhibit myrosinase activity which converts progoitrin into the goitrogenic agent goitrin (Oginsky, Stein and Greer 1965).

An unusual theory has been proposed by Beres (1969) who concluded that goitre in Hungary was associated with wet areas and high levels of magnesium, calcium and potassium ions. He proposed that the goitre of these areas was caused by algae, particularly *Microcystis*, which removed iodine from water and secreted antithyroid compounds including thiourea, thiouracil,

methyl mercaptan and cyanides.

This theory however, is unlikely to be important, since the amount of iodine usually provided by water in the diet is not considered significant under normal conditions, and thus water iodine levels would not be important unless other sources of dietary iodine were insufficient to satisfy requirements.

NORMAL IODINE METABOLISM

The normal iodine cycle within the body is presented as a prelude to describing thyroid problems related to hereditary thyroid malfunction and goitrogenic agents. A summary of the normal iodine cycle is shown in Fig.1:6.

Under normal conditions iodine enters the body either from water or food as iodide which is rapidly absorbed from the gastrointestinal tract into the blood. The thyroid and kidneys compete actively for iodine in the blood, Isler *et al.* (1958) showed in mice that approximately the same amounts of iodine were excreted in the urine as were accumulated by the thyroid gland.

Iodide is transported into the thyroid against a concentration gradient, probably by active transport, (Halmi 1964) there it enters a general pool of iodide derived from either extrathyroidal sources or deiodination of iodotyrosines.

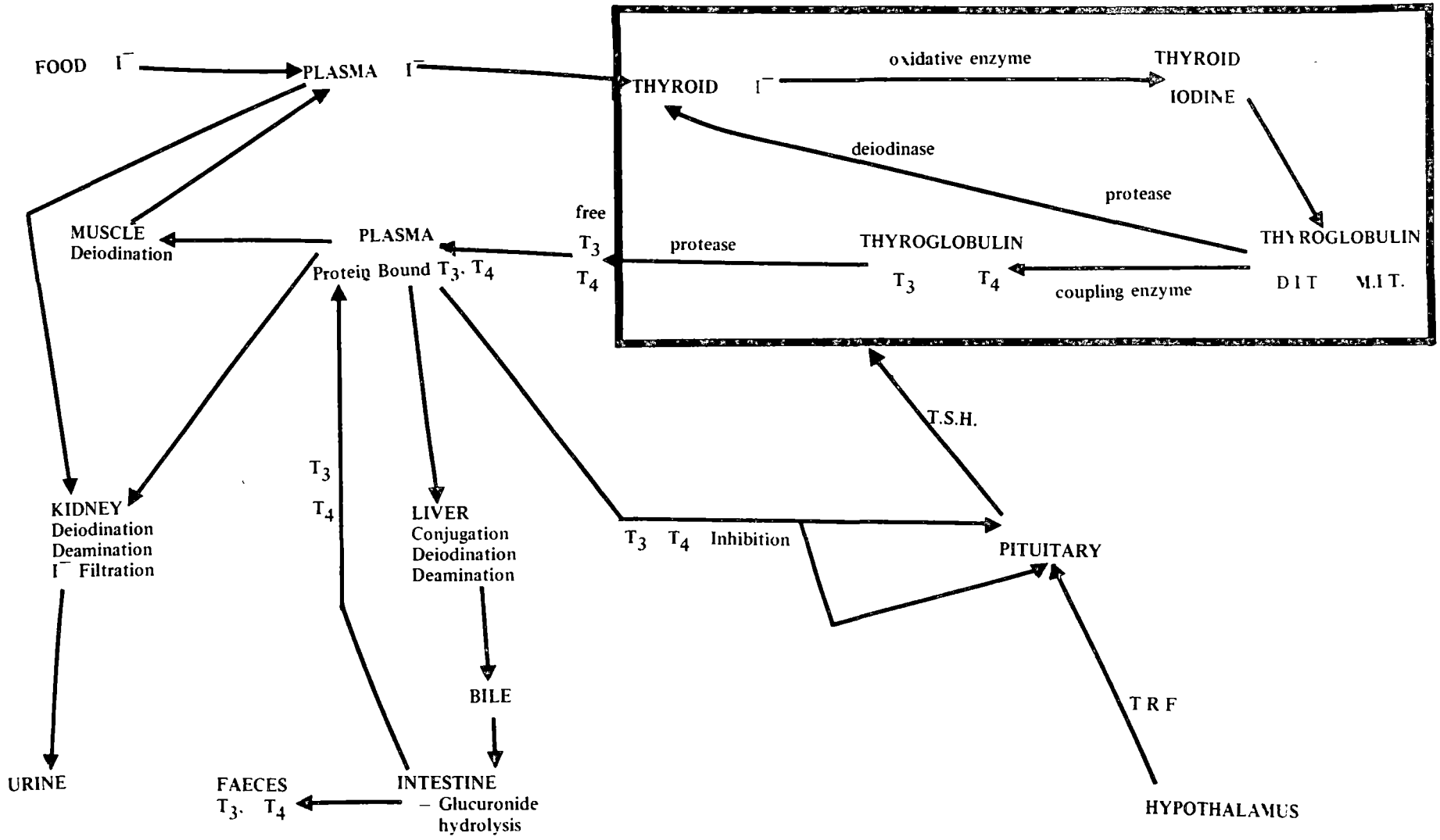
The sequence of events leading to formation of thyroid hormones has been described by Pitt-Rivers and Cavalieri (1964):

(1) Iodide is converted to an active form by an oxidative enzyme system.

FIGURE 1:6

Normal Cycle of Iodine Metabolism

(Reactions bounded by the black rectangle occur within the thyroid gland)


FOOD I⁻
PLASMA I⁻
THYROID I⁻
oxidative enzyme
THYROID
IODINE
deiodinase
protease
MUSCLE
Deiodination
PLASMA
Protein Bound T3. T4
free
T3
T4
protease
THYROGLOBULIN
T3 T4
THYROGLOBULIN
DIT MIT.
coupling enzyme
T.S.H.
KIDNEY
Deiodination
Deamination
I⁻ Filtration
T3
T4
LIVER
Conjugation
Deiodination
Deamination
T3 T4 Inhibition
PITUITARY
BILE
TRF
URINE
FAECES
T3. T4
INTESTINE
– Glucuronide
hydrolysis
HYPOTHALAMUS

(2) Tyrosine molecules are iodinated to form monoiodotyrosine (MIT) and diiodotyrosine (DIT)

(3) Thyroxine is formed by coupling of two molecules of DIT and triiodothyronine by the coupling of one molecule of MIT and one of DIT.

Reactions 2 and 3 occur within the thyroglobulin molecules, the most likely site being at the follicular cell - colloid interphase (Tong *et al.* 1962).

Before release of iodothyronines into the blood, thyroglobulin is degraded by a protease enzyme which appears to be closely associated with the thyroglobulin (McQuillan and Trikojus 1953), and which hydrolyses linkages between two aryl amino acids (McQuillan, Stanley and Trikojus 1954).

Released iodothyronines are secreted into the blood, probably by diffusion (Tata 1964), and iodotyrosines are deiodinated, the iodine being returned to the thyroid iodide pool.

Within the blood, thyroid hormones are rapidly bound to proteins, these are either gamma globulin (thyroxine binding globulin), thyroxine binding prealbumin or serum albumin. Thyroxine binding globulin is the major component and triiodothyronine is bound less strongly than thyroxine (Tata 1964). The hormones are unable to enter cells in the bound form, and therefore a reversible equilibrium exists between bound and free circulating thyroid hormone (Tata 1964).

Within the bodily organs three patterns of accumulation of radioactive thyroid hormones can be distinguished.

a) Organs such as the liver and kidney which have a rapid turnover of thyroid hormone.

b) Tissues such as skeletal muscle which slowly accumulate thyroid hormone.

c) Organs such as the brain in which there is little buildup of

hormone.

(Brown-Grant and Tata, 1961)

Within the cell there does not appear to be any specific sub-cellular site of thyroid hormone concentration. Tata *et al.* (1962) published the distribution shown in Table 1:6.

Metabolism of the thyroid hormones has been classified according to the major reactive groups of the iodothyronine molecule, the pathways being deiodination, phenolic conjugation and oxidative deamination or transamination. Deiodination is the major metabolic pathway of thyroid hormones and results in return of iodine to the plasma (Tata 1964). In the kidney, however, Shimoda and Greer (1972) demonstrated that deiodination of triiodothyronine resulted in loss of iodine directly into the urine without it re-entering the blood. The authors suggest thyroxine would behave simsimilarly.

The production of β-glucuronides in the bile has been demonstrated by Taurog *et al.* (1952) after administration of thyroxine or triiodothyronine. These phenolic conjugates undergo intestinal hydrolysis and the free hormone is either excreted in the faeces or reabsorbed into the circulation (Tata 1964). Other conjugates, such as sulphate esters particularly of triiodothyronine, have also been found in the tissues and blood (Roche *et al.* 1959).

The alanine side chain of thyroxine and triiodothyronine may undergo deamination or transamination, the products being excreted in the bile or deiodinated like the parent amino acid (Tata 1964).

The relative roles of thyroxine and triiodothyronine are not fully understood. Triiodothyronine has been estimated to be 3.5 to 5 times as active as thyroxine (Hemming and Holtkamp 1952; Gross and Pitt-Rivers 1953), and since approximately 17-30% of secreted thyroxine is converted to triiodothyronine (Schwartz *et al.* 1971; Sterling 1970), the conversion

TABLE 1:6

I^{131} distribution (%) within the cell

Fraction	Liver	Muscle
Nuclei and debris	22.3	30.0
Mitochondria	19.3	10.5
Microsomes	15.8	11.8
Cell sap	39.5	43.9

(Source Tata *et al.* 1962)

could account for 50 to 100% of the metabolic activity of thyroxine. Sterling (1970) suggests that in current research the question remains as to whether thyroxine has a primary action or is effective only after conversion to triiodothyronine.

Thyroid hormone secretion is controlled by thyrotropin, a glycoprotein with a molecular weight of 26,000 - 30,000 (West *et al.* 1966) produced by the pituitary. Thyrotropin production is controlled by circulating thyroid hormone levels via a negative feedback mechanism. Shadlow *et al.* (1972) suggested that triiodothyronine was the controlling hormone, since it was bound much more strongly than thyroxine by the pituitary. They also found pituitary binding sites with a low capacity and high affinity for triiodothyronine but could not demonstrate limited capacity binding sites for thyroxine. The same authors also suggested that at normal endogenous levels of circulating hormone the binding sites are nearly saturated and the pituitary works in an intermittent fashion. Thyrotropin production is also stimulated by thyrotropin releasing factor, a compound with the structure (pyro) Glu-His-Pro (NH_2) (Bowers *et al.* 1970, Bowers *et al.* 1967), this compound needing 0.3 µg triiodothyronine and 2.7 µg thyroxine for complete inhibition of the stimulation (Bowers *et al.* 1967).

HEREDITARY GOITRE

Iodine deficiency is now well established as probably the most important single factor in goitre production. However, a residual prevalence of about 4% goitre is found in non-endemic areas (Greer 1962a). Hereditary

malfunctions of the thyroid gland have been identified as contributing to this residual goitre to some degree.

Stanbury and Chapman (1960) reported a human patient whose thyroid, salivary glands and gastric mucosa all failed to concentrate iodide. Only small fractions of injected I^{131} entered the gland and became protein bound, and the authors interpreted this as indicating that the hypothyroidism and consequent goitre resulted from a genetic defect in the iodine concentrating mechanism (Fig.1:7).

A family of goitrous cretins studied by Stanbury and Hedge (1950) were shown to accumulate iodide normally into the thyroid, but the levels of thyroid hormone in the serum were very low. In addition, immediately after the administration of potassium thiocyanate the accumulated iodide was discharged. They suggested that the patients were able to extract iodide from the blood, but were unable to convert it into an organically bound form (Fig.1:8).

A case of congenital goitre was examined by Werner *et al.* (1957) who found equal amounts of monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (T_3) and thyroxine (T_4) in the serum. They also found a high level of serum inorganic iodide. Despite the high levels of MIT and DIT the dehalogenase activity of the thyroid gland appeared to be normal. These results indicated that there was a defect in coupling of the iodotyrosines, resulting in a concentration of iodotyrosines in the thyroid gland which caused leakage into the serum and loss of the iodine in the urine (Fig.1:9). There was some T_4 formation in this patient and, probably because of the large size of the gland, production of T_4 was sufficient for the body's needs.

Another defect was reported by De Groot and Stanbury (1959) and Dowling, Ingbar and Freinkel (1961), who discovered an abnormal iodinated substance in the blood and urine of some goitrous patients. There

NOTE

Figures 7 to 11 are taken from Murray and McGirr (1964)
The normal iodine cycle is outlined in each case with heavy lines up to the point at which the defect occurs.

FIGURE 1:7

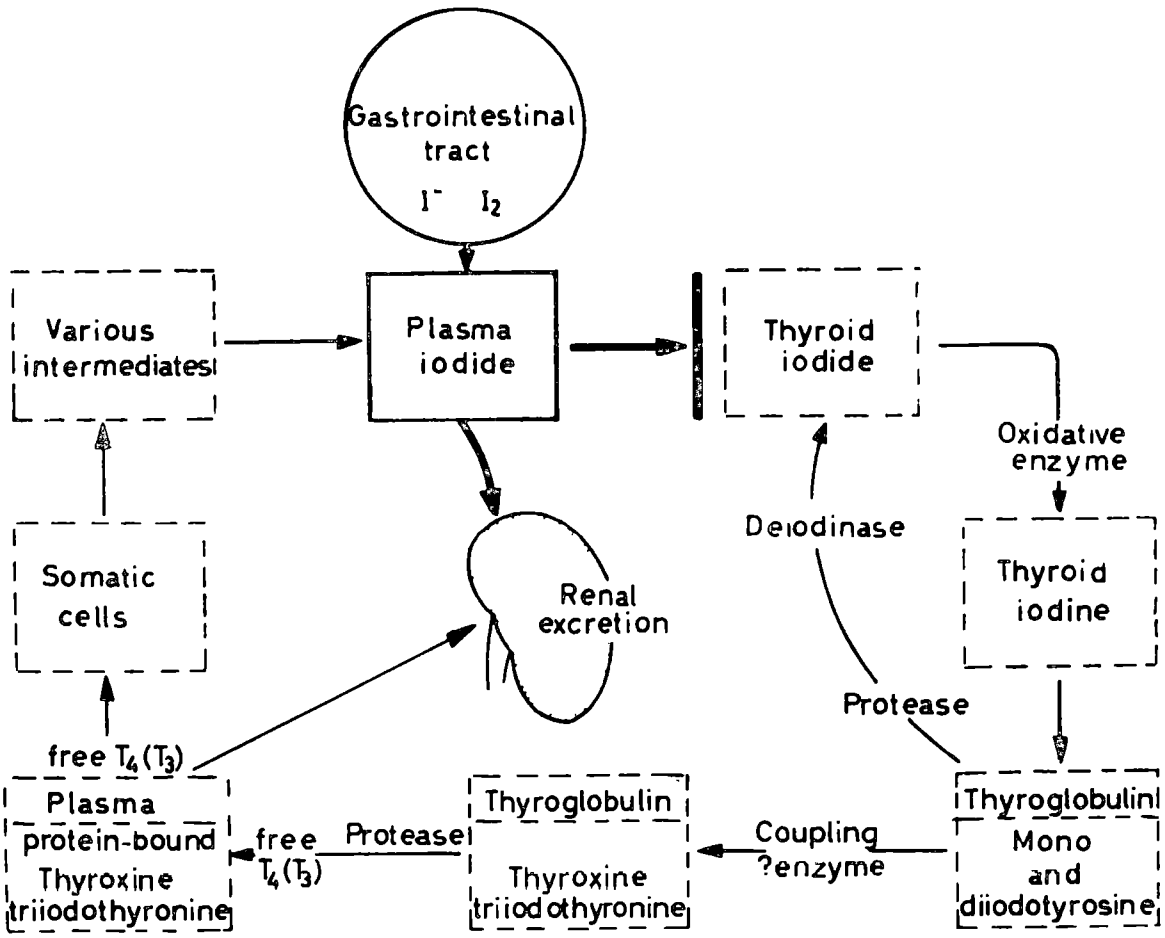
Trapping defect

The thyroid lacks the ability to concentrate iodine and it is excreted in the urine.

FIGURE 1:8

Organification defect

The thyroid can accumulate iodide, but there is a failure in the step which iodinates tyrosines.


Gastrointestinal tract
I⁻ I₂
Various intermediates
Plasma iodide
Thyroid iodide
Oxidative enzyme
Deiodinase
Somatic cells
Renal excretion
Thyroid iodine
Protease
free T₄(T₃)
Plasma protein-bound Thyroxine triiodothyronine
free T₄(T₃)
Protease
Thyroglobulin
Thyroxine triiodothyronine
Coupling ?enzyme
Thyroglobulin
Mono and diiodotyrosine

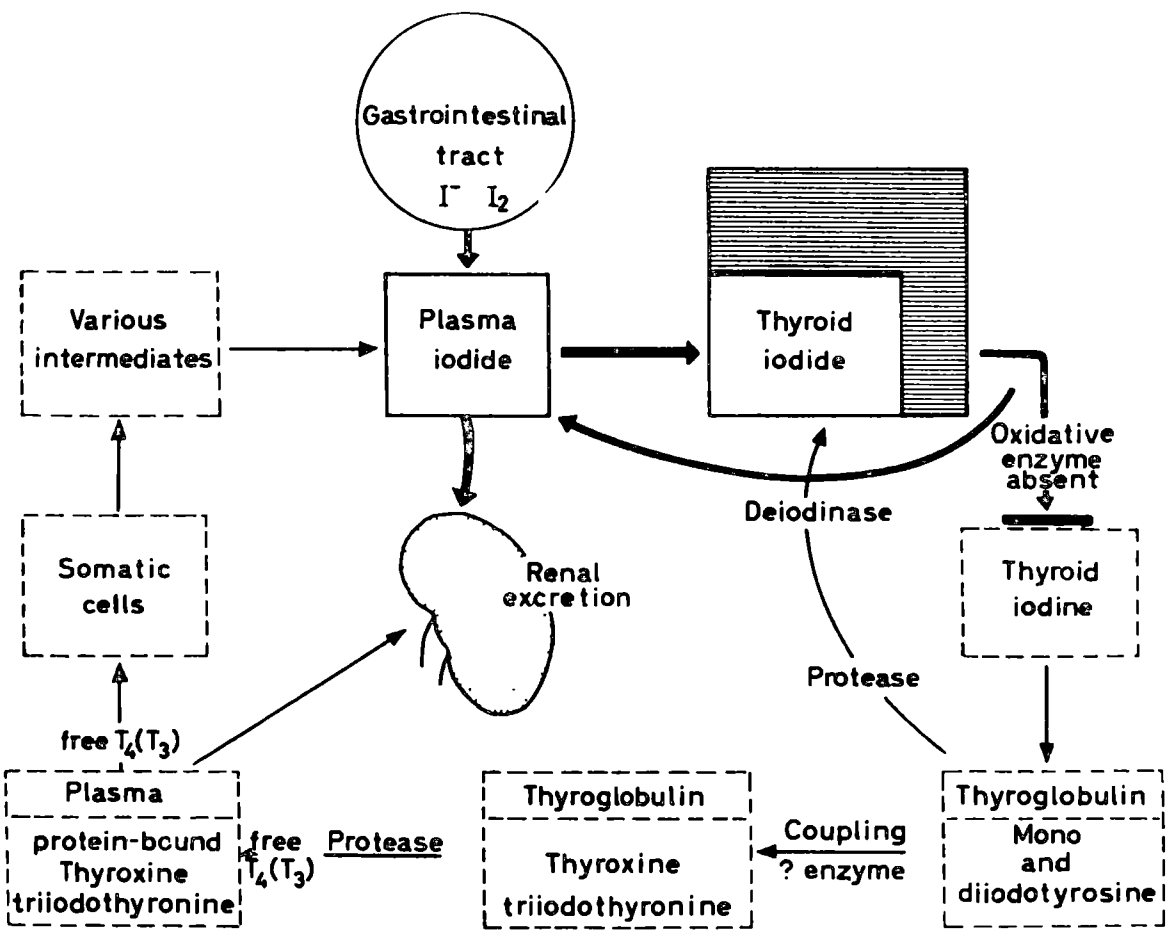

Gastrointestinal tract
I⁻ I₂
Various intermediates
Plasma iodide
Thyroid iodide
Oxidative enzyme absent
Deiodinase
Somatic cells
Renal excretion
Thyroid iodine
Protease
free T₄(T₃)
Plasma protein-bound Thyroxine triiodothyronine
free T₄(T₃)
Protease
Thyroglobulin
Thyroxine triiodothyronine
Coupling ? enzyme
Thyroglobulin
Mono and diiodotyrosine

FIGURE 1:9

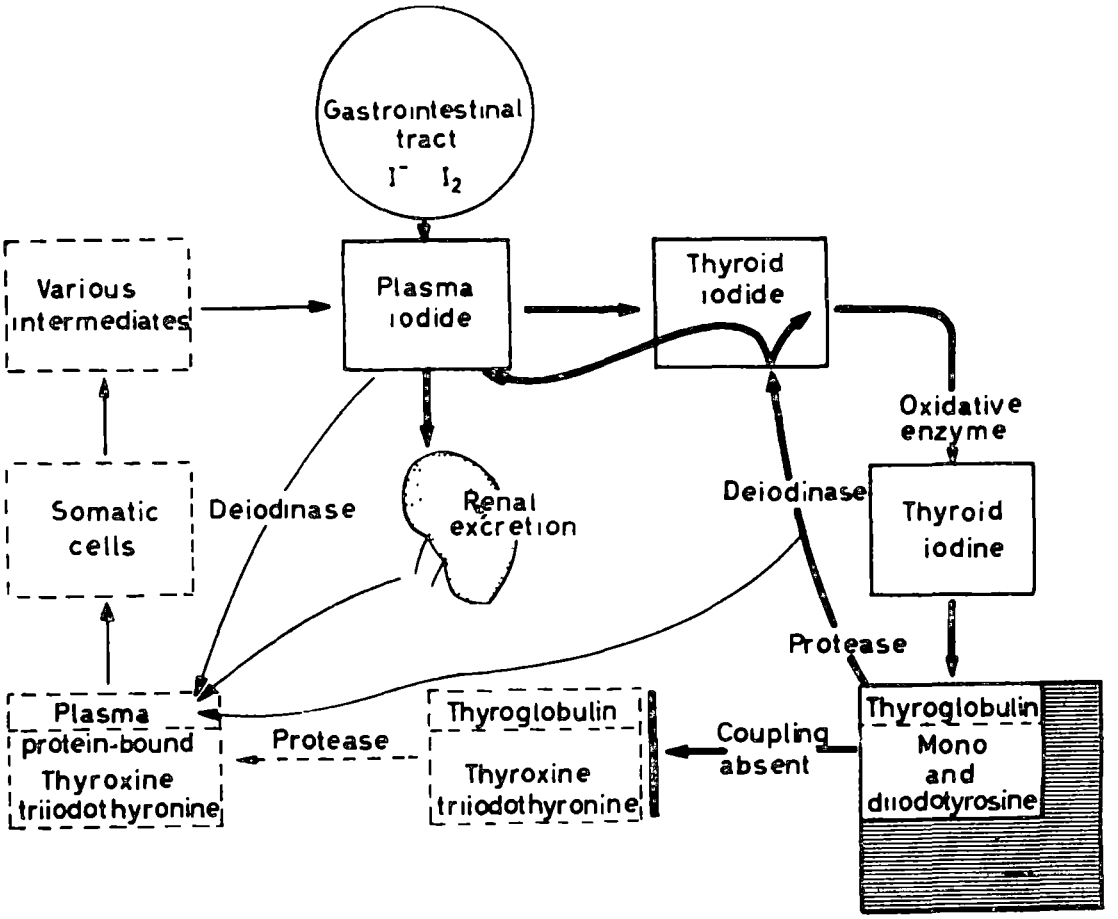
Coupling defect

Iodotyrosines fail to condense to form iodothyronines within the thyroid. There appears to be increased recycling, and a rapid flux, of iodine into and out of the thyroid.

FIGURE 1:10

Abnormal Iodoprotein defect

An unusual iodinated polypeptide is produced and secreted into the circulation. This compound is inactive and excreted in the urine.


Gastrointestinal tract
I⁻ I₂
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Oxidative enzyme
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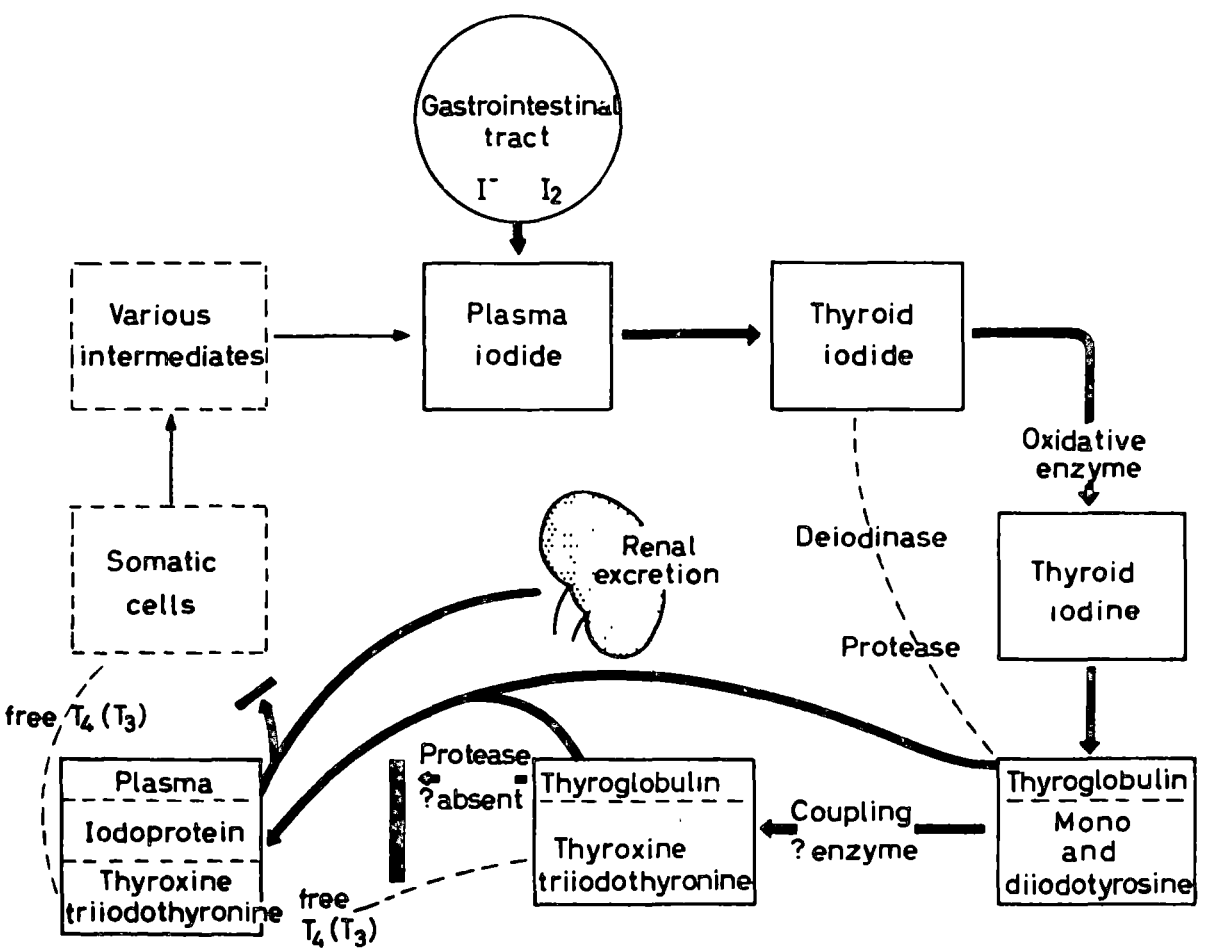
Deiodinase
Renal excretion
Deiodinase
Thyroid iodine
Protease
Plasma protein-bound Thyroxine triiodothyronine
Protease
Thyroglobulin Thyroxine triiodothyronine
Coupling absent
Thyroglobulin Mono and diiodotyrosine


Gastrointestinal tract
I⁻ I₂
Various intermediates
Plasma iodide
Thyroid iodide
Oxidative enzyme
Somatic cells
Renal excretion
Deiodinase
Thyroid iodine
Protease
free T_4 (T_3)
Plasma Iodoprotein Thyroxine triiodothyronine
Protease ?absent
Thyroglobulin Thyroxine triiodothyronine
Coupling ?enzyme
Thyroglobulin Mono and diiodotyrosine
free T_4 (T_3)

was an increased I^{131} uptake by the thyroids of these patients and no release when treated with potassium thiocyanate. The deiodinase activity was also normal. The abnormal compound was detected when it did react in a chemically similar manner to normal thyroid hormones. It was suggested that this compound was of thyroidal origin and was iodinated instead of thyroxine. The compound appeared to be an albuminous type of protein (Fig.1:10).

Stanbury, Meijer and Kassenaar (1956) reported six related patients with goitre, all of whom had labelled MIT, DIT, T_3 and T_4 in the serum following the administration of I^{131}. Intravenously administered DIT was excreted unchanged in the urine. The authors suggested that an explanation for this goitre was excessive loss of iodine through loss of MIT and DIT following defective deiodination of these compounds (Fig.1:11).

As well as the reported cases of hereditary goitre in humans there have been some reports of hereditary goitre in animals, the best documented case occurring in Merino sheep in South Australia. Goitre occurred on properties where the absence of goitrogens, and adequacy of iodine supply were confirmed (Mayo and Mulhearn 1969). Most of the affected lambs died at, or soon after birth, but those which survived had increasingly large thyroids.

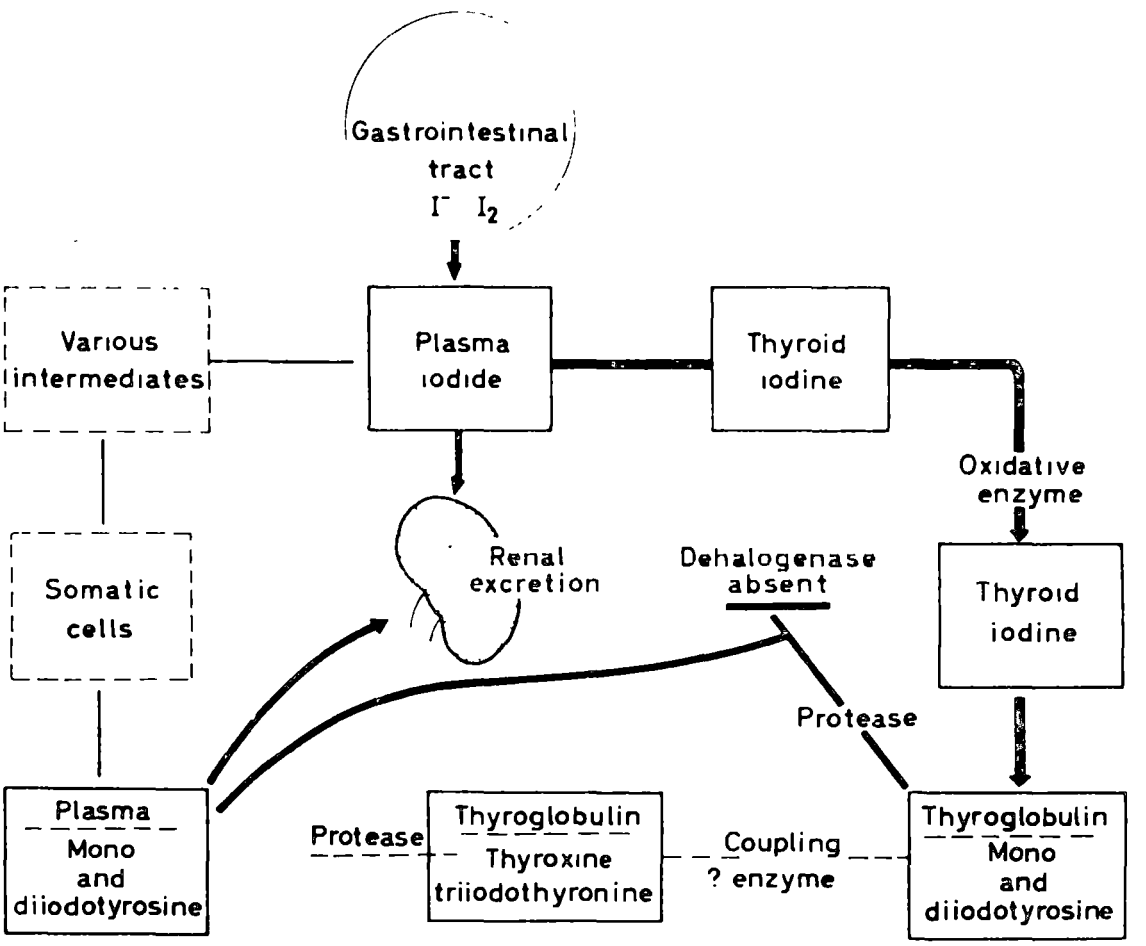
A biochemical investigation undertaken by Falconer (1966a, 1966b) showed that the goitrous animals had higher serum protein-bound iodine levels, but lower butanol-extractable I^{131} than normal sheep. The non-hormonal iodine fraction of the goitrous sheep appeared to contain iodotyrosines and iodoprotein. The similarity of half lives of injected thyroxine in both normal and goitrous sheep indicated no extra-thyroidal defect in thyroxine metabolism, while the short half life of injected iodotyrosines, conbined with the continuous presence of iodotyrosines in the serum, indicated that the iodotyrosine deiodinase system was active.

When Falconer examined the goitrous thyroid tissue he found a greater

FIGURE 1:11

Dehalogenase defect

The absence of dehalogenase results in iodotyrosines being released from the thyroid, with a consequent impairment of iodothyronine production. This is aggravated by the continual loss of iodotyrosines in the urine due to a lack of peripheral dehalogenase.


Gastrointestinal tract
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Thyroid iodine
Protease
Plasma
Mono and diiodotyrosine
Protease
Thyroglobulin
Thyroxine triiodothyronine
Coupling
? enzyme
Thyroglobulin
Mono and diiodotyrosine

rate of uptake and release of radioiodine, and greater iodotyrosine deiodinase activity than normal. There appeared to be no protein of thyroglobulin size present and the incorporation of radioactive leucine and proline into the thyroglobulin fraction *in vitro* was markedly reduced (leucine and proline being normal constituents of thyroglobulin). From the results obtained it was concluded that the goitre was due to compensatory hypertrophy of the gland resulting from a defect in the ability to synthesise thyroglobulin.

Mayo and Mulhern (1969) suggested that this outbreak of goitre was due to an autosomal recessive gene with high penetrance and variable expressivity. A similar case in South Australia was reported by Rac *et al.* (1968), the conclusions drawn being similar to those of Falconer (1966a, 1966b).

An outbreak of goitre in three Dorset-Horn flocks in Britain was examined by Watson (1962). He found that the goitre occurred only in lambs sired by one particular ram, indicating that genetic factors were involved, but unfortunately no biochemical studies were undertaken to isolate the factors responsible.

There is some evidence of breed differences in susceptibility to goitre in both sheep and goats. George, Farleigh and Harris (1966) reported that the incidence of goitre in Dorset-Horn lambs (50%) was greater than in crossbreds (44%) or Merinos (21%). However, the three flocks were not grazed together, so iodine intakes may have differed. A survey of goitre in sheep in Britain showed the incidence of goitre in Dorset-Horns was higher than in other breeds (Table 1:7) (Watson 1962).

An interesting outbreak of goitre was reported by Rajkumar (1970) in Uttar Pradesh, India, a zone of endemic human goitre. A goat farm was established in this area, presumably to upgrade the quality of the local goats by the introduction of improved breeds. It was found that the incidence of

TABLE 1:7

Incidence of goitre in British sheep
(source Watson 1962)

Breed	No. Owners Questioned	% With Goitre
Dorset Horn	82	12.2
Dorset Down	48	2.2
Hampshire Down	50	0.0

goitre in the new-born young of introduced goats and their crosses was much higher than that in the local goats (Table 1:8).

Treatment of the pregnant does with potassium iodide reduced the incidence of goitre in all breeds to zero, indicating an iodine deficiency goitre from which the local goats did not suffer. In Australia, goat breeders generally agree that Nubian kids are more prone to goitre than other breeds (Bray, 1973 pers. comm.).

It is not clear whether the breed differences noted are due to differences in the body iodine cycle, in the retention of iodine, or minor differences in grazing habit which provide different iodine intakes. Under natural grazing conditions it seems logical that susceptibility to goitre would be selected against, as animals with goitre are less fertile and have weaker young than normal. Both of these factors would select against genetic combinations which predispose animals to goitre.

ANTITHYROID COMPOUNDS

Antithyroid agents are generally thought to produce goitre by depressing the formation of thyroid hormone to a point where the blood thyroid hormone levels are decreased. The lowered level of circulating thyroid hormone reduces negative feedback to the hypothalamus and pituitary, resulting in increased release of thyrotropin which, in turn, stimulates the thyroid cells to proliferate and the gland to become enlarged and more efficient.

Although the general mode of action of antithyroid compounds is known, the precise manner in which each of them operates is not fully understood.

TABLE 1:8

Incidence of goitre in goats
(source Rajkumar 1970)

Breed	Number	No. With Goitre	% Goitre
Barbari	29	16	55.2
Local	186	1	0.5
Barbari x Local	57	4	7.0

They are divided into several groups according to their apparent site of action. General groupings have been proposed by Greer *et al.* (1964) as:

a) Substances which interfere with thyroidal iodine concentration.

b) Compounds which inhibit organic binding of iodine within the thyroid.

c) Substances which accelerate loss of iodine from the body.

a) SUBSTANCES WHICH INTERFERE WITH IODINE CONCENTRATION

Wyngaarden *et al.* (1952) identified members of this group in decreasing order of potency with respect to the thyroid as - perchlorate, thiocyanate, periodate, iodate, chlorate, triodate, hypochlorite and nitrate. Fluoroborate also belongs in this group (Yamada and Jones 1968). A dose response study reported by Wyngaarden *et al.* (1952) showed that perchlorate is ten times and nitrate 1/30th as active as thiocyanate in inhibiting radioiodine uptake.

These compounds appear to competitively inhibit uptake of iodine by the thyroid gland and discharge any unbound iodine already within the thyroid (Greer, Kendall and Smith 1964). At normal goitrogenic levels of these compounds, there is little uptake of the goitrogen into the thyroid (Chow *et al.* 1969) therefore their activity must be upon the external membranes of the gland.

These goitrogens also act on other iodine concentrating organs within the body. In the mammary glands, for example, a dose of 4 mg/kg body wt. of perchlorate reduced the radioiodine content of goats milk by 70% (Djurdjevic and Lengemann 1970).

Yamada and Jones (1968) found that, within the body, several of the antithyroid compounds (perchlorate, thiocyanate, fluoroborate, nitrate and chlorate) increased the displacement of thyroxine from its serum binding proteins and increased uptake of thyroxine by muscle within the body. It is not known whether the latter result is dependent on the former reaction.

Although these compounds are classed as inhibitors of iodine concentration they may also inhibit incorporation of thyroidal iodine into organic

molecules at higher levels of intake. Butler *et al.* (1957) demonstrated that *in vitro* thiocyanate inhibited iodine incorporation in sheep thyroids, and Greer, Stott and Milne (1966) reported that thiocyanate, perchlorate and nitrate all behaved similarly in rat thyroids. Perchlorate and thiocyanate also inhibited thyroidal incorporation of iodine in whole rats when injected at concentrations of 50 - 100 µg/animal, thiocyanate being the most potent.

Thiocyanate and nitrate have been studied extensively in agricultural research as they occur naturally in plants. Substances giving rise to thiocyanate are present in plants as either thioglucosides (glucosinolates) (van Etten *et al.* 1969) or cyanogenic glucosides (Corkill 1940).

The natural thioglucosides are the likely source of goitrogens in brassica plants. These compounds are hydrolysed when wet, unheated plant material is crushed, the reaction being catalysed by myrosinase (thioglucosidase). The general thioglucoside structure is of the form

$$\begin{array}{l} R - C - S - \text{GLUCOSE} \\ \quad\;\; \| \\ \quad\;\; N - O - SO_2 - O^- \end{array}$$

and it will hydrolyse (van Etten *et al.* 1969) to form either organic isothiocyanate (R - N$=$ C $=$ S), organic nitrite (R - C$\equiv$ N) or organic thiocyanate (R - S - C$\equiv$ N).

The thiocyanates and nitrites are further degraded to release thiocyanate into the serum, and the goitrogenic effect of glucobrassicin, for example, is thought to be due to such liberation of thiocyanate (Michajlovskij and Langer 1967).

If a hydroxyl group is present on the 2-carbon position of the organic aglucone, the isothiocyanate formed by hydrolysis cyclises to produce a goitrogen which primarily inhibits organic binding of thyroidal iodine (van Etten *et al.* 1969).

Although allyl-, methyl- and butyl- isothiocyanates have been shown to inhibit uptake of iodine into the thyroid (Langer and Stolc 1965; Langer

and Greer 1968), lower levels than those shown to inhibit iodide concentration inhibit incorporation of iodine into organic molecules within the thyroid. Langer and Greer (1968) suggested that the effect on the concentrating mechanism may be due to irreversible cell damage, thus the actual antithyroid activity is primarily inhibition of iodide incorporation.

The range of thioglucosides found in plants can be exemplified by those found in Tasmanian cruciferous weeds and chou-mollier by Bachelard and Trikojus (1963a) (Table 1:9).

Examinations of the antithyroid effect of most of these have not been made, but one, glucocheirolin, which is the main thioglucoside of Queensland turnip weed *Raphistrum rugosum*, was found after hydrolysis to act in a manner similar to thiocyanate (Bachelard, McQuillan and Trikojus 1963).

The hydrolysis product, cheirolin, when incubated with bovine rumen liquor was converted into the substituted thioureas, dicheirolin thiourea and monocheirolin thiourea (Bachelard and Trikojus 1963b)—compounds which were inactive in depressing radioiodine uptake in both rats and humans (Bachelard, McQuillan and Trikojus 1963). The authors suggest that this degradation pathway may not be generally applicable to isothiocyanates, although benzyl-isothiocyanate, one of the compounds tested, was converted to dibenzyl thiourea, a known goitrogen. It would appear that this pathway, if general, may result in potent goitrogens being formed in the rumen from non- or mildly-goitrogenic compounds, or other goitrogens being rendered inactive, depending upon the specific thioglucoside present.

The cyanogenic glucosides are characterized by release of HCN when crushed in the presence of the enzyme linamarase. They are widespread in nature, having been isolated from approximately 1000 plant species covering 92 families (Conn 1969). The most important cyanogenic glucosides in agriculture are linamarin and lotaustralin. These may occur together in the same plant as in white clover (Corkill 1940), or separately as in

TABLE 1:9

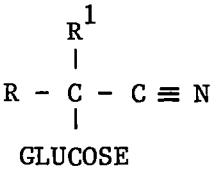
Distribution of thioglucosides in Tasmanian cruciferous weeds

Plant	Name of Thioglucosides Identified	R- Group
Brassica campestris L. (wild turnip)	Gluconapin*	$CH_2:CH(CH_2)_2-$
	Glucobrassicanapin	$CH_2:CH(CH_2)_3-$
	Glucoiberin	$CH_3SO(CH_2)_3-$
	Glucoberteroin ε	$CH_3S(CH_2)_5-$
	Progoitrin ε	$CH_2:CHCH(OH)CH_2-$
Sisymbrium officinale L. (hedge mustard)	Glucoputranjivin*	$(CH_3)_2CH-$
	Glucocochlearin	$CH_3CH_2CH(CH_3)-$
	Sinigrin	$CH_2:CHCH_2-$
	Glucocheirolin ε	$CH_3SO_2(CH_2)_3-$
S. orientale L. (Indian hedge mustard)	Glucoputranjivin*	$(CH_3)_2CH-$
Raphanus raphanistrum L. (wild radish)	Unknown*	-
	Glucoraphenin	$CH_3SOCH:CH(CH_2)_2-$
	Glucoalyssin	$CH_3SO(CH_2)_5-$
	Glucocheirolin ε	$CH_3SO_2(CH_2)_3-$
Sinapis arvensis L. (charlock)	Gluconapin*	$CH_2:CH(CH_2)_2-$
	Glucoarabin	$CH_3SO(CH_2)_9-$
	Sinalbin ε	$p\text{-}HOC_6H_5CH_2-$
	Glucoiberin ε	$CH_3SO(CH_2)_3-$
B. oleracea var acephala D.C. (Chou mollier)	Progoitrin	$CH_2:CHCH(OH)CH_2-$
	Gluconapin	$CH_2:CH(CH_2)_2-$
	Glucoiberin	$CH_3SO(CH_2)_3-$
	Sinigrin	$CH_2:CHCH_2-$
	Glucobrassicanapin ε	$CH_2:CH(CH_2)_3-$

* Main thioglucoside component. ε Occurring in trace amounts.
(Taken from Bachelard and Trijojus 1963a)

linseed (Care 1954).

The general formula for the cyanogenic glucosides is



with linamarin having R = R^1 = CH_3 and lotaustralin R = CH_3, R^1 = C_2H_5 (Conn 1969).

In ruminants it has been found that the plant enzyme is not necessary for release of HCN as the rumen microflora can hydrolyse both lotaustralin and linamarin (Coop and Blakley 1949).

When cyanogenic plants are ingested and hydrolysed the rate of absorption of HCN from the rumen is extremely rapid, with 75% of an administered dose being cleared within 15 minutes (Coop and Blakley 1949). Blakley and Coop (1949) could detect only small amounts of HCN in urine or saliva, and even the serum cyanide level remained low (of the order of 0.5 µg/ml) after dosing a sheep with 91 mg of HCN. However serum thiocyanate reached higher levels (2.2 µg/ml) two hours after treatment.

Blakley and Coop (1949) studied the production of thiocyanate from HCN in the presence of sheep liver extract. With cystine present it appeared that the reaction proceeded through formation of an α-amino-β-thiocyano-ϕproprionic acid, but the production of thiocyanate was more rapid when inorganic sulphate was added to the liver extract and it was suggested that H_2S from the rumen could be an important sulphur donor to the HCN detoxification process. The amount of thiocyanate excreted into the urine suggest that this may be the major detoxification pathway of HCN in sheep, as 75% of administered cyanide doses were recovered as thiocyanate (Blakley and Coop 1949).

In cows however, Worker (1957) reported that the level of HCN intake did not appear to affect the serum thiocyanate level. He attributed this partly to a more efficient urinary excretion of thiocyanate in cows and

postulated the existence of an alternative mechanism for detoxifying ingested cyanide in this species.

Experiments where high and low cyanide clover strains have been fed, demonstrate that the high cyanide strains are goitrogenic to rats and guinea pigs (Flux *et al.* 1956), and the thyroid iodine content of sheep was significantly reduced by short term grazing (43 days) of high cyanide clover pasture (Butler *et al.* 1957).

Plant nitrate has been implicated as the causative agent of congenital goitre in sheep where high levels of nitrogenous fertilizer have been applied to pasture (Reid *et al.* 1969). In general, however, as nitrate is a much less potent goitrogen than thiocyanate (Wyngaarden *et al.* 1952), its action is much less predictable and can be prevented by relatively low iodine intakes. Lee *et al.* (1970) reported that in rats 0.4% dietary KNO_3 induced a thyroid weight of 27.7 g and thyroid epithelial cell height of 8.67 μ with a dietary iodine intake of 0.08 ppm, but the thyroid weight was only 19.8 g and cell height 3.67 μ when the dietary iodine was raised to 0.68 ppm.

Some animals have been shown to adapt to an increase in dietary nitrate. With lambs, Arora *et al.* (1968) showed that after 50 days on a diet containing 3.8% KNO_3 the thyroid secretion rate was similar to that in control animals, but after only 1 day on the high nitrate diet the thyroid secretion rate of a similar group of lambs was depressed by 20%. Later growth studies showed that the lambs adapted to a high nitrate diet in 14 to 43 days. Rats also were found by Bloomfield *et al.* (1962) to adapt to an increased nitrate intake (2.5% KNO_3). These animals had reduced thyroidal uptake for approximately 2 weeks. The same authors also suggested from unpublished data that rats could compensate for an increased nitrate load under usual environmental conditions but not when they were also under cold stress. An outbreak of goitre amongst weaned lambs was also attributed to cold stress and high nitrate under field conditions by

Wallach (1965). Feed iodine levels were not reported, so the possibility of iodine deficiency goitre cannot be excluded. In this case the goitre was eliminated by use of iodised salt licks.

Cattle may be less susceptible to nitrate as a goitrogen, since Janudeen *et al.* (1965) reported that doses of 660 mg/kg body weight daily had no effect on thyroid function.

b) COMPOUNDS WHICH INHIBIT ORGANIC BINDING OF IODINE

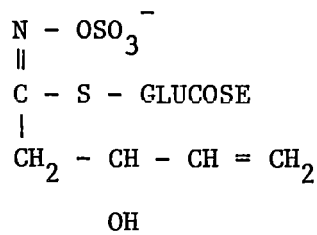
The discovery by Chesney *et al.* (1928) that goitre could be caused by a cabbage diet stimulated other workers to emulate and extend this line of research. Some workers however, were unable to produce any thyroid enlargement under experimental conditions similar to those of Chesney (Spence *et al.* 1933; Heyman 1934; Hercus and Purves 1936). The latter group, working on the belief that the goitrogenic agent may have been a glucoside, fed seeds of some Brassica plants to rats. They found that cabbage seed, rape seed and steamed black and white mustard seed all induced goitre in experimental animals, but steamed rape seed had no effect.

The authors concluded that, in rape, enzymes are essential for production of the active agent while in cabbage they are not. Hercus and Purves (1936) also tested turnips, as Hopkirk *et al.* (1930) had reported an outbreak of congenital goitre after ewes were fed a winter supplement of turnips. It was found that turnips from one area and one particular year were goitrogenic, but that others were not. The authors suggested that the sporadic nature of goitrogenic activity of turnips may contribute to the production of goitre epidemics in stock.

Subsequent work (Griesbach *et al.* 1947, Kennedy and Purves 1941) showed that iodine was partially effective against rape seed goitre, but thyroxine injections or hypophysectomy prevented goitre development.

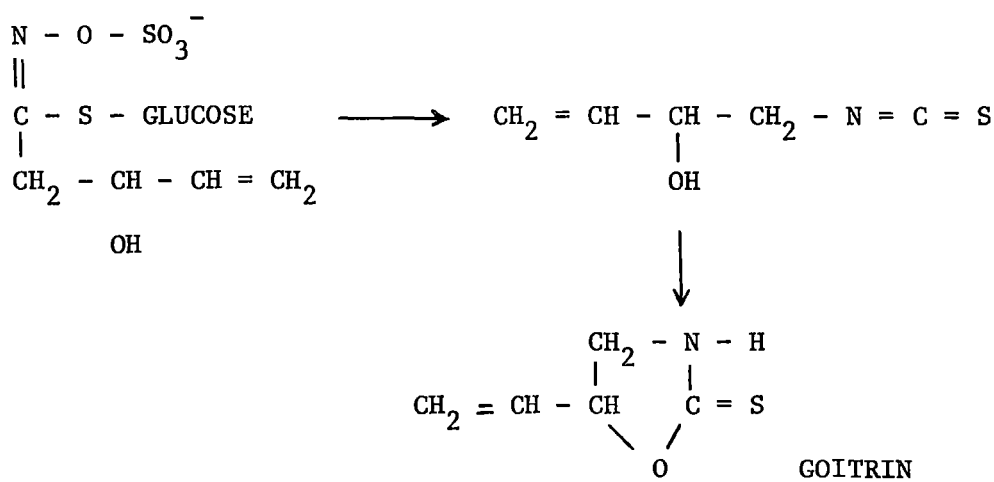
This goitrogen which was resistant to iodine prophylaxis was isolated by Astwood *et al.* (1949) from roots and seeds of several crop plants, and

was identified as L-5-vinyl-2-thiooxazolidone, and named goitrin. In the course of studies it became apparent that goitrin did not occur in the free, active form. Boiling the intact plant tissue destroyed its goitrogenic activity, but if the seeds or roots were finely ground and soaked in water the activity was not destroyed by boiling. It was proposed that heating destroyed an enzyme essential for liberation of goitrin, and it was shown that an extract of ground inactive seed treated with a purified enzyme preparation (obtained from seeds) produced goitrin (Greer 1962a). The precursor of goitrin was subsequently isolated from rutabaga *(Brassica napobrassica* (L.) Mill) and turnip roots and identified as a mustard oil glucoside with the formula:



(Greer 1962b)

Conversion to goitrin is catalysed by the enzyme myrosinase (thioglucodisase) via an unstable intermediate (van Etten *et al.* 1969)



GOITRIN

Further work revealed that progoitrin, when administered to animals in the absence of myrosinase, appeared to have antithyroid activity. This was found to be due to conversion of progoitrin to goitrin by bacteria of the lower intestinal tract (Greer *et al.* 1961), particularly *Escherichia coli* and *Proteus vulgaris* (Oginsky *et al.* 1965).

Because of the ability of intestinal bacteria to activate progoitrin, any progoitrin-containing food must be considered as being potentially goitrogenic. On the other hand, only rutabaga and turnip have been shown to possess clinically significant amounts of progoitrin (Greer 1962a).

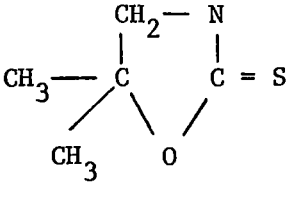
The natural variability of Brassicae in content of thioglucoside and thiocyanate could explain the contradictory results obtained by early workers investigating cabbage goitre. Leaf analyses have revealed that rape leaves contain between 0.1 and 0.6% progoitrin on a dry weight basis, young plants containing less than mature plants (van Elten and Daxenbichler 1971). Altamura *et al.* (1959) found leaves of several varieties of cabbage in America contained an average of 12.5 µg progoitrin per 100 g , while a more extensive investigation in Czechoslovakia produced the following values (Michajlovski *et al.* 1969)

Cabbage progoitrin content, µg/100g leaf (fresh wt.)

Cabbage	Isothiocyanate	Goitrin	Thiocyanate
Winter 1965	6.11	2.54	-
" 1966	11.78	1.97	3.09
Summer 1967	12.57	-	2.02

The type and amount of goitrogen would thus appear to depend on the stage of maturity and season of the year in which the plants were grown.

Other naturally occurring thio-oxazolidone-forming thio-glucosides having goitrogenic activity have been isolated. One of these is glucoconringiin, which forms conringiin, which has approximately the same antithyroid activity as goitrin and has the structure

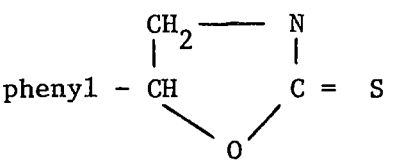


(Astwood *et al.* 1945)

It is potentially an important goitrogen as it is found in a common

North American weed, Hare's Ear Mustard (*Bupleurum rotundifolium* L.).

A more recently isolated compound is barbarin, which has approximately half the goitrogenic activity of goitrin in rats and humans (Greer and Whallon 1961). This compound is found in high concentrations as its thio-glucoside, gluco-barbarin, in the green leaves of members of the genus Barbarea which is widespread in Northern Europe. The structure of barbarin is



As most of the naturally occurring compounds which block organic binding of iodide in the thyroid were not produced until recently in large quantities, much of the experimental work has been carried out using artificial goitrogens. The most common of these being thiouracil, thiourea and some of the sulpha drugs.

Investigations with rats showed that increasing doses of propylthiouracil inhibited first the formation of iodothyronines, then diiodotyrosine and finally monoiodotyrosine (Richards and Ingbar 1959; Slingerland *et al.* 1959; Iino *et al.* 1961). The last step appeared to be much more resistant to blocking and even the relatively large amount of 180 mg of propylthiouracil per rat failed to block all monoiodotyrosine production, whereas 1 mg prevented thyronine and diiodotyrosine formation (Iino *et al.* 1961).

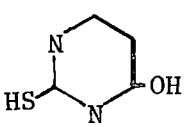
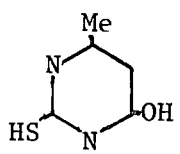
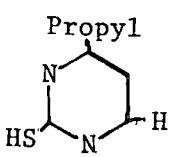
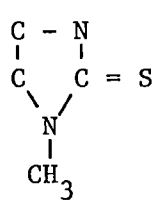
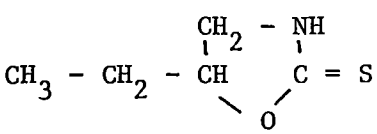
Shimoda and Greer (1966) obtained similar results when using rat thyroids *in vitro* and they discovered that propylthiouracil entered the gland rapidly enough to prevent organic binding of any iodide previously contained within the thyroid.

A range of compounds tested for antithyroid activity with isolated thyroids, thus removing differences due to metabolism in the intact animal, gave the results shown in Table 1:10 (Iino 1961).

These results differ somewhat from those of Astwood *et al.* (1945) who

TABLE 1:10

Structure and activity of some goitrogens

Goitrogen	Activity (Relative to 2 thiouracil)	Structure
Thiourea	0.5	$S = C(NH_2)_2$
2-thiouracil	1.0	
6-methyl-2-thiouracil	0.5	
6 propyl-2-thiouracil	0.66	
1 methyl-2-mercaptoimazol	1.66	
Goitrin	0.66	

(From Iino 1961)

used live animal tests. They found propylthiouracil was 11 times as effective as thiouracil, and approximately 100 times as active as thiourea. These differences may have been due to structural modification of the drugs within the animal body.

Rimington (1961) tested a range of sulphur containing compounds and concluded that the thione configuration R_2 C = S was responsible for a much greater degree of antithyroid activity than the thiol R - SH.

Sulphadiazine was shown to behave in a manner similar to propylthiouracil in rats, but it was only 1/5000th as active (Milne and Greer 1962). Astwood *et al.* (1943) discovered that sulphadiazine had the greatest antithyroid activity of seven sulpha drugs tested, and was 250 times as potent as the least active drug when administered orally. These seven compounds, therefore, cannot be considered as a group of very active antithyroid agents. The antithyroid activity of sulphadiazine is however increased over 1000 fold when iodide is administered concurrently (Milne and Greer 1962). This synergistic effect is unusual, as other antithyroid agents have decreased activity when iodide is also used.

High iodine intakes have been put forward as the causative agent in some cases of congenital goitre in humans and horses. A woman with an intake of 234 g of potassium iodide over the full pregnancy gave birth to a child with a thyroid weighing 60 g. (normal weight 1.5 - 2.5 g). In another case, a child was born with a thyroid weight of 30 g to a woman who had ingested 324 g of potassium iodide during pregnancy (Galina *et al.* 1962). Baker and Lindsay (1968) reported congenital goitre in foals born to mares fed a diet in which the iodine intake was more than 48 mg/day. In none of the three cases above was there any effect on the maternal thyroid.

The mode of action of iodide as a goitrogen is controversial. Wyngaarden *et al.* (1953) considered it to be due to partial saturation of the concentrating mechanism, whereas Wolff and Chaikoff (1948c) stated

that the concentrating mechanism remained active, and there was blocking of incorporation of iodide into organic molecules. This may have been by iodide ions binding with the active iodide in the thyroid gland (Fawcett and Kirkwood, 1953).

Wolff and Chaikoff (1948b) maintained rats on a diet containing 300 µg/kg I^- and gave additional doses of 5, 10, 50 and 100 µg I^{131}. They found that diiodotyrosine production and the conversion of diiodotyrosine to thyroxine were depressed by the two higher doses. When given injections of between 10 and 500 µg I^- thyroid hormone production ceased in rats until the plasma iodine dropped below 20-35 µg/100 ml of blood (Wolff and Chaikoff 1948a; Wolff and Chaikoff 1948c). The time taken for thyroid hormone production to recommence varied between 5 hours (50 µg dose), and 25 hours (500 µg dose) after injection. They later showed however (Wolff *et al.* 1949) that excess iodide had only a temporary effect and, despite the continued maintenance of a high level of plasma iodine, formation of thyroid hormones began again after 26 hours. This may explain why Galina *et al.* (1962) and Baker and Lindsey (1968) found there was no change in maternal thyroids when excess iodide caused congenital goitre in the young.

Using both thyroid slices and isolated thyroid follicular cells Shimoda, Inoue and Greer (1966) observed that intact thyroid follicles were not necessary for the inhibitory action of excess iodide to occur and concluded that the inhibition was probably exerted directly at the level of the thyroid epithelial cell. These cells have been found to concentrate iodide and incorporate it into iodo-tyrosines and iodothyronines in the absence of colloid and follicular structure (Tong *et al.* 1962).

It appears that excess iodide may act in a manner similar to the other agents, which block incorporation of iodine into organic molecules, but its effect is only transitory.

c) SUBSTANCES WHICH ACCELERATE IODINE LOSS FROM THE BODY

As the kidneys and thyroid compete for serum iodide, any factor which increases urinary iodine content would reduce thyroidal iodide uptake and, depending upon the iodine supply, tend to induce goitre. Isler *et al.* (1958) demonstrated with mice that a single injection of 30 mg of sodium chloride increased urinary iodine excretion by a factor of 10. Mice fed a diet containing 1% NaCl for 63 days developed enlarged thyroids with reduced amounts of colloid, the authors attributing this condition directly to increased urinary iodide loss. Taylor (1954) suggested that calcium may behave in a similar manner, as rats fed a low iodine diet containing 2% calcium carbonate developed goitre although the thyroid function was not impaired.

Iodine depletion may also occur through the loss of thyroxine from the body. Van Middlesworth (1957) demonstrated that thyroxine may be lost from the body via the faeces, and this may be related to the faecal mass excreted, as the addition of 30% cellulose or bran to a control diet increased faecal dry matter excretion, and faecal thyroxine loss increased by 300%.

The authors suggested several possibilities for this increased faecal loss:

(1) Mechanical, the faecal mass being so great that faecal contents move too rapidly to allow reabsorption of biliary thyroxine.

(2) The rate of production of bile or concentration of thyroxine in the bile may be altered by certain dietary substances.

(3) The capacity of the intestine to reabsorb thyroxine secreted into it may be chemically altered by some substances.

(4) Thyroxine remaining in the intestine for prolonged periods may be broken down by bacteria with the release of the thyroxine iodide, making that iodide more readily available for reuse by the thyroid.

From the experimental results obtained, the promotion of faecal thyrox-

ine excretion by increased cellulose intake would favour the first and last alternatives. However, it has been shown that the addition of walnuts to a rat diet increases faecal thyroxine loss (Linazasoro *et al.* 1970). Such an effect may be due to substances which affect the biliary secretion or intestinal reabsorption of thyroxine. Hillier (1971) demonstrated that biliary thyroxine is predominantly bound to bile salt micelles, the free thyroxine being similar in concentration to the serum thyroxine level; therefore any substance which stimulated increased bile flow would probably increase biliary secretion into the intestine. Whether or not it is reabsorbed from the intestine would depend upon some of the other factors proposed by van Middlesworth (1957).

THYROID ACTIVITY DURING PREGNANCY AND FOETAL LIFE

The thyroid gland has some influence on almost every stage of reproduction, many of the effects probably being due more to general metabolic effects brought about by the thyroid hormones (Myant 1964).

Robertson and Falconer (1961) reported that in ewes there was a general rise in serum protein-bound iodine and release of iodine from the thyroid during oestrus. Falconer (1963a), however, showed this was not essential for conception as thyroidectomy had no apparent influence on either the oestrus cycle or conception. During pregnancy there is a slight increase in rate of thyroxine turnover in sheep, but this is of doubtful importance in view of the minor increase involved (Annison and Lewis 1959; Robertson and Falconer 1961). Bruce and Sloviter (1957) found in mice, and Chou (1944) in rabbits, that there is a prolongation of gestation if thyroid

deficiency is initiated before pregnancy begins. This may be related to an effect on parturition (Chou 1944) or a slight reduction in the size of litters (Bruce and Sloviter 1957) which would reduce the effect of one of the factors which initiate parturition.

Maternal thyroid hormones do not appear to be important in early foetal development, as they do not pass across the placenta during the first part of pregnancy (Hall and Myant 1956; Contopoulos *et al.* 1964), and only slowly during the latter period (Osorio and Myant 1960; Grumbach and Werner 1956; Myant 1958). This increase in hormone transport across the placenta with advancing pregnancy may be a result of

(1) Increasing permeability of the placental tissue.

(2) An increase in surface area of the placental blood vessels.

(3) An increase in blood flow in the placenta.

(4) A change in thyroxine-binding proteins.

(Osorio and Myant 1960)

Evidence for change in the foetal thyroxine-binding proteins has been presented by Myant and Osorio (1959) who reported the absence of albumin and gamma globulin in foetal rabbits until the nineteenth day of pregnancy. Following their formation, these compounds gradually changed until they were similar to the adult form. Foetal thyroxine binding proteins had only 10% of the binding capacity of the adult, but showed 5 times the affinity for thyroxine.

Although the passage of thyroid hormones across the placenta is limited, iodide is actively accumulated by the foetus, (Gorbman *et al.* 1952; Contopoulos *et al.* 1958; Crone and Waagϕ1961; Miller *et al.* 1967), possibly by a method similar to thyroidal iodide uptake, as the placental iodide transfer can be inhibited by perchlorate (Crone and Waagϕ1961).

Because of the lack of thyroxine derived from maternal sources, some attention has been centered upon the stage when the foetal thyroid becomes

active. Hogben and Crew (1924), using metamorphosis of amphibians as a biological test for thyroxine, discovered that the foetal sheep thyroid produced hormone in the third month of gestation, while production began in the ox foetus in the fourth month. The pituitary gland of both species began secretion in the third month. When radioiodine became available Barnes *et al.* (1957) demonstrated uptake of iodine by foetal sheep thyroids on the 50th day of gestation and formation of thyroid follicles on the 52nd day.

Koneff *et al.* (1949), working with calf thyroids, reported that between the 58th and 70th days of gestation the thyroid consists of proliferating masses of branching epithelial plates or cords, one to three cells wide and surrounded by well developed blood and lymphatic capillaries. There were many nuclei undergoing mitosis and the cytoplasm contained no visible colloid. Colloid first appeared between the 75th and 88th days, it moved to the cell borders and eventually passed into intercellular spaces marking the centre of future follicles. Follicular growth began at 120 days and continued until 205 days after conception. Organically bound iodine compounds appeared between the 53rd and 70th days, but these may not have been thyroxine or triiodothyronine, as Beierwaltes (1967) showed in dog thyroids, that diiodotyrosine and monoiodotyrosine were formed earlier than the thyronines. It is still most likely that the evidence of Hogben and Crew (1924) is the more accurate for the time of initiation of thyronine production, as the test they used was specific for the presence of thyroxine.

Uptake of radioactive iodine by the foetal sheep thyroid was found to increase rapidly during the fourth month of pregnancy by both Barnes *et al.* (1958) and Wright and Sinclair (1959), as shown in Table 1:11, but these authors used different criteria for thyroid function. Barnes *et al.* estimated the relative storage of the two thyroids by equilibrating the maternal and foetal thyroids with daily radioiodine doses over

TABLE 1:11

Radioactivity/g foetal thyroid as a ratio of maternal activity

	Days of Pregnancy				
	70	85	100	115	120
Barnes *et al.* (1958)	0.2	0.3	0.7	1.5	2.5
Wright & Sinclair (1959)	1.2	1.2	1.2	3.0	4.6

long periods, while a single dose as administered by Wright and Sinclair allowed an estimate of rate of turnover. The latter authors suggested the difference in activity to be due to the foetal thyroid having an inferior iodine binding capacity early in foetal life.

Wright and Sinclair (1959) also found that the fourth month of pregnancy was most important in development of goitre in lambs born to kale-fed sheep. Table 1:12 demonstrates the degree of goitre in lambs born to ewes fed on kale for different periods during pregnancy.

Administration of thiouracil was also shown to markedly reduce uptake of iodine by the foetus during the fourth and fifth months of pregnancy (Wright 1959). In mice thiouracil has been demonstrated to act by supressing formation of colloid and thyroid follicles in the foetal thyroid (Kauffman *et al.* 1948), and it is likely that a similar result would be obtained with other animals.

Concentration of radioiodine in the foetus occurs in organs other than the thyroid. The foetal calf stomach has been reported to accumulate more iodine than the foetal serum (Miller *et al.* 1967), and similar results have been recorded in rabbits (Crone and Waagϕ 1961). The foetal part of the placenta concentrates iodine in rabbits (Crone and Waag 1961), cows (Miller *et al.* 1967), pigs and sheep (Contopoulos *et al.* 1964).

Perchlorate inhibits accumulation of radioiodine in both the placenta and foetal stomach, indicating that there may be a form of active transport operating in these organs (Crone and Waagϕ 1961).

There is a large accumulation of iodine within the foetus towards parturition, possibly because the only route of excretion is back across the placenta (Gorbman *et al.* 1952).

Following birth there is a rapid decrease in both total and protein-bound iodine content of the serum, suggesting a high rate of both faecal and urinary iodine loss (Miller *et al.* 1967). Little is known of the need

TABLE 1:12

Size of lamb thyroids after ewes have grazed kale

Period on kale (days of pregnancy)	nil	30 - 90	60 - 90
Mean lamb thyroid wt.	1.0 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
Period on kale (days of pregnancy)	90 - 120	90 - 142	120 - 142
Mean lamb thyroid wt.	2.5 ± 0.5	3.2 ± 1.1	1.6 ± 0.1

(From Wright and Sinclair 1959)

for high iodine levels at birth, but one factor may be related to the initiation of breathing. Redding *et al.* (1972) discovered that in the rat, thyroxine is a potent regulator of lung surfactant metabolism; increased thyroxine being associated with greater availability of surfactant. This surfactant lowers surface tension at the alveolar fluid-air interface, facilitating inflation of air sacs with relatively small changes in air pressure. Inadequate surfactant production, or excessive degradation may be responsible for respiratory distress in the newborn.

Although the thyroid does not appear to be necessary in oestrus or conception, thyroid deficiency in the ewe reduces pre- and post-natal viability of the lamb (Falconer 1965), despite the presence of an apparently adequate foetal thyroid, as Table 1:13 indicates.

During lactation, the mammary gland becomes an active concentrator of iodide. Falconer (1963b) with sheep, showed that 40-60% of a dose of radioiodine appeared in the milk as I^- within 140 hours, and Potter *et al.* (1959) recovered 50% of a radioiodine dose from the milk and mammary tissue of rats within 24 hours. Milk collected from dogs following an injection of I^{131} contained 30-60% of the radioiodine in a protein-bound form (van Middlesworth 1956), while milk from rats contained up to 86% of the iodine in ~~iodinated~~ organic form, mainly as monoiodotyrosine and diiodotyrosine (Potter *et al.* 1959). Injection of radioactive iodothyronines resulted in very small amounts of radioiodine being accumulated in the milk of both rats (Potter *et al.* 1959) and rabbits (Brown-Grant and Galton 1958). In both rats and cows (Potter *et al.* 1959; Lengemann 1965) perchlorate was found to be a potent inhibitor of iodine accumulation by the mammary gland, reducing milk iodine levels below those of the plasma, but not blocking incorporation of iodine into organic molecules within the gland. Propylthiouracil however did not block iodine accumulation but prevented formation of iodinated tyrosines (Potter *et al.* 1959). The mammary gland thus appears to be analogous to the thyroid in activity.

TABLE 1:13

Effect of thyroid on lamb viability

	No. ewes	No. lambs	Dead at birth	Died within 1 week
Thyroidectomised Ewes	9	6	4	2
" " with regenerated thyroid	3	4	2	-
Sham operated controls	12	8	-	1

(From Falconer 1965)

On the basis of the published literature, a series of experiments were designed to examine the nature of congenital goitre in Tasmanian sheep. These experiments were primarily to determine the major cause of goitre in these sheep, including identification of any goitrogenic agents present in a known goitrous area, and examination of the iodine requirements, and supply, in a closed flock bred in this goitrous area.

MATERIALS, METHODS AND RESULTS

Each experimental section (field work, plant growth studies and animal house sheep experiments) will be dealt with separately, but as some analyses are common to each of the sections the methods used will be detailed first. The results of a survey to determine the distribution of congenital goitre in sheep in Tasmania will also be presented.

GOITRE SURVEY

In order to assess the extent of congenital goitre in Tasmania, veterinarians of the Tasmanian Department of Agriculture were asked to provide information on the location of properties on which lambs with goitre had been found in the past twenty years.

From this survey, the distribution of ovine goitre in Tasmania was determined (Fig. 2:1). These outbreaks, when contrasted with the number of properties with breeding ewes in each municipality* (Table 2:1, Commonwealth Bureau of Census and Statistics 1971) showed that congenital goitre was not randomly distributed throughout the breeding ewe population. It was found that three municipalities (Bothwell, Hamilton and Longford) had a significantly ($P < 0.01$) higher incidence of goitre than the Tasmanian average (statistical analysis performed by Dr. N. McGlashan, Uni. of Tas.).

The basis of this non-random distribution is not readily apparent as these regions do not have any obvious climatic or geological similarities that distinguish them from non-goitrous areas.

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*Breeding ewe data could only be obtained on a municipal basis.

Figure 2:1

DISTRIBUTION OF OVINE GOITRE IN TASMANIA.

(From information supplied by veterinarians of Tas. Dept. of Agriculture)

Properties with diagnosed goitre

TABLE 2:1

Distribution of Breeding Ewes and Congenital Goitre by Municipality

Map No.	Municipality	Prop. with Breeding Ewes	No. Properties with Goitre Observed	Expected*
1	Kingborough	39	1	0.56
2	Hobart	4	-	0.06
3	Glenorchy	20	1	0.29
4	New Norfolk	105	5	1.52
5	Brighton	104	-	1.51
6	Clarence	81	-	1.17
7	Sorell	125	-	1.80
8	Green Ponds	78	2	1.12
9	Richmond	152	1	2.19
10	Spring Bay	56	-	0.81
11	Glamorgan	59	-	0.85
12	Bruny	21	-	0.30
13	Esperance	21	-	0.30
14	Port Cygnet	26	1	0.37
15	Huon	42	3	0.61
16	Tasman	50	-	0.72
17	Launceston	2	-	0.03
18	King Island	82	-	1.18
19	Circular Head	53	-	0.76
20	Wynyard	106	-	1.54
21	Burnie	67	-	0.97
22	Penguin	76	-	1.09
23	Ulverstone	175	-	2.52
24	Kentish	198	-	2.86
25	Deloraine	265	1	3.82
26	Devonport	59	-	0.85
27	Latrobe	95	-	1.37
28	Beaconsfield	132	-	1.90
29	Georgetown	46	-	0.66
30	Lilydale	110	-	1.58
31	Scottsdale	92	-	1.33
32	Ringarooma	70	1	1.01
33	Fingal	80	1	1.15
34	Portland	43	-	0.62
35	Flinders	125	-	1.80
36	Westbury	246	1	3.54
37	Longford	211	11	3.04**
38	St. Leonards	88	-	1.27
39	Evandale	74	3	1.07
40	Hamilton	136	13	1.96**
41	Bothwell	53	11	0.76**
42	Campbell Town	73	1	1.05
43	Ross	34	-	0.49
44	Oatlands	247	-	3.57
45	Waratah	1	-	0.01
46	Zeehan	-	-	
47	Queenstown	-	-	
48	Gormanston	-	-	
49	Strahan	- -	-	
	Total	4022	58	

*If goitre was not associated with a specific area then the incidence should be 1.4% of the population (i.e. total number of properties divided by number of properties with goitre).

ANALYTICAL AND SAMPLING TECHNIQUES

IODINE ANALYSIS

The major difficulty in analysis of plant and tissue iodine is to prevent the loss of iodine by volatilization during oxidation of organic matter. A number of standard methods were tested using I^{131} tracer techniques. The method of Mitchell (1965), which involves combustion of the sample under pressure, was found to be erratic in that recovery of standards was not reproducible. Modifications of serum iodine analyses, such as chloric acid digestion (Hoch *et al.* 1964), and alkaline incineration (Brown *et al.* 1953; Acland, 1957 and Grossman and Grossman, 1955) were also found to give variable I^{131} recoveries.

The method finally adopted was a modification of that developed by Borst Pauwels and van Wesemael (1961). This is a wet digestion method, and the iodine is determined by its catalytic action on a cerium-arsenic reaction. The method is as follows (Chemical brand names, where given, were the only reagents which were found not to inhibit the reaction).

Reagents

a) Sulphuric acid, B.D.H. Aristar

b) Iodine free water made by distilling glass-distilled water from a flask containing 50g of Na_2CO_3 per 20 litres of water (Grossman and Grossman, 1955).

c) Newman acid: 1 vol. conc. HNO_3 (A.R. grade) plus 1 vol. conc. H_2SO_4

d) Perchloric acid (A.R. grade)

e) Acid arsenic: Dissolve 9.8g arsenic trioxide in 14ml 10N NaOH, then add 600ml water, neutralize with 10N H_2SO_4, add 42ml conc. H_2SO_4, followed by 10ml conc. HCl (A.R. grade), dilute to 1 litre.

f) Ceric solution: Dissolve 5g ceric ammonium sulphate, low in other rare earths, (Ajax Chemical Co. or Merck Chemical Co.) in 70 ml of 5N H_2SO_4 with gentle heating. After filtering, dilute with distilled water to 100 ml. For use, this solution was diluted with 3 volumes of 3.5N H_2SO_4. The ceric

ammonium sulphate was previously purified by washing 15 g with 75 ml of 96% ethanol (redistilled).

Method

Plant material dried at 65° C to constant weight was ground to pass a 2 mm sieve, and a 200 mg sample was taken and placed in a 50 ml Pyrex volumetric flask together with 7.5 ml Newman acid and 1.0 ml perchloric acid. The flasks were fitted with lead collars and heated to 180°C by submerging in a heated oil bath.

After cooling, the digests were diluted with water to 40 ml (with constant shaking). One ml of acid arsenic was then added and the digest diluted to 50 ml with water. The digests were allowed to stand overnight, prior to 10 ml being pipetted into a test tube containing 1.0 ml of acid arsenic. The tubes were equilibrated in a water bath at 30° C for approximately 30 minutes, then 2.0 ml aliquots of ceric solution were added to each tube in sequence at intervals of 1 minute. The transmittance of each solution was measured in a spectrophotometer at 420 nm exactly 20 minutes after addition of the ceric solution.

Two blank determinations were included in each run. Following blank correction the iodine content of standard plant samples was reproducible between runs to within 2%. Iodine levels were calculated by reference to a standard curve of range 0-1000 µg/kg.

Soil, water and urine analyses could be performed by this method, using approximately 100 mg of soil, 0.5 ml of water or 0.2 ml of urine in the initial digestion.

Precautions

(1) In preparation of the standard curve it was necessary to constantly agitate the standard iodine solution when dispensing aliquots for digestion.
(2) Glassware was cleaned by soaking in 95% conc. H_2SO_4: 5% conc. HNO_3 for at least 48 hours before initial use. Between digests it was sufficient to rinse several times with double distilled water.

This method was not suitable for the determination of protein bound iodine (P.B.I.) of serum because of an undetermined inhibition of colour development in the arsenic-cerium step.

A number of methods of estimating serum P.B.I. were attempted, including the methods of Brown *et al.* (1953); Grossman and Grossman (1955); Acland (1957); Yee *et al.* (1967), and the method developed by workers in C.S.I.R.O. (Thorburn, 1971 pers. comm.) which estimates thyroxine content.

None of these methods gave reproducible results due to loss of iodine during digestion or incomplete binding to resins, and thus it was not possible to measure serum P.B.I. levels of ewes or lambs in later field work.

THIOCYANATE ANALYSIS

Thiocyanate was analysed by the method of Brown (1971 pers. comm.).

Reagents

a) Tricarboxylic acid (T.C.A.) 20% in water

b) Arsenious acid 2% in water (saturated)

c) Bromine water (saturated)

d) Pyridine - 60 ml pyridine + 10 ml conc. HCl + 30 ml water.

e) Colour reagent - mix pyridine solution with aniline (98:2, v/v) immediately before use

Method

Two ml serum, 3.0 ml H_2O and 5.0 ml T.C.A. were added to a centrifuge tube. After 10 minutes this was centrifuged and 2.0 ml of supernatant were transferred to a test tube, followed by 0.1 ml of bromine water. After mixing, 0.25 ml of arsenious acid was added and the bromine vapour removed with a stream of air. Three ml of colour reagent was added, the

solution was mixed and allowed to stand for 5 minutes to allow colour development. The final colour was measured in a spectrophotometer at 495 nm, and thiocyanate level was determined from a standard curve, of range 0-10 µg/ml.

NITRATE ANALYSIS

Plant nitrate was determined by the method of Baker and Smith (1969), using an Orion Nitrate Electrode. Serum nitrate was determined by the method of Woolley *et al.* (1960) as modified by Menary and Carseldene (Menary, 1971 pers. comm.).

PASTURE SAMPLING

Collections of plant material were made at approximately six-weekly intervals throughout each year. Each species was collected randomly throughout each plot to minimise any effects of minor variations of soil iodine levels. Specimens were collected by cutting individual plants close to the ground. Each species was stored in a plastic bag to reduce transpiration, dried at 65^{o} C as soon as possible (within 4 hours), ground to pass a 2 mm sieve and stored in airtight glass containers for later analysis.

Pasture composition was estimated on a percentage basis by visual estimation of 100 quadrats (area of 30.5 cm square) distributed randomly in

each plot. These estimations were carried out during May and October of 1970 and 1971.

EWE AND LAMB BLOOD SAMPLING

At approximately monthly intervals, blood samples were taken from the jugular vein of each ewe into evacuated glass containers. The samples were allowed to coagulate, centrifuged and the serum collected and frozen for later analysis.

Samples of lamb blood were taken by heart puncture and then treated similarly to the ewe blood samples.

THYROIDAL RADIOIODINE UPTAKE

The counting equipment consisted of a Nuclear Enterprise Scalar Spectrometer, with a probe containing a 5 cm sodium iodide crystal. The scintillation probe was enclosed within a lead collimator which projected 6 cm beyond the crystal face.

Ewes were injected i.p. with approximately 20 µl of I^{131} carrier free in phosphate buffer. Prior to injection the activity of iodine in each syringe was measured by counting it in position in a phantom thyroid consisting of a paraffin block with a syringe position 1 cm from the edge. The

activity of iodine remaining in each syringe was measured after injection, so that the activity of the injected dose could be calculated.

Thyroid radioactivity was measured at 2, 6 (or 7) and 24 hours post injection. The thyroid was located by palpation where possible, but if the gland could not be located the point of maximum counting rate was used. Three counts of 10 seconds each were made and the mean determined.

Thyroid radioactivity was corrected for decay and expressed as a percentage of the injected dose.

LAMB EXAMINATION

During each lambing season every lamb was weighed and its thyroid palpated within 10 hours of birth. The thyroid of any lamb which died within 4 days of birth was removed and fixed in 5% formalin.

The classification by palpation of thyroid enlargement was as follows:-

n normal

\+ slightly enlarged, possible to feel by palpation

++ enlarged to 2.5-5 cm in length

+++ enlarged to 5-7.5 cm in length

++++ grossly enlarged, visible without palpation

(Based on Sinclair and Andrews, 1954)

Histological sections were prepared by courtesy of Mr. R. Mason, Tas. Dept. of Agric., Mt. Pleasant Lab. from thyroids of animals which had died.

Examples of thyroids from these classification groups are shown in Fig. 2:2 and representative histological sections are shown in Figs. 2:3 to 2:7.

Figure 2:2

THYROID GLAND SIZE RANGE AND CLASSIFICATION

GLAND	A	B	C	D
THYROID CLASSIFICATION	N	+++	++	++++
WEIGHT	0.8 g	26.9 g	4.9 g	65.5 g

Figure 2:3

Normal Lamb Thyroid Section. Thyroid wt. 0.8 g.

Note regular follicular shape and cell size, colloid filled follicles.

Mag X 750


A
B
C
D

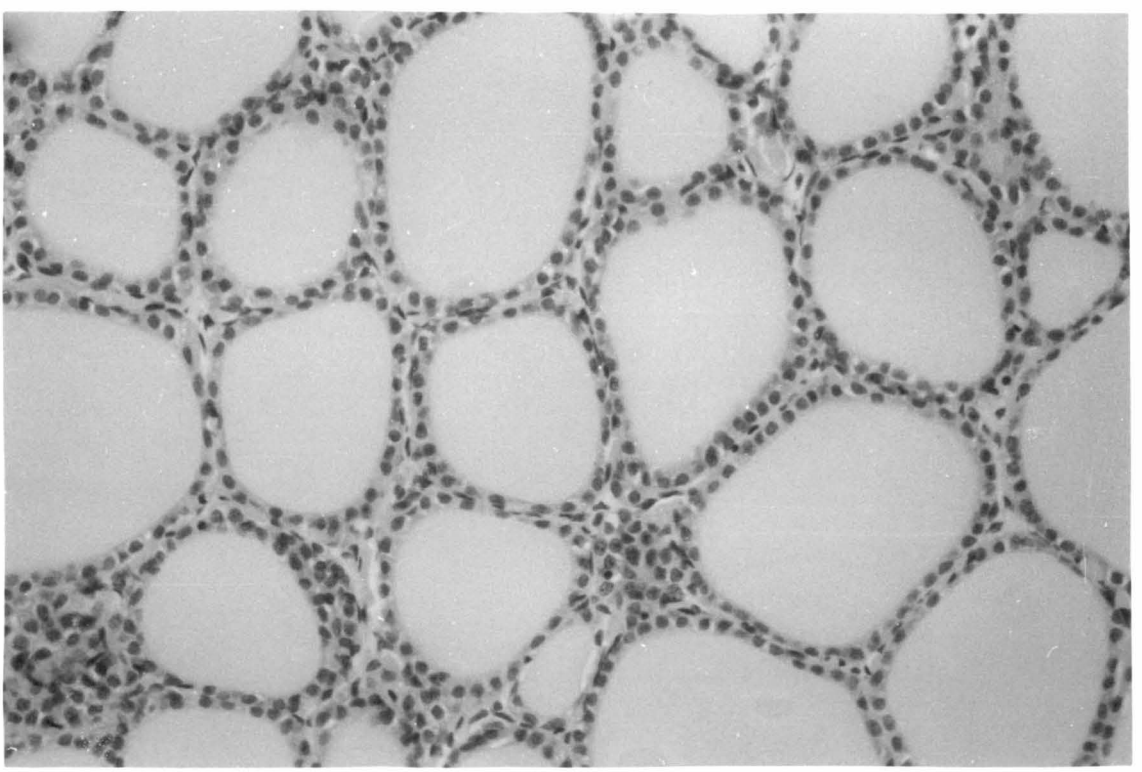


Figure 2:4

Lamb Thyroid Section: classification +; weight 2.7 g

Note some excess cell division and cell enlargement.

Follicles variable in size and colloid filled.

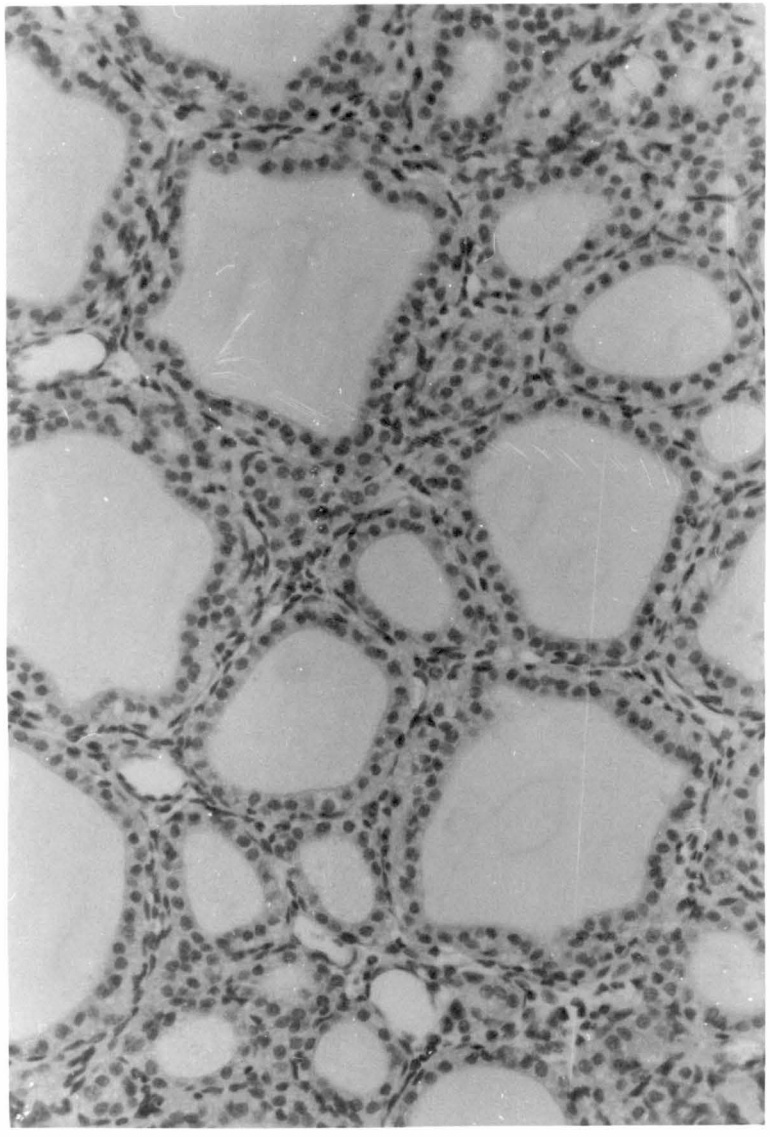
Mag X 750

Figure 2:5

Lamb Thyroid Section: classification ++; Thyroid wt. 4.9 g. Note colloid depletion in follicles.

Excessive cell division and cell growth so that follicles are becoming invaginated.

Mag X 750



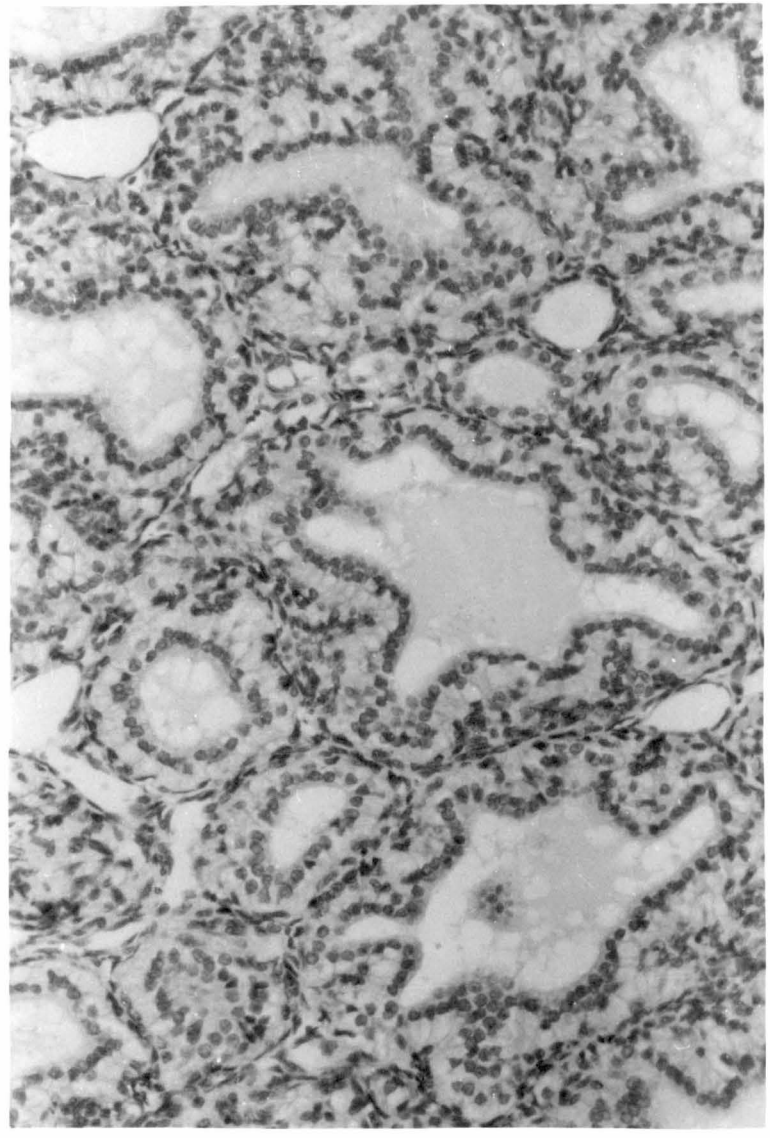


Figure 2:6

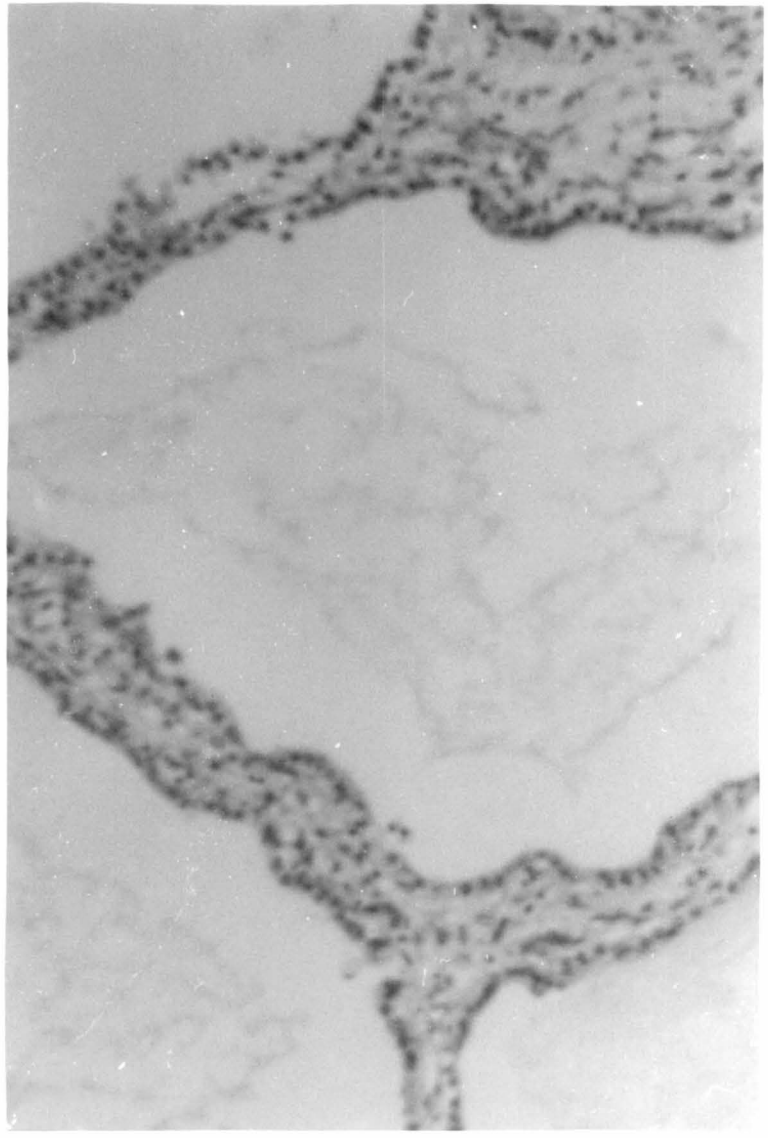
Lamb Thyroid Section: classification +++; Thyroid wt. 26.9 g. Note colloid depleted follicles.
Excess cell division and cell enlargement causing invagination of follicles and collapse of smaller follicles.

Mag X 750

Figure 2:7

Lamb Thyroid Section: classification ++++; Thyroid weight 65.5 g. (Partly autolysed). Note extremely large size of follicles. Only remnants of colloid.
Cells enlarged.

Mag X 750



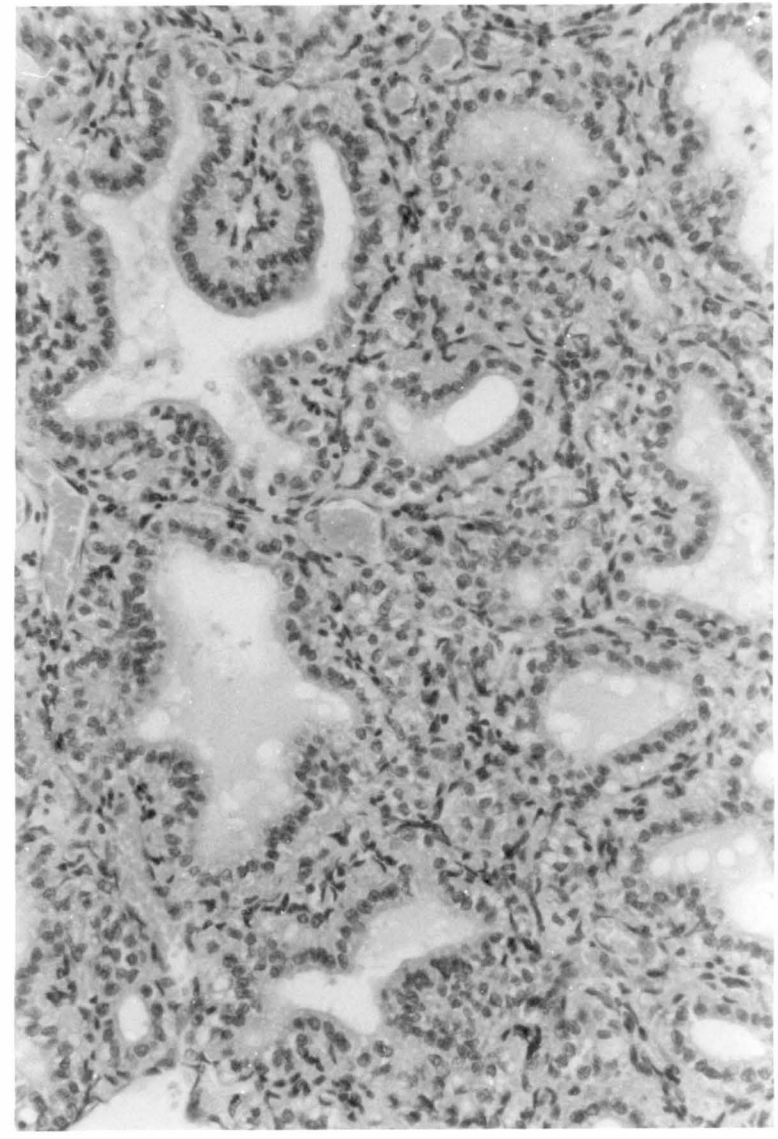


Figure 2:6

Lamb Thyroid Section: classification +++; Thyroid wt. 26.9 g. Note colloid depleted follicles.
Excess cell division and cell enlargement causing invagination of follicles and collapse of smaller follicles.

Mag X 750

Figure 2:7

Lamb Thyroid Section: classification ++++; Thyroid weight 65.5 g. (Partly autolysed). Note extremely large size of follicles. Only remnants of colloid.
Cells enlarged.

Mag X 750

Lambs from feeding experiments in the animal house were weighed within 8 hours of birth then killed with an injection of 15 ml of Nembutal (Pentobarbitone sodium) into the heart. The thyroid was removed and fixed in 5% formalin for later histological examination.

GRAZING TRIAL

Since congenital goitre in sheep in Tasmania can be prevented by iodine supplements, it can be argued that thiouracil type goitrogens are not important as causative agents. Thus goitrogens, if present, would be of the thiocyanate type. If this latter type of goitrogen is implicated, then thiocyanate or nitrate might appear to be the most likely compounds involved under grazing conditions.

A grazing trial was established in May 1970 at Dennistoun, approximately 3 miles north of Bothwell. This property was chosen since extensive congenital goitre was found in the general farm flock during the 1968 lambing season. In the same season ewes in a Dept. of Agriculture stocking rate trial established on sandy soil at Dennistoun had up to 80% lamb mortality, associated with a high incidence of goitre (R.R. Shepherd, 1970 pers. comm.).

The present grazing trial was established to investigate the cause of this goitre outbreak, and also to examine the suggestion of Cunningham (1955) that the sporadic nature of goitre in lambs may be related to increased cyanogenic glucosides of clover in some years in an environment of marginal iodine deficiency.

The extent of the contribution of thiocyanate (from cyanogenic glucosides) and nitrate to the incidence of goitre in lambs was studied in the first two years of the trial. Following this, the grazing trial was used to measure the relative importance of contributions of iodine from food and water to the pregnant ewe's iodine nutrition.

DISTRICT AND CLIMATE

Average monthly rainfall at Dennistoun, Bothwell (40 yr. average) and monthly rainfall for 1970-73 are shown in Fig. 2:8 (from data provided by G.B. Edgell). The mean daily maximum and minimum temperatures for each month (7 yr. average, Hobart Meterological Bureau) and daily average maxima and minima for 1970-73 are shown in Table 2:2.

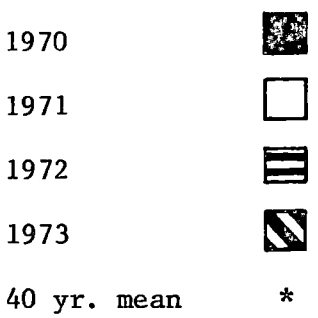
It can be seen from Fig. 2:8 that rainfall is distributed throughout the year, but during two periods, December to mid February and June to September, other factors reduce the effectiveness of rain in promoting pasture growth. In winter, (June-September) temperatures are generally below those necessary to allow grass growth (10^{o} C, Alberda 1966), while in summer the evaporation rate is high enough to prevent any normal rainfall being effective in promoting plant growth.

Effective autumn rainfall occurs in the period February to May, with the amount of rain in the earlier part of this period determining, to a large extent, the amount of autumn growth and, therefore, feed availability during the period of ewe pregnancy (April to September).

Rainfall during the autumn growing season was particularly good in 1970, 1971 and 1973, with 204, 205 and 250 mm of rain respectively (40 yr. average 156 mm). In 1972 autumn rain was limited to 71 mm, and this depressed plant growth which in turn limited feed availability in August and September. Figure 2:9 shows representative pasture heights in the field trial in August 1971 and 1972. In 1971, the pasture was 4.8 cm in height, with dead material remaining from the previous summer. In 1972 the pasture was less than 2 cm in height and almost all the dead material had been eaten.

Figure 2:8

Recorded Rainfall Distribution at Dennistoun, Bothwell



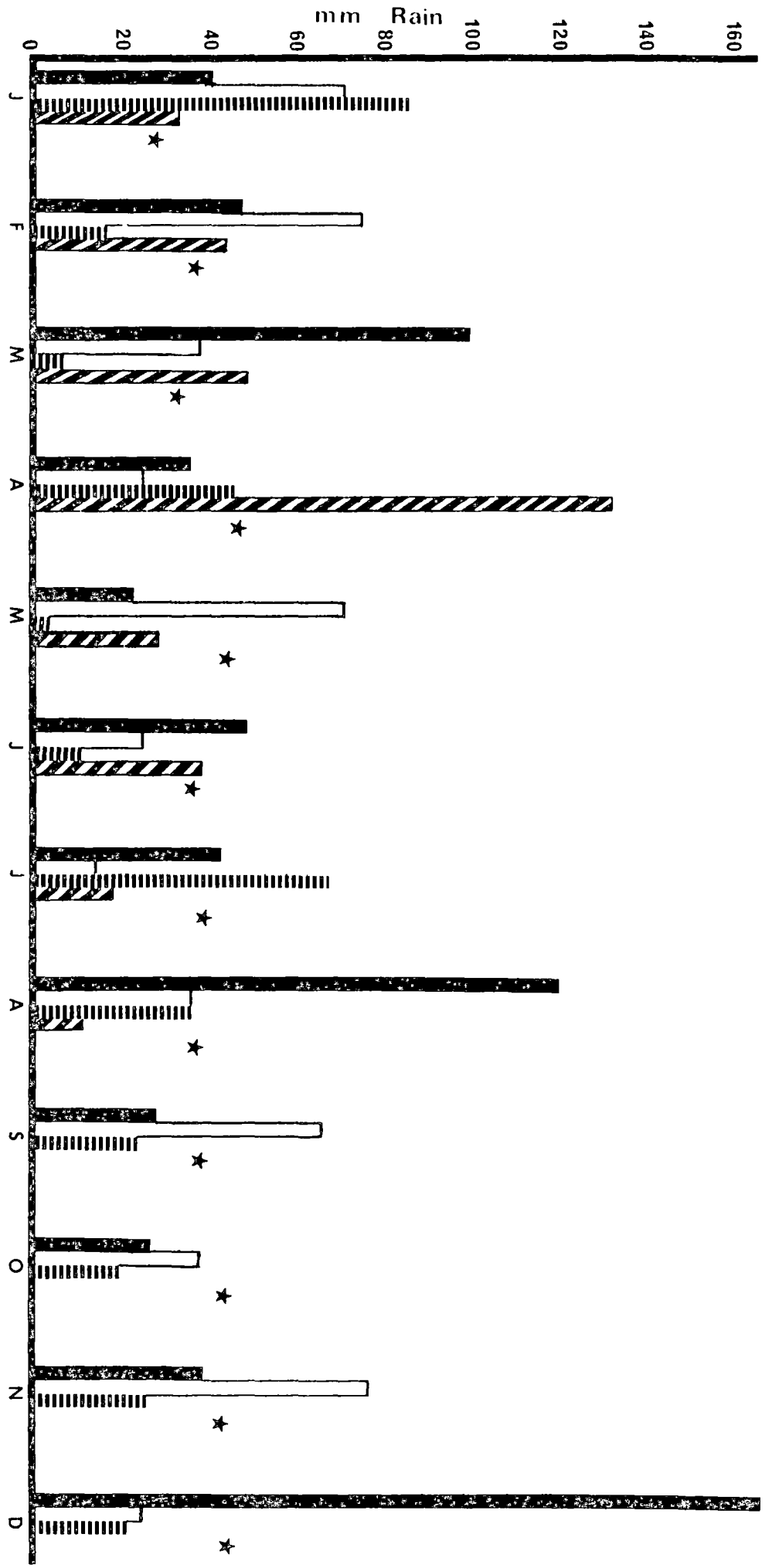

mm Rain
0
20
40
60
80
100
120
140
160
J
F
M
A
M
J
J
A
S
O
N
D

TABLE 2:2

Bothwell Township Average Daily Maximum and Minimum Temperature

Maximum ^{o}C

	J	F	M	A	M	J	J	A	S	O	N	D
MEAN	22.6	23.0	20.6	16.8	13.0	11.2	10.9	11.7	12.9	16.5	17.6	19.9
1970	21.8	21.8	20.1	17.8	12.3	11.4	10.4	10.3	11.1	15.5	18.4	18.6
1971	21.8	24.1	22.0	18.5	13.0	11.3	11.3	11.1	12.7	-	16.8	20.7
1972	22.0	25.9	18.0	18.1	14.5	11.3	9.8	12.3	15.5	17.5	19.2	20.0
1973	24.5	22.8	18.1	17.5	13.1	9.4	10.9	12.0	14.7			

Minimum ^{o}C

	J	F	M	A	M	J	J	A	S	O	N	D
MEAN	7.3	7.2	6.4	3.9	1.7	-0.1	-0.2	0.3	1.6	2.6	5.2	6.1
1970	9.0	5.1	5.9	5.4	0.5	0.4	1.1	0.8	1.1	3.1	5.3	6.1
1971	10.4	9.3	6.8	3.2	0.9	-0.2	-2.3	-0.8	1.8	3.1	5.9	6.7
1972	6.5	8.9	3.9	5.0	0.8	-1.8	-0.2	0.7	4.6	3.6	4.8	7.0
1973	8.6	8.8	6.4	5.4	4.9	-0.5	-0.7	0.4	3.2			

Figure 2:9

A

Photo taken August 1972, showing pasture height following a poor autumn growing season

B

Photo taken August 1971 showing pasture height following a good autumn growing season.
Both photos are from Plot A (sand soil) at the grazing trial, Bothwell.

SITE AND ANIMAL MANAGEMENT

Two grazing plots were established on each of two soil types: plots A and B on a sand adjacent to a Dept. of Agriculture stocking rate trial in which congenital goitre had occurred and plots C and D on a nearby clay soil for comparative purposes. The plot areas were 2.0 ha each on the sand and 1.6 ha on the clay.

The soil types chosen were both alluvial deposits, the first being a sand (loamy) to 28 cm depth overlying yellow sand, and the second, a fine sandy light clay to 18 cm overlying heavy clay.

The sand had a pH of 4.8 and an organic matter content of 1.7% (method of Walkley and Black, as described by Piper, 1947) while the clay had a pH of 5.4 and organic matter content of 5.0%. As is normal on this type of property, water was supplied to the sheep from excavated water holes in the clay soils, and earth channels from the Clyde River to a concrete trough in the sandy areas.

All plots had a stocking rate of 12.5 Polworth ewes per hectare. These ewes were chosen at random from the four-tooth age group in a closed flock comprising the majority of sheep on this property. The pasture in both areas was a 30 year old sward of ryegrass *(Lolium perenne* L.*)* and subterranean clover *(Trifolium subterraneum* L.*)* with a high level of weed infestation, the principal weeds being barley grass *(Hordeum leporinum* Link*)*, Great Brome *(Bromus diandrus* Roth*)*, and vulpia *(Vulpia bromoides* (L.) S.F. Gray*)*.

The plots were set stocked with ewes that had mated and not returned to service by 4.5.71. They were removed for shearing and dipping for only one week in early September. Lambs were born during the first two weeks of October and were weaned in mid-December.

Replacement ewes were added to each plot on 16.3.71 to make up for losses from deaths in the previous year. One 6-tooth Polworth ram from

the closed flock, fitted with a sire-sine harness (Radford *et al.* 1960) was placed with the ewes in each plot on 1.5.71 for six weeks.

The ram in plot C (clay soil) was apparently infertile as normal mating took place, but ewes failed to conceive. Lambing and weaning in the remaining plots was normal and occurred at times similar to the previous year. On 15.3.72, 12 ewes from plot A (sand) were drenched with 250 mg KI to examine the effect of a premating iodine drench on congenital goitre development.

At the same time ewes in plot D (clay) were prevented from drinking the water from the water hole, and rain water from Dennistoun household tanks was supplied in its place. This was done to assess the relative contribution of plants and water to the animals' iodine intake in the clay soil plots, as the water in these plots contained much more iodine (150 µg/l) than that supplied to the sand plots (18 µg/l). This factor may well have been affecting the difference in goitre incidence between the plots, as results from the previous two years indicated that the goitre in lambs was due to iodine deficiency.

Rams were run with the ewes from 7.4.72 for six weeks. On 24.5.72 ewes which had not been marked by rams were removed from each plot and the stocking rate adjusted to 10 ewes/ha, because the relatively poor autumn growing season had resulted in reduced pasture production and it was thought the pastures could not support 12.5 ewes/ha.

At this time, 8 ewes from plot B on the sand were exchanged with a similar number from plot C on the clay soil to examine the effect of a change in the pasture-water iodine intake during the period of pregnancy, on goitre incidence. It was subsequently found that the ewes in plots A and D were not pregnant.

Lambing occurred in September and the ewes and lambs were replaced with four-tooth ewes from the closed breeding flock on 2.12.73, to ensure that all ewes had a previous iodine status as similar as possible. Drink-

ing water was provided in plot A (sand soil) by a water tank, using water from the water hole in plot D (150 µg/l). Plot B (sand soil) had the previously supplied river water (18 µg/l). Plot C (clay soil) ewes were allowed access to the water hole (150 µg/l) while plot D (clay soil) were offered only river water (18 µg/l) provided from a tank.

The intention was to see if ewes drinking water high in iodine during summer could retain enough iodine to influence goitre production in lambs during pregnancy. There appears to be very little information available on the whole body storage of iodine by animals in iodine deficient environments. This could be an important factor in determining the severity of a goitre outbreak in sheep, as it may be possible for a high iodine intake during one period of the year to protect an animal during a later period of low iodine intake.

One 6-tooth Polworth ram with a sire-sine harness was run with each plot of ewes from 1.5.73 until 22.5.73, the plot rams then being interchanged to minimise any problem of ram infertility. On 12.6.73 all rams were removed and the ewes reduced to a stocking rate of 8.5/ha by removal of non-mated ewes and some which had returned to service.

This reduction in ewe numbers was done with the intention of maximising goitre incidence, as Healy *et al.* (1972) had reported higher goitre incidence at a lower stocking rate under New Zealand conditions. The stocking rate of 8.5 ewes/ha was selected because it is average for the Bothwell district.

The ewes were removed for one week in August for shearing and drenching, and lambing began in late September.

RESULTS

LAMBING RESULTS

In 1970 there was a significantly higher goitre incidence ($p < 0.01$; $X^2 = 21.3$) among lambs born in the sand plots than those in the clay plots (Table 2:3). There was however no difference between the groups in lamb mortality, or average birth weight.

In 1971 the results (Table 2:4) were similar to 1970 (Table 2:3) but the degree of goitre in the sand plots was more marked, both in the percentage of goitrous lambs (40%, 1970; 94%, 1971) and the palpable size of the glands (1 +++, 1970; 7 +++, 2 ++++, 1971). Both of these results could be due to the ewes being maintained for different periods (4 months 1970; 16 months 1971) on either a low iodine pasture, or pasture containing antithyroid agents.

At the 1972 lambing (Table 2:5) two groups, A and D were found to be non-pregnant, despite the fact that they had mated normally (as shown by use of a sire-sine harness on each ram). No data ~~is~~ are therefore available from the ewes in these plots. In the other two groups half the ewes on each plot were interchanged, resulting in four treatments, one maintained on each of sand and clay throughout, one maintained on sand during 1970 and 1971 and clay after mating in 1972 and the other maintained on clay during 1970 and 1971, then changed to sand after mating in 1972.

Due to the small numbers involved, no statistical analysis of the data could be carried out. There is strong evidence of a difference in the incidence of goitre, however, as no ewe transferred to the clay soil had a goitrous lamb, whereas 6 out of the 11 lambs born to those maintained on the sand were goitrous. Similarly, no statistical comparison could be made between the extent of goitre in plot B (sand) in 1971 and the ewes maintained in plot B in 1971. Here again, the difference in degree of goitre found (94% in 1971, 55% in 1972) tends to indicate a difference in iodine intake by the ewes in the two years, although the iodine

TABLE 2:3

1970 Lambing results from Bothwell field trial

	Plot			
	A	B	C	D
Soil	sand	sand	clay	clay
Ewes lambed	24	24	19	20
Lambs born	27	31	23	25
Classification				
N	15	20	22	25
+	9	9	1	-
++	3	1	-	-
+++	-	1	-	-
++++	-	-	-	-
No. dying within				
4 days	7	5	6	1
Average birth wt.(kg)				
a) normal	3.7	3.7	4.0	4.0
b) goitrous	3.6	3.7	5.2*	-

* 1 lamb only

TABLE 2:4

1971 Lambing results from Bothwell field trial

	Plot			
	A	B	C	D
Soil	sand	sand	clay	clay
Ewes lambed	23	16	NIL	16
Lambs born	33	21		22
Classification				
N	2	2	INFERTILE RAM	22
+	11	11		
++	15	4		
+++	3	4		
++++	2	-		
No. dying within 4 days	13	5		2
Average birth wt. (kg)				
normal	2.7*	3.5*		3.6
goitrous	3.6	3.9		-

* 2 only

TABLE 2:5

Lambing results from Bothwell field trial, 1972

	Plot			
	B		C	
Plot Soil 1970, 71	sand	sand	clay	clay
Plot Soil 1972	sand	clay	sand	clay
Ewes lambed	8	10	5	7
Lambs born	11	13	9	9
Classification				
N	5	13	8	9
+	2	-	1	-
++	3	-	-	-
+++	-	-	-	-
++++	1	-	-	-
No. dying within 4 days	1	-	1	-
Average birth wt. (kg)				
normal	3.6	3.5	3.2	3.1
goitrous	3.4		5.0*	

* one only

content of pasture plants was similar in both years.

In 1973, lambs from ewes on the sand soil had significantly (X^2 = 28.1; p <0.01) more goitre than those on the clay soil (Table 2:6). There was not a significant difference in lamb goitre incidence between plots in which the ewes had high iodine drinking water and those with low iodine water. This indicates that under field conditions the iodine content of the drinking water of ewes did not appear to be an important factor in iodine nutrition during pregnancy. This was possibly related to the low water intake of grazing ewes during winter, which has been measured at less than 200 ml/day (Brown and Lynch 1972).

All of the thyroid sections from dead lambs were similar to those shown in figures 2:3 to 2:7, with varying degrees of cell hyperplasia and hypertrophy, follicular invagination and almost complete colloid depletion in the more severe cases of goitre.

The mean weight and weight range of excised thyroids of each classification group were:-

N	1.4 g	RANGE	0.8 - 1.9
+	2.8 g	"	2.4 - 3.4
++	10.7 g	"	4.9 -18.9
+++	27.6 g	"	26.9, 28.9 (2 only)
++++	73.9 g	"	65.4, 82.4 (2 only)

All glands were firm and well vascularized, except those in the last group which were soft and spongy to touch. In these glands the follicles were very large compared to normal (see Fig. 2:7).

This classification approximates to the size classification of Andrews and Sinclair (1962), who found normal lambs had thyroids of less than 1.3 g and goitrous lambs greater than 2.8 g. The intermediate group were classed as suspect. In the present classification the top of the "suspect" group has been classed as probably goitrous and the lower part classed as normal. However, classification by thyroid size alone may not indicate the true ext-

TABLE 2:6

1973 Lambing results from Bothwell field trial

	Plot			
	A	B	C	D
Soil	sand	sand	clay	clay
Water	Low I	High I	High I	Low I
Ewes lambed	17	17	14	14
Lambs born	17	18	16	20
Classification				
N	1	1	16	18
+	9	4		2
++	4	8		
+++	2	5		
++++	1			
No. dying within 4 days	2	3	3	4
Average birth wt. (kg)				
normal	5.9*	6.3*	4.2	4.2
goitrous	4.4	4.3		4.3**

* 1 only ** 2 only

ent of thyroid abnormality. In 1970 and 1971 five of the lambs which died in plots A and B (sand) were classified normal by palpation, although upon histological examination, two were found to have abnormal thyroid structure. These lambs' thyroids had follicular invaginations and excessive cell division, and histologically they resembled +++ classification animals. Because of this, it is possible that some of the living lambs classified as normal may have been physiologically goitrous, as Kossila *et al.* (1970) considered thyroid structure to be a more accurate guide to abnormality than size. This effect did not appear to occur in plots C and D on the clay soil in 1970, 71 or 72. In 1973, five lambs from each plot were killed within 24 hours of birth to examine the extent of this condition (Table 2:6a). As there was extensive goitre in lambs in plots on the sand soil (A and B), only 1 normal lamb from this soil type was killed (B5). This lamb however, had a thyroid which was histologically abnormal. The thyroids of lambs from the clay plots were almost all classified normal by palpation, but 6 of the 10 showed some histological abnormality, although this was slight.

The results found from this experiment confirm the observations made in previous years that lamb thyroids, while being normal in size may be histologically abnormal. Although the numbers killed in each plot were small, there is an indication that the lambs in plot D (clay soil, low iodine water) had a higher degree of thyroid abnormality than those in plot C (clay soil, high iodine water). These differences were only slight, being represented by increased cell division and enlarged follicular cell size, but they were similar to the changes induced by a lowered iodine intake in a ewe feeding trial (Table 2:26). These changes may therefore be related to the difference in iodine intake due to the different water iodine contents in the two plots.

TABLE 2:6a

Comparison of the degree of goitre measured by field palpation and histological examination of thyroid sections from lambs in each plot of the field trial at Bothwell, 1973.

Plot	Degree of goitre by palpation	Thyroid wt (g)	*Mean follicular diameter (nm)	Histology
A1	+	2.9	110	Some excess cell division, cells cuboidal, colloid present.
2	++	8.3	110	Excess cell division, follicles invaginated, cells cuboidal.
3	+	2.4	65	Excess cell division, some follicles cell filled, colloid depleted, follicles invaginated, cells cuboidal.
4	++	14.2	65	As for A3.
5	+	3.0	70	As for A3.
B1	++	8.1	78	Excess cell division, little colloid cells H=2w**
2	+++	22.5	160	Some excess cell division, cells cuboidal colloid depleted.
3	++	3.7	100	Excess cell division, colloid depleted, cells H= 2-3w, minor invaginations in most cells.
4	++	5.5	90	Some excess cell division, follicles colloid filled, cells cuboidal, minor follicular invaginations.

Cont.

Plot	Degree of goitre by palpation	Thyroid wt (g)	*Mean follicular diameter (nm)	Histology
5	N	1.8	60	Excess cell division, most follicles filled with cells, colloid depleted, cells, cuboidal.
C1	N	1.2	65	Colloid deeply stained cells cuboidal, some excess cell division.
2	N	1.2	78	Normal cells W = 2H
3	N	2.0	70	As C2.
4	N	1.5	80	As C2.
5	N	1.7	75	Some excess cell division, otherwise normal, cells W = 2H.
D1	N	0.9	60	Some excess cell division, colloid deeply stained, cells cuboidal.
2	N	1.0	60	As D1.
3	+	2.3	60	Normal cells W = 2H.
4	N	1.5	60	As D1.
5	N	1.3	55	As D1.

* Mean follicular diameter was calculated by counting the number of follicles across the diameters of 10 random fields of a calibrated microscope.

** Cell shape is expressed as the relative length of the axis measured towards the centre of the follicle (H) and the one at right angles to this (W).

THYROIDAL RADIOIODINE UPTAKE

Thyroidal radioiodine uptakes were measured to determine whether differences in thyroid activity existed between ewes grazing different pastures on the two soil types.

In 1971, 8 sheep from each of Plots B and C were used to determine radioiodine uptake levels approximately 1 month prior to lambing; the results are summarized in Table 2:7. (Full figures Appendix 1:3; Statistical Analysis, Appendix 2:3).

Although this comparison was invalidated to some extent by the ewes in plot C not being pregnant there is still quite an interesting comparison between the apparently normal uptake pattern of ewes in plot C, and the much more rapid initial uptake found in ewes from plot B, indicating a greater iodine need in the plot B ewes. There was a highly significant difference between the rates of uptake at 2 and 7 hours, but this difference was not apparent at 24 hours.

In 1972, radioiodine uptake measurements were made on ewes from all plots in early (1.6.72) and late pregnancy (24.8.72). Ewes in plots A and D were later shown to be non-pregnant and the uptake results from these ewes were used only to determine whether a change in radioiodine uptake occurred between the two determinations. Statistical analysis of the 6 and 24 hour uptakes for Group A (2 hr uptake were not done in August due to Scalar battery failure) and all 3 uptakes for Group D showed no difference between the two uptake periods existed (Full figures Appendix 1:4; statistical analysis Appendix 2:4). This indicates that there was a similar iodine supply at both times.

In each of plots B and C there were effectively two sub-groups, as each group had been split and half interchanged with the other group seven days prior to the first uptake measurement.

The mean uptakes for each sub-group are shown in Fig. 2:10 (Full figures Appendix 1:4; statistical analysis Appendix 2:4).

TABLE 2:7

Mean radioiodine uptake of ewes from plots B and C, 1971. (% of injected dose).

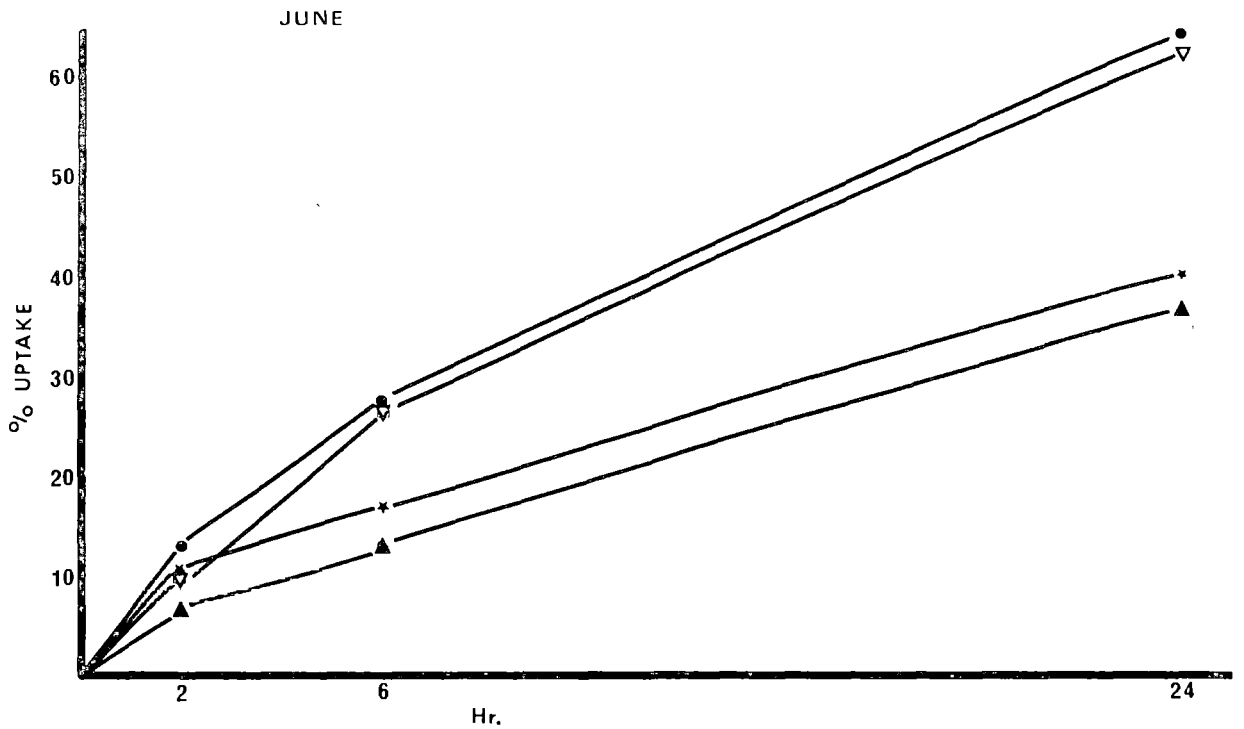
	Time (hr)		
	2	7	24
Sheep on plot B (sand)	25.0	53.2	53.2
Sheep on plot C (clay)	11.8	29.9	47.8

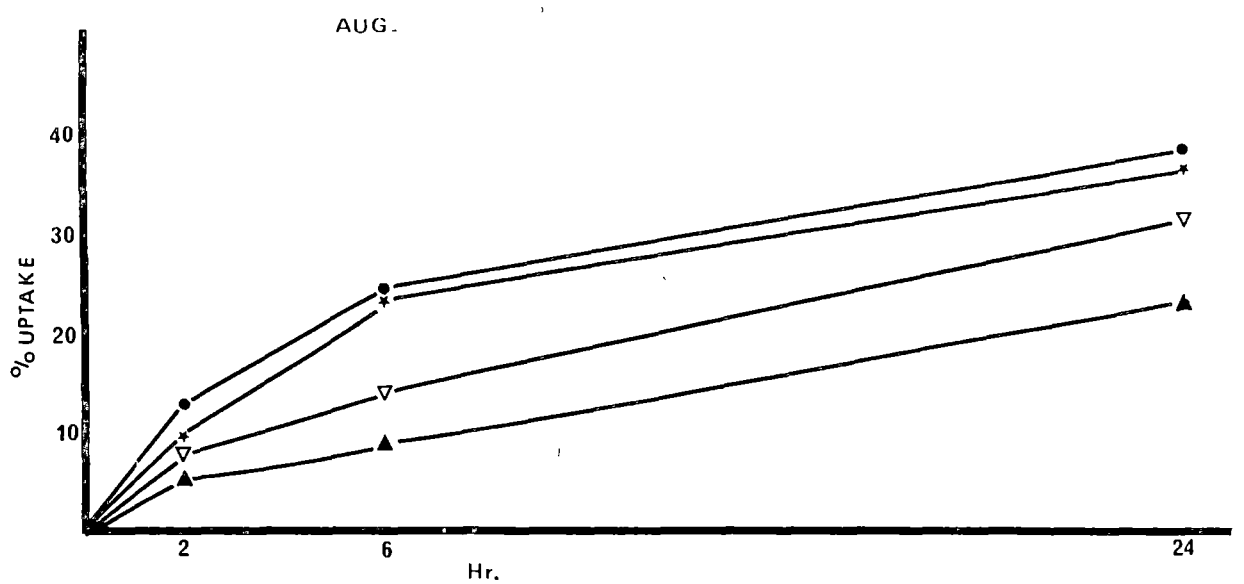
Figure 2:10

Radioiodine uptake of ewes at two stages of pregnancy in Bothwell Field Trial 1972.

The four groups represent ewes maintained on sand or clay soil throughout pregnancy, and also ewes transferred from sand to clay and vice versa.

LEGEND	PLOT 1972	PLOT 1971
●	B (sand)	B (sand)
▲	C (clay)	C (clay)
✱	B (sand)	C (clay)
▽	C (clay)	B (sand)


JUNE
60
50
40
30
20
10
% UPTAKE
2
6
24
Hr.


AUG.
40
30
20
10
% UPTAKE
2
6
24
Hr.

When all the pregnant ewes were considered as a group, it was found that there was a significant difference in radioiodine uptake at 2 hours ($p < 0.05$) and at 24 hours ($p < 0.01$) between the two sampling dates, with the June measurement being greatest in both cases. This difference in uptake between determinations indicates either a general increase in iodine supply over the intervening twelve weeks, or a decrease in the amount of radioiodine available to the maternal thyroid in August. Since this was the fourth month of pregnancy, the foetal thyroid would be actively accumulating iodide and therefore would be competing with the maternal thyroid for circulating iodine (Barnes *et al.* 1958; Wright and Sinclair, 1959).

The lack of change of radioiodine uptake in the non-pregnant ewes, plus the lack of increase in plant and water iodine supplies indicates that there was not a general increase in iodine supply. To the contrary, however, the actual lambing results of 1972 compared to those of 1971 indicate that there was an increase in iodine supply during the intervening year, as the degree of goitre in the sand plot (B) in 1972 was below that found in 1971, although in both years there was little difference in plant iodine level.

In general, transfer of ewes between plots after mating had a significant effect on the thyroidal radioiodine uptake. In June, the transferred ewes had radioiodine uptake patterns similar to those exhibited by the ewes which had remained on the same soil. In both cases the transferred ewes had significantly different uptakes from the ewes on the plots to which they had been transferred.

In August the reverse had occurred, the sheep on each soil type had uptakes similar to one another but between soil types the uptakes were significantly different ($p < 0.01$), with the ewes on the sand plot having the greater uptake.

Plans to measure radioiodine uptakes of ewes in all plots in the final month of pregnancy in 1973 were not carried out because our agent failed to supply radioiodine at that time, and lambing took place before supplies were available.

PLANT AND SOIL MATERIAL

Pasture composition estimates were carried out in 1970 and 1971 (Appendix 1:1), but were discontinued in 1972 as it was felt they were of little use in estimating the composition of feed eaten by the ewes.

Visual estimates by two observers at the times of pasture collection indicated that the two major species grazed were subterranean clover and ryegrass. Ryegrass was eaten throughout the year, but there was a period of approximately 2 months during January and February when subterranean clover was absent from the pasture. Germination of this annual species took place after substantial rain in February.

The annual grasses, barley grass, bromus and vulpia, were grazed as small plants during June and July, but their acceptability rapidly decreased. The two perennial grasses Yorkshire fog *(Holcus lanatus* L.*)* and Tussock Poa *(Poa australis)* tended to be grazed during periods of feed shortage in summer (December to early February) and late winter (October) in preference to the annual species. Plant iodine levels are presented in Appendix 1:2, but as it was considered that ryegrass and subterranean clover were the predominant species grazed (from visual observation of the pasture), only the iodine levels of these two species were analysed for statistical differences (Appendix 2:1), the iodine levels at each harvest being shown in Table 2:8. The plants grown on the clay soil had significantly ($p < 0.01$) higher levels of iodine than those on the sandy soil. Despite this difference, it was only at a few of the harvests from the clay plots that the plant iodine content was as high as that deemed necessary to prevent goitre (300 µg/kg) by Butler and Johnson (1957). A significant difference was also shown between iodine content at different harvest dates and an examination of the mean iodine content of each species at each harvest date showed that in subterranean clover there was a tendency for autumn-winter iodine content to be lower than that found in spring, with the reverse situation in ryegrass (Appendix 2:1), but neither of these trends was

TABLE 2:8

Iodine content of ryegrass and subterranean clover (µg/kg dry weight) collected from the Bothwell Field Trial 1970-73.

Harvest Date	Species	Plot: Sand A	Sand B	Clay C	Clay D
10.6.70	ryegrass	285	170	198	275
	sub clover	120	185	207	243
21.8	ryegrass	172	280	276	246
	sub clover	204	160	220	155
18.10	ryegrass	52	63	290	140
	sub clover	160	66	274	295
12.1.71	ryegrass	170	170	186	350
	sub clover	-	-	-	-
5.3	ryegrass	125	160	173	135
	sub clover	156	102	149	111
13.5	ryegrass	453	327	657	579
	sub clover	460	315	584	593
25.6	ryegrass	262	120	168	251
	sub clover	95	167	242	205
2.8	ryegrass	192	230	257	223
	sub clover	136	141	282	146
24.9	ryegrass	93	167	450	189
	sub clover	158	106	212	137
22.11	ryegrass	58	42	286	127
	sub clover	133	88	250	585
20.12	ryegrass	44	52	260	469
	sub clover	143	247	272	601
9.2.72	ryegrass	157	146	171	323
	sub clover	-	-	-	-
14.4	ryegrass	274	227	587	245
	sub clover	202	135	149	125
18.5	ryegrass	205	286	243	180
	sub clover	192	85	141	56

Cont.

Harvest Date	Species	A	B	C	D
30.6	ryegrass	177	155	318	335
	sub clover	190	40	56	103
10.8	ryegrass	169	153	312	339
	sub clover	163	119	197	242
22.2.73	ryegrass	176	158	202	328
	sub clover	219	237	224	240
12.4	ryegrass	273	214	294	179
	sub clover	200	148	230	264
12.6	ryegrass	199	170	330	344
	sub clover	84	76	102	115
27.7	ryegrass	189	154	321	388
	sub clover	148	106	192	134
16.9	ryegrass	135	147	355	307
	sub clover	158	121	235	134

marked. It was found however, that in both species the plant iodine content at the harvest on 13.5.71 was significantly higher than any other. This harvest was preceded by an application of superphosphate at a rate of 190kg/ha on 14.4.71. Subsequent analysis of superphosphate for iodine revealed that it contained between 19 and 36 ppm iodine. At the next harvest (25.6.71) the plant iodine content had returned to previous levels, possibly because of heavy rain in May washing superphosphate dust from the plants.

Field (1964) suggested that the soil may provide nutrients directly to the grazing animal by ingestion during periods of low feed availability. To examine the iodine content of each soil in the grazing trial, five soil samples were taken at random from the A horizon of each plot and each sample was separated into two fractions, the first being the top 1.5 cm which included approximately 1 cm depth of decaying organic matter, and the second the remainder of the horizon. Examples of this decaying organic matter mat can be seen in Figs. 2:11 and 2:12. Each sample was thoroughly mixed, dried at 65^{o} C and subsampled for iodine analysis, the results being shown in Tables 2:9 and 2:10.

Analysis of variance (Appendix 2:2) shows that there was no significant difference between the soil types for the top 1.5 cm, but in the remainder of the A horizon the clay was significantly higher in iodine ($p < 0.01$). It is also noticeable that the top 1.5 cm of soil is much more variable in iodine content than the remainder of the horizon.

The organic matter of the surface soil was measured by weight loss on ignition. Four samples from each plot were heated to 600^{o} C and the amount of organic matter lost was calculated (Table 2:11, statistical analysis Appendix 2:2). Small errors may be introduced in this method, due to loss of water from between clay particles, but with high levels of organic matter these losses are negligible (Piper 1947).

Shacklette and Cuthbert (1967) found that one of the most important factors in determining the amount of iodine in a soil is the quantity of

Figure 2:11

Organic matter mat on clay soil from grazing trial
(marker in cm intervals)

Figure 2:12

Organic matter mat on sand soil from grazing trial.

TABLE 2:9

Iodine content of the surface 1.5 cm of soil taken at 5 random sites in each of the plots of the Bothwell grazing trial (values in µg/kg dry weight).

	Plot			
	Sand		Clay	
	A	B	C	D
	1554	661	1436	1565
	770	1484	1430	1688
	1240	977	922	1360
	820	926	833	832
	1312	1045	755	983
Mean	1121	1019	1075	1285

TABLE 2:10

Iodine content of the A horizon, excluding the top 1.5 cm, of soil from 5 random sites in each of the plots of the Bothwell grazing trial (values in µg/kg dry weight).

	Plot			
	Sand		Clay	
	A	B	C	D
	228	246	904	920
	298	285	800	853
	230	254	947	875
	260	238	928	816
	247	255	870	903
Mean	253	256	890	873

TABLE 2:11

Percentage weight loss on ignition of samples of surface 1.5 cm of soil from each plot in the Bothwell grazing trial.

	Plot			
	Sand		Clay	
	A	B	C	D
	33	11	19	26
	21	46	30	32
	36	22	35	19
	14	18	16	28
Mean	26	24	25	26

organic matter in it. It seems likely that the variability in iodine content of the surface soil in the grazing trial (Table 2:9), could be due to variability in organic matter content (Table 2:11).

The difference in iodine content between the surface 1.5 cm (Table 2:9) and the remainder of the horizon (Table 2:10) could also be due largely to differences in organic matter content, as the surface layer had a mean organic matter content of approximately 25%, while lower in the horizon the mean organic matter content was 1.7% in the sand and 5.0% in the clay.

ANTITHYROID AGENTS

Antithyroid agents have been implicated in both human and animal goitre in Tasmania (Clements and Wishart 1956; Clements 1957). In congenital goitre of lambs in Tasmania, the curative properties of iodine supplements to ewes would preclude the thiouracil-type goitrogen as a causative agent. However, goitrogens of the thiocyanate type may be involved. Screening tests for the two most likely compounds under field conditions, thiocyanate and nitrate, were carried out.

Ewe serum thiocyanate levels were monitored in 1971. In 1972, pasture nitrate levels were measured at approximately six-weekly intervals. In addition, some ewe and lamb serum nitrate levels were determined.

Blood samples were taken from all ewes on 4.7.70 and 20.8.70 (approximately 65 and 115 days pregnant) and analysed for thiocyanate content as described previously. These two dates were chosen to represent one where the foetus is not well developed, and one in the fourth month, which is the most important period for goitre development in lambs (Wright, 1959).

Correlation of ewe serum thiocyanate levels with the presence or abs-

ence of goitre in the newly born lamb was tested and shown in Table 2:12 (full figures, Appendix 1:5).

The only significant difference in serum thiocyanate levels between ewes which had normal or goitrous lambs occurred in plot B, where ewes which subsequently bore goitrous lambs in September had significantly higher serum thiocyanate levels in July than those which had normal lambs ($p < 0.05$, Student's t test). Since there was not a significant difference in the August thiocyanate levels, it would seem unlikely that this factor was involved in goitre production. The fact that there was a general rise in thiocyanate level in ewes in the plots in which goitre developed, however, suggested that further examination of this factor was necessary. It may be that the effect of thiocyanate was compounded with variation in iodine intake of individual animals, and thus may have contributed to goitre production if the ewe had a low iodine intake.

Plant material collected during 1971 was analysed for nitrate content, but as the major species grazed were subterranean clover and ryegrass, values for these species were separated (Tables 2:13a and 2:13b) and statistically analysed (Appendix 2:5). The nitrate contents of all species are presented in Appendix 1:6.

In both ryegrass (Table 2:13a) and subterranean clover (Table 2:13b) the plants growing in sand were significantly higher in nitrate than those growing on the clay soil ($p < 0.05$). Both species also had varying nitrate contents between harvests. In clover, the nitrate content at the first harvest (March) was significantly higher than any of the others, while the ryegrass was significantly higher in nitrate at the March, May and June harvests ($p < 0.01$; Duncan's Multiple Range test).

The drop in nitrate content of both species with time may have been related to the age of leaf harvested and activity of soil organisms which release nitrate. Wright and Davidson (1964) reported that nitrate content of leaves increases until plants are mature, then decreases after flowering.

TABLE 2:12

Mean ewe serum thiocyanate levels (µg/ml)
of ewes in Bothwell field trial,
July and August, 1970.

	Plot							
	A		B		C		D	
	N*	G**	N	G	N	G	N	G
July	2.1	2.2	1.2	1.6	0.3	-	0.5	0.3+
August	3.2	3.1	1.8	1.8	0.3	-	0.6	0.2+

N* Ewes which subsequently had normal lambs

G** Ewes which subsequently had goitrous lambs

\+ One lamb only

TABLE 2:13a

Nitrate content of ryegrass from Bothwell Field Trial, 1971 (p.p.m.)

	Plot				
	Sand		Clay		
	A	B	C	D	Mean
5.3.71	1756	1430	613	1165	1241
13.5.71	1135	933	680	1049	949
25.6.71	2551	1246	805	1058	1415
2.8.71	752	385	315	455	477
24.9.71	723	411	360	514	502
Mean	1383	881	555	848	

TABLE 2:13b

Nitrate content of sub. clover from Bothwell Field Trial, 1971 (p.p.m.)

	Plot				
	Sand		Clay		
	A	B	C	D	Mean
5.3.71	1347	2349	1153	947	1449
13.5.71	1052	1139	181	696	792
25.6.71	573	624	691	965	714
2.8.71	467	364	135	493	365
24.9.71	461	325	158	406	338
Mean	780	960	464	701	

The plants harvested would tend to be more mature as the year progressed, due to lack of growth during the winter. The same authors also reported that activity of soil bacteria is a factor determining nitrate availability for uptake by plants, and cold weather would reduce the activity of these organisms to a minimum, thus reducing the nitrate supply.

The higher levels of nitrate in herbage grown on sand may have been sufficient to be one of the factors operating in goitre development in the sheep on the sand plot, although the levels found were less than half those reported in the literature to have an antithyroid effect. Bloomfield *et al.* (1961) for example found that 3100 ppm dietary nitrate depressed thyroidal I^{131} uptake by 25%, and Arora *et al.* (1968) showed that 2500 ppm nitrate depressed thyroxine secretion.

In order to examine the effect of the different herbage nitrate levels on ewe and lamb serum nitrate, blood was collected from all ewes on 20.8.71 and analysed for nitrate content. This date was chosen as it was in the fourth month of pregnancy, the time when the effect of goitrogens should have the greatest effect on the foetal thyroid development (Wright, 1959). Serum from 10 ewes taken at random from each plot on the 4.6.71 and 13.7.71 was also analysed to ensure that there had not been high ewe serum nitrate levels early in pregnancy.

The range in ewe serum nitrate was from 2.0 to 3.8 ppm, irrespective of date of collection, soil type on which the ewes were grazed or degree of goitre subsequently recorded in the lambs (detailed results Appendix 1:7).

The serum nitrate levels found in lambs with different degrees of goitre are shown in Table 2:14. Only a few animals could be sampled as the lamb's blood had to be collected within a few minutes of birth to ensure that it was as close as possible to the pre-birth state. From an examination of Appendix 1:7, and Table 2:14 it is evident that there is no relationship between the occurrence of goitre in lambs, and the nitrate level of either their own, or their dam's serum.

TABLE 2:14

Lamb serum nitrate levels, (ppm w/v) from samples taken at birth and compared with the degree of palpable goitre in the lamb (Rothwell grazing trial, 1971).

Degree of Goitre	Serum Nitrate
N	19, 21
+	22, 18
++	18, 19, 22, 19, 19
+++	22

PLANT GROWTH EXPERIMENT

Discussion with veterinarians of the Tasmanian Department of Agriculture revealed that goitre was a problem only in years when autumn rainfall was above average. This variation in goitre incidence between years may have been due to depressed pasture iodine content associated with the dilution effects of high soil moisture. Consequently, a pot trial was designed to examine the effect of varying watering levels on the iodine content of ryegrass and subterranean clover, grown on both sand and clay soils, during the autumn-winter period.

Soil was collected from within the grazing plots at Bothwell, each sample being from a single site. At each site the top 1 cm of material was removed (as this was almost completely organic matter) and samples taken to a depth of 10 cm in both the clay and sand soils.

The samples were allowed to air dry, sieved through a 6.4 mm sieve, thoroughly mixed and weighed into 15 cm pots lined with plastic bags to prevent water loss. Each pot contained 2.5 kg soil on an oven dry basis.

The permanent wilting point (15 atmos. suction) of each soil was measured using a pressure membrane apparatus, and the field capacity estimated by wetting each soil to excess and allowing to drain for 24 hours.

The weights and water content of each soil were:

	Clay	Sand
Oven dry wt.	2500 g	2500 g
Field capacity	3700 g	3580 g
Permanent wilting point	2800 g	2610 g
Variation in available water	900 g	820 g
Variation in soil water content during treatments		
Light watering*	675 g	615 g
Heavy watering	225 g	205 g

*Light watering - watering when 75% of available water utilized.

Heavy watering - watering when 25% of available water utilized.

The species used were perennial ryegrass (Tas. No. 1) and subterranean clover (Mt. Barker), both as single species and a mixture of the two.

Seeds were germinated before planting, and then planted(6.4.71) to give all combinations of; the two soil types, two levels of watering and three species mixtures for each of 5 harvest dates, a total of sixty pots. Due to lack of available covered space this could not be replicated, and the pots were therefore arranged as a single randomized block.

To prevent rain adding excess unmeasured water to the pots they were placed on tables (80 cm high) beneath a clear polythene cover 1.7 m above the table and extending beyond the pots by 1 m in each direction.

Twenty days after planting, each pot was thinned to a total of 14 plants, and in the case of the mixed species this consisted of seven plants of each species. Water treatments could not be applied until all the soil depth was penetrated by roots, to allow the plants to fully exploit available moisture. For the first seven weeks, therefore, light watering of all pots was carried out at intervals of approximately three days.

The first harvest was taken 11 weeks after planting, and subsequent harvests were made at intervals of 4 weeks. At each harvest, the plants were cut at ground level and the total wet weight of each species in each pot was recorded. The plant material was dried at 65^{o} C, ground to pass a 2 mm sieve and stored for later iodine analysis. After each harvest the remaining pots were rerandomized to eliminate any position effects. Throughout the experiment all pots were weighed each alternate day and water was added when necessary. It was necessary to water to field capacity at each watering to ensure even wetting of all soil in the pot.

Iodine analysis was performed by the method detailed previously and the results are shown in Table 2:15. Statistical analysis of the iodine contents of these species (Appendix 2:6) revealed no significant difference between any of the treatments or harvest dates. This tends to negate to a large extent suggestions that increased autumn rainfall and the subsequent

TABLE 2:15

Iodine content of plants grown under differing water regimes on Bothwell soils, 1971 (µg/kg D.M.).

Soil	Watering	Sp.	Harvest No. 1	2	3	4	5
Sand	Heavy*	Ryegrass	104	132	70	132	127
"	"	Clover	144	95	113	116	95
"	"	Mixture***	106	120	120	103	105
"	Light**	Ryegrass	79	66	107	102	140
"	"	Clover	79	70	78	104	112
"	"	Mixture	131	95	107	107	80
Clay	Heavy	Ryegrass	98	74	112	166	123
"	"	Clover	56	89	124	168	112
"	"	Mixture	145	116	130	89	110
"	Light	Ryegrass	124	87	110	226	152
"	"	Clover	72	111	54	104	97
"	"	Mixture	123	103	82	94	106

* Heavy watering - rewatering when moisture level dropped to 25% of available water used.

** Light watering - rewatering when moisture level dropped to 75% of available water used.

*** Mixture iodine content calculated as the mean iodine content x weight of the two species in that pot.

increase in pasture growth is involved in goitre production due to dilution of iodine taken up by the plants.

An interesting feature of this experiment was that there was no significant difference between the iodine content of plants grown on the two soil types, whereas under field conditions, there was significantly more iodine in the plants grown on the clay soil (Table 2:8). It is possible that drying and sieving the soil may have affected the binding of iodine to soil particles and made them more resistant to release. In the field, the plants may have penetrated into the B horizon of the clay soil, which was a heavy clay which may have had a higher iodine content.

The amount of plant growth throughout the experimental period as measured by the dry matter production at harvest 5 differed markedly (Table 2:16). Clover produced significantly more dry matter than ryegrass, with an intermediate level of production being found in the mixture. All three were significantly different from one another at the 0.01 level (Appendix 2:6). Plants growing on clay produced more dry matter than those growing on sand ($p < 0.01$) and those plants receiving more water also produced significantly more dry matter ($p < 0.05$).

The difference due to soil reflects the greater nutrient supplying capability of the clay, and the effect of watering treatment indicates that the degree of moisture stress imposed limited growth, although it did not affect uptake of iodine per unit of dry matter produced.

When total iodine uptake throughout the period was considered (i.e. iodine content x dry matter production at harvest 5) significant differences were found between both the two soils and the different species (Table 2:17; statistical analysis, Appendix 2:6). These differences in total iodine were generally similar to those found in dry matter production except that watering level had no significant effect.

TABLE 2:16

Total dry matter produced at harvest 5 in watering trial, 1971 (g/pot).

Soil	Watering	Ryegrass	Mixture	Clover
Sand	Heavy	3.8	13.8	20.9
"	Light	3.9	11.6	15.2
Clay	Heavy	7.6	22.1	25.1
"	Light	6.3	17.9	23.2

TABLE 2:17

Total iodine content of ryegrass and clover at harvest 5 of watering trial, 1971 (ng/pot).

Soil	Watering	Ryegrass	Mixture	Clover
Sand	Heavy	481	1294	1968
"	Light	546	496	1702
Clay	Heavy	935	1548	2811
"	Light	957	1656	2250

EWE FEEDING TRIALS

GOITROGENS

In 1970, ewes in plots A and B grazing pasture on sandy soil had higher levels of blood thiocyanate (Table 2:12) and more goitrous lambs (Table 2:3) than those on the clay soil. It was considered that, although these levels were low compared to normal goitrogenic levels (e.g. Butler *et al.* 1957), they could be important in inducing congenital goitre in lambs if the ewes were marginally iodine deficient during pregnancy. An animal feeding trial, involving different intakes of iodine and thiocyanate, was undertaken to examine this hypothesis.

A group of Polworth ewes were run with a harnessed ram from 6.5.71 to 13.5.71, the marked ewes being identified and returned to the ram whose raddle colour had been changed. On 11.6.71 ewes which had not re-mated were removed from the flock and laparotomies performed (by Mr. T. Mather, Tas. Dept. of Agric., Hobart) to test for pregnancy.

Twelve ewes, known to be pregnant were housed in individual cages on 14.6.71, ewe numbers being restricted by animal house space.

The animals were fed a diet consisting of 45% oaten chaff, 54% oat grain and 1% mineral mix (Table 2:18), the proportions of chaff and grain being chosen, after iodine analysis, to give a basal iodine level of 121 µg iodine/kg dry weight in the diet.

The ewes were offered 940 g/day (1.25 x maintenance) for the first 10 weeks and 1400 g/day (1.8 x maintenance) for the remaining 8 weeks (Coop, 1961).

The 12 animals were divided randomly into three groups of four with the following treatments:-

(1) Basal ration

(2) Basal ration + 250 mg KCNS/day (150 mg CNS^-)

(3) Basal ration + 250 mg KCNS + 328 µg KI/day (250 µg I^-)

This resulted in daily iodine and thiocyanate intakes as shown in

TABLE 2:18

Composition of mineral mix used in ewes diet.

Mineral Mix	%	*Trace Element Mix	%
$CaCO_3$	50	$FeC_6H_5O_7.5H_2O$	54.65
$Ca_3(PO_4)_2$	25	$CuSO_4.5H_2O$	21.96
$NaHCO_3$	23	$MnSO_4.H_2O$	3.38
Trace *	2	$CoCl_2.6H_2O$	2.29
		$ZnSO_4.7H_2O$	13.95
		H_2SeO_3	0.21
		$NaMoO_4.2H_2O$	3.56

Table 2:19.

The lowest iodine concentration fed to the sheep was below levels found in the pasture at Bothwell, the rationale being to accentuate any thiocyanate effect that may occur. The higher level represented an intake of approximately 30% above that suggested by Butler and Johnson (1957) to be the lower limit for normal thyroid development in the absence of goitrogens.

Feed was offered at 9.00 am each morning, KCNS and KI being administered orally in 30 ml of distilled water at 9.30 am. Ewes on the basal diet had 30 ml of distilled water administered in a similar manner to equalize any effects of handling the animals daily. Drinking water was tap water (0.6 µg iodine/l, Connolly, 1971 pers comm.) offered *ad lib.*

Feed refusal occurred to varying degrees throughout the experiment, and at each feeding the iodine content of the uneaten feed was calculated and a small amount of lucerne chaff, (iodine content 1280 µg/kg) with equivalent iodine content to the feed refused, was offered before the next day's feed. This had the effect of maintaining the iodine intake at the correct level, and the lucerne chaff also stimulated the ewes' food intake, this latter aspect being important to avoid pregnancy toxaemia which had been experienced with this ration in other feeding trials. In two cases, however, complete food refusal occurred at week 12 and symptoms of pregnancy toxaemia developed, both ewes eventually dying despite intravenous glucose infusions and glucose drenches.

Blood iodide levels were not monitored through lack of a suitable analytical technique, but blood thiocyanate levels were measured at 3 and 24 hours after dosing in week 20, these sampling times representing the approximate maximum and minimum blood thiocyanate levels of ewe previously drenched with 0.5 g KCNS (Appendix 1:8).

The results obtained (Table 2:20) indicated that the blood thiocyanate levels of the dosed ewes reached higher maximum levels than those in the field, but were not greatly dissimilar during the greater part of the day.

TABLE 2:19

Daily thiocyanate and iodine intake of ewes in goitrogen trial.

	Week 5 - 15		Week 16 - 23	
Group	Iodine (µg)	Thiocyanate (mg)	Iodine (µg)	Thiocyanate (mg)
1	114	-	170	-
2	114	150	170	150
3	364	150	420	150

TABLE 2:20

The concentration of blood thiocyanate in ewes in the animal house thiocyanate experiment measured 3 and 24 hours after dosing (values in µg/ml).

Group	Mean CNS^- Intake					
Basal	nil	Ewe no.	4	5	11	
		3 Hr	0.3	0.4	0.6	
		24 Hr	0.2	0.4	0.4	
Basal + KCNS	150 mg	Ewe no.	2	8	10	
		3 Hr	6.7	5.8	5.7	
		24 Hr	1.5	1.5	2.9	
Basal + KCNS + KI	150 mg	Ewe no.	3	6	7	9
		3 Hr	7.9	5.7	10.8	7.2
		24 Hr	1.7	1.2	1.4	7.9

Radioiodine uptake of the thyroid was measured on 2.8.71 to estimate the relative thyroid activity of the ewes. The means for each group are shown in Table 2:21, with complete results in Appendix 1:9.

Statistical analysis (Appendix 2:7) at each uptake period indicated a significant difference in radioiodine uptake between groups at 6.5 and 24 hours. Differences between the basal and KCNS groups were not significant, but the KI treated group was significantly lower ($p < 0.01$) than the control at 6.5 hours, and significantly lower than both other groups at 24 hours ($p < 0.01$).

The ewes lambed over a period of 2 weeks, from Sept. 29th to October 11th. The lambs were killed within 8 hours of birth and the thyroids were removed and fixed for histological examination.

The lamb weights, thyroid weights, histological comments and mean follicular diameters are presented in Table 2:22. Mean follicular diameter was calculated for each gland by counting the number of follicles along the diagonals of each of 10 fields on a calibrated microscope and averaging the total of 20 counts.

Analysis of variance (Appendix 2:8) indicated that there was no difference between the groups in either lamb weight, thyroid weight or thyroid to body weight ratio, but the mean follicle diameter of the basal group was significantly greater than either of the other two ($p<0.05$) although this was mainly due to the lamb from ewe 4.

It might be noted, nevertheless, that the data in Table 2:22 suggests a more goitrous condition amongst lambs in the basal and KCNS treated groups than in the group treated with both KI and KCNS.

Although the number of animals in each group was small, it is obvious that there was no effect of thiocyanate treatment in increasing the degree of goitre of animals with a sub-optimal iodine intake. This is evident in both the uptake of radioiodine by ewes and the thyroids histology of the lambs, and suggests that the levels of blood thiocyanate found in ewes

TABLE 2:21

Mean radioiodine uptake (% of injected dose) of ewes in final month of pregnancy in thiocyanate experiment.

	Sampling time (hr)		
Treatment	2	6.5	24
Basal	19.4	34.5	51.2
Basal + KCNS	11.4	23.2	40.6
Basal + KCNS + KI	3.1	4.7	9.2

TABLE 2:22

Lamb characters measured in thiocyanate treatment trial, 1971.

Group	Ewe No.	Lamb Wt. (kg)	Thyroid Wt. (g)	T/B* Ratio	Mean Follicle Diam (μ)	Histology
Basal	4	4.05	37.3	9.2	330	Follicles all markedly invaginated colloid depleted, cells H=4W**, excessive cell division.
	5	2.25	3.2	1.4	150	Follicles colloid filled, cells H=1-2W.
	11	3.53	7.4	2.1	110	Little follicular invagination, colloid filled follicles, cells H=2-3W, some excess cell division.
Basal + KCNS	2 A	2.42	6.2	2.6	120	Follicles invaginated, colloid depleted, cells H=1-2W, some excess cell division.
	B	2.73	2.3	0.8	100	Follicles with little lumen, and invaginated, some excess cell division, cells H=2-3W
	8 A	2.59	1.6	0.6	-	Autolysed (Born dead).
	B	2.30	1.6	0.7	80	Follicles colloid filled, Cells H=1.5W.
	10 A	2.90	14.2	4.9	110	Follicles with no lumen, cells H=3-4W, large amount of excess cell growth.
	B	2.13	15.1	7.1	110	As for 10 A.
Basal + KCNS + KI	3	3.08	0.5	0.2	70	Normal, cells 2H=W, follicles colloid filled, regular in shape.
	6 A	2.20	0.9	0.4	90	Normal as 3.
	B	2.24	0.8	0.3	90	Normal as 3.
	7 A	3.22	1.0	0.3	90	Normal cells cuboidal.
	B	2.69	1.0	0.3	80	Normal cells cuboidal.
	9 A	1.56	0.4	0.3	60	Normal as 3.
	B	1.72	0.4	0.2	40	Normal as 3.

* T/B ratio = $\frac{\text{Thyroid wt. (g)}}{\text{Lamb wt. (kg)}}$

** Estimate of follicular cell shape
H = cell width measured on the diameter of the follicle
W = cell width measured at right angles to H

grazing the plots on sandy soil in 1970 were not likely to have contributed to goitre production in the lambs. There was considerable variability within each group in the animal house experiment, ewe no. 4 in the basal group being particularly divergent. This animal had an abnormally high radioiodine uptake (Appendix 1:9), even though its iodine intake was similar to the other group members. The lamb of this animal also exhibited a more marked degree of goitre than any other. The variability between animals may be a reflection either of previous iodine nutrition or efficiency of iodide retention within the body, with some animals having greater iodine excretion rates than others.

IODINE NUTRITION EXPERIMENT

In 1972, an experiment was conducted to estimate the iodine requirements of pregnant ewes necessary to prevent congenital goitre, and to examine the iodine balance of ewes during pregnancy. This experiment was necessary, as the ewes used in the field trial were from a closed flock which had been on iodine deficient pastures for over 20 years. As a consequence, they may have become genetically adapted to a lower iodine environment, as was found in goats in India by Rajkumar (1970). A possible lower iodine requirement was suggested by the fact that some ewes on the sand plots did not have goitrous lambs, although the herbage iodine content was less than that suggested as a minimum by Butler and Johnson (1957).

Rams with sire-sine harnesses were run with a group of 200 Polworth ewes, taken from the same closed flock as the ewes in the field trial, from 21.4.72 to 19.5.72 the raddle colour being changed on 5.5.72. On 24.5.72 20 ewes which had not returned to the rams after the first mating were shorn

and transported to the animal house.

These animals were fed a diet of 1:1 oat grain and oaten chaff for seven days prior to being caged in individual metabolism crates. This initial feeding as a group was to prevent later feed refusal which often accompanies initial caging of flock sheep, as indicated by previous experience.

The ewes were caged on 31.5.72 and divided into four groups of five animals per group, the members of each group being caged at random. The basal diet fed is shown in Table 2:23. To this basal diet, potassium iodide was added at levels of 80, 160 and 240 µg I^-/kg of feed, resulting in four diets containing A, 117; B 197; C 277 and D, 357, µg iodine/kg feed. The iodide was sprayed onto the food in a solution containing urea, molasses, Na_2SO_4, trace elements and iodide. After 21.8.72, glucose was added to the feed to prevent pregnancy toxaemia.

To prevent loss of iodine from the feed by volatilization, feed was mixed at two weekly intervals. Analyses were made of total iodine content within each period to check for iodine loss.

Each ewe was offered 60 g dry wt. of feed/day/kg body wt.$^{0.75}$, with body weights being measured at two weekly intervals (Appendix 1:10). Glass distilled water was offered *ad lib.*

Faeces were collected in open mesh nylon bags, which allowed the urine to pass through, and this was collected in trays under each cage. This method may have resulted in contamination of faeces by urine and probably some transfer of iodine from one to the other.

The ewes were fed at 9.00 am, following the collection of uneaten food, faeces and urine. Subsamples of 10% of each were taken, the urine being bulked in glass containers and stored at 2° C. Feed and faeces samples were dried for 24 hours at 65° C and bulked. All bulk samples were collected over a seven day period, and at the end of this time, the feed and faeces samples were ground to pass a 2 mm sieve and all samples were analysed in duplicate for iodine content. Some samples were reanalysed in later weeks

TABLE 2:23

Basal diet fed to ewes in iodine balance experiment.

Component	% in Diet	Iodine (µg/kg)	Iodine/kg mixed feed (µg)
Oat grain	55.0	39.4	21.7
Oaten chaff	42.1	223.0	93.9
Urea	1.0	30.0	0.3
Molasses	0.5	251.0	1.2
Na_2SO_4	0.4	-	-
Mineral mix (as in Table 2:13)	1.0	-	-
Glucose (after 21.8.72)	1.0	-	-
			117.1

This diet had a digestibility of 65%, measured by feeding to ewes.

to ensure there was no change with time in reagents employed in the analytical procedure, and analyses performed in different periods were consistent.

Thyroidal radioiodine uptake measurements were made on all ewes on 3.6.72 and 22.8.72. Lambing began on 24.8.72 and continued over a 4 week period. Lambs were killed and the thyroids fixed for later examination. One ewe in group A was not pregnant and therefore, was excluded. The balance study was concluded for each ewe at the end of the collection period prior to lambing. Iodine intake for each ewe was calculated on a weekly basis from the iodine in food offered minus iodine in feed refused. Iodine output was calculated from the total faecal plus urinary iodine output. These values were not separated into the urinary and faecal components because of cross contamination of urine and faeces during collection. An iodine balance was calculated for each sheep over each seven day period. Although lambing continued until the 15th week of the experiment, statistical analyses were carried out only over the period up to the beginning of lambing (i.e. the first 9 weeks), as taking values beyond this time greatly increased the complexity of the analytical procedure. Iodine balance figures for each group, however, were plotted (Fig. 2:13) until half the group had lambed. With less than half of the ewes in each group, individual variation may have a large effect on group mean values. Appendix 1:11 contains weekly iodine intake, excretion and balance data for each sheep.

Analysis of variance of the iodine balance data over the 9 week period (Appendix 2:9) indicated that there was an effect of time, due mainly to fluctuation of iodine balance between weeks, but the most significant effect found was the difference between groups, each group being significantly different from the others ($p < 0.01$, t test), with group D having the most positive balance, followed by groups C, B and A.

Comparison of iodine intake and excretion graphs (Fig. 2:14 - 2:17) with iodine balance (Fig. 2:13) within any one group indicates a greater dependence of iodine balance on excretion, rather than intake, and this was

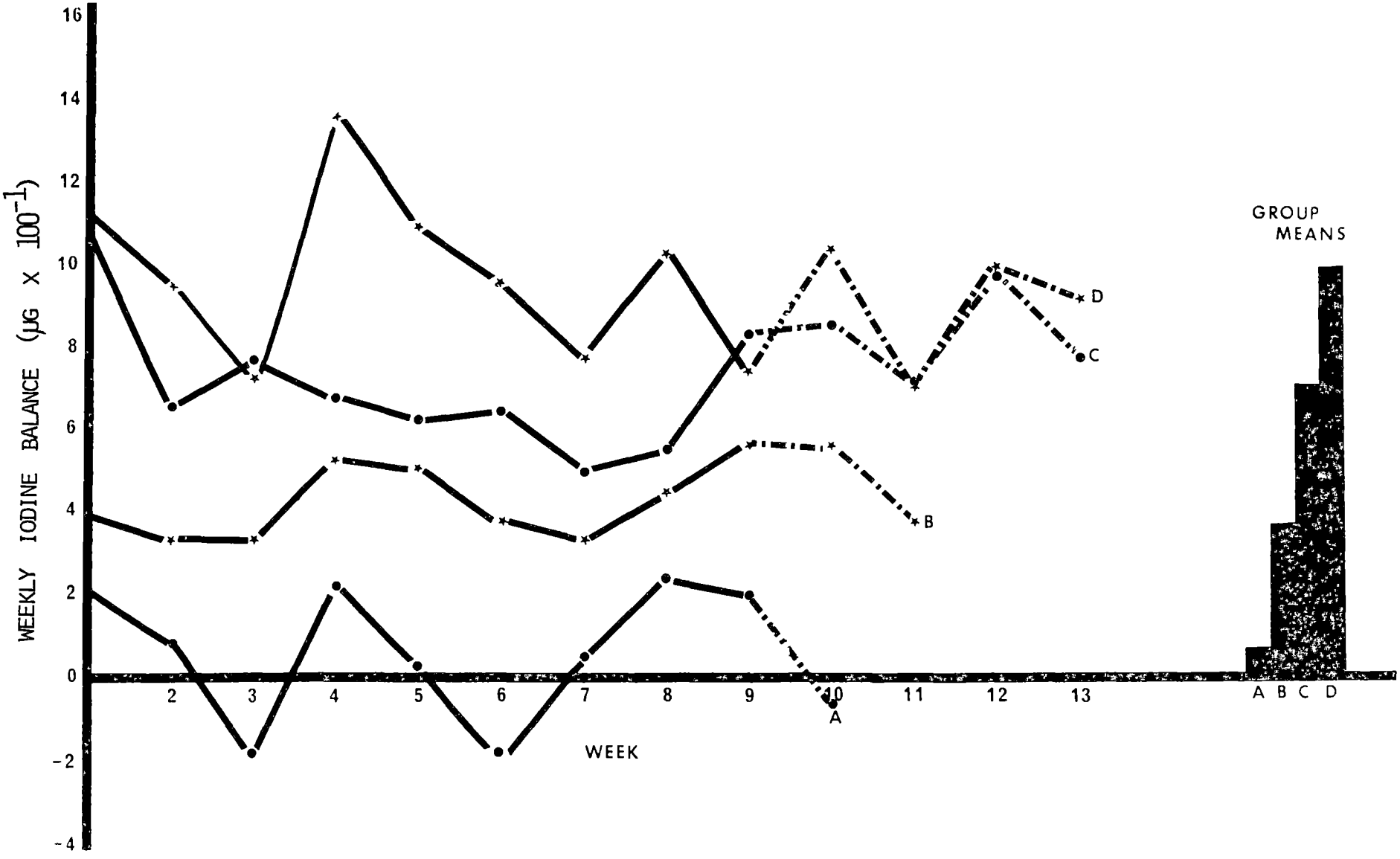
Figure 2:13

Mean iodine balance (on a weekly basis) for each group of ewes in the iodine balance study.

Solid line - Statistically analysed

Broken line - Remainder of period until half the ewes in each group had lambed.

The group mean balances, calculated over weeks 1 - 9 are also presented.


WEEKLY IODINE BALANCE (μG X 100⁻¹)
16
14
12
10
8
6
4
2
0
-2
-4
2
3
4
5
6
7
8
9
10
11
12
13
WEEK
A
B
C
D
GROUP MEANS
A B C D

Figures 2:14 - 2:17

Mean iodine intake and excretion values for each group of ewes in the iodine balance experiment, taken up to the point where half of each group had lambed.

FIG. 2:14

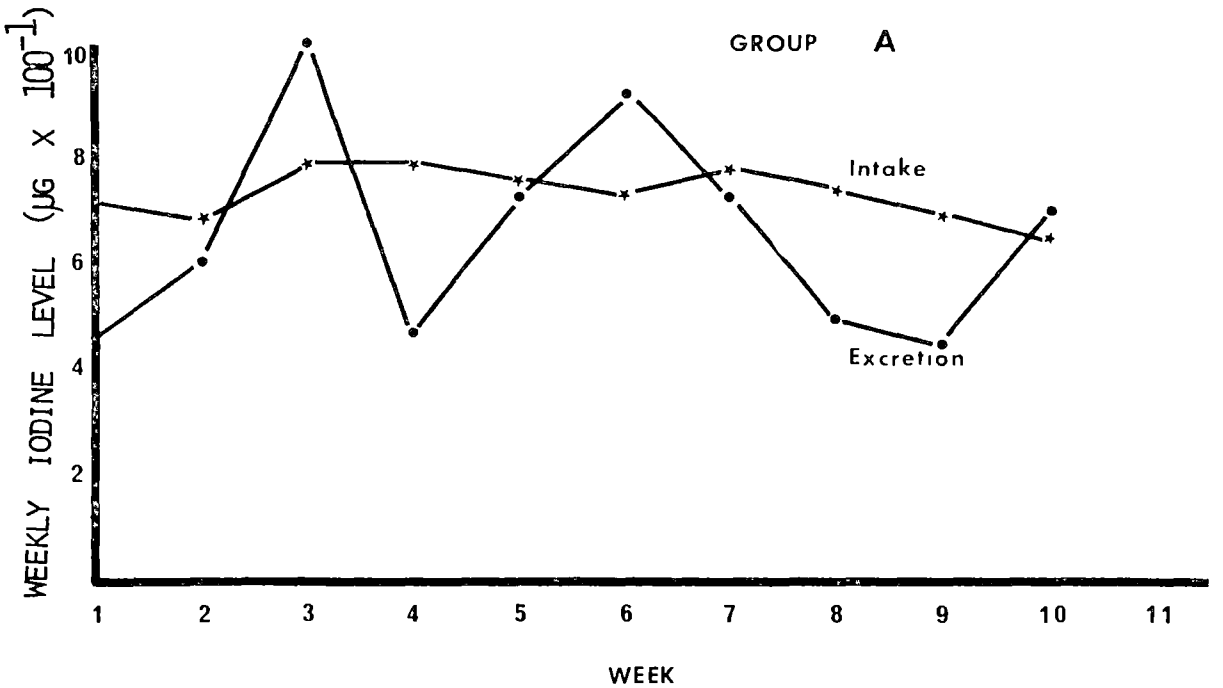


FIG. 2:15

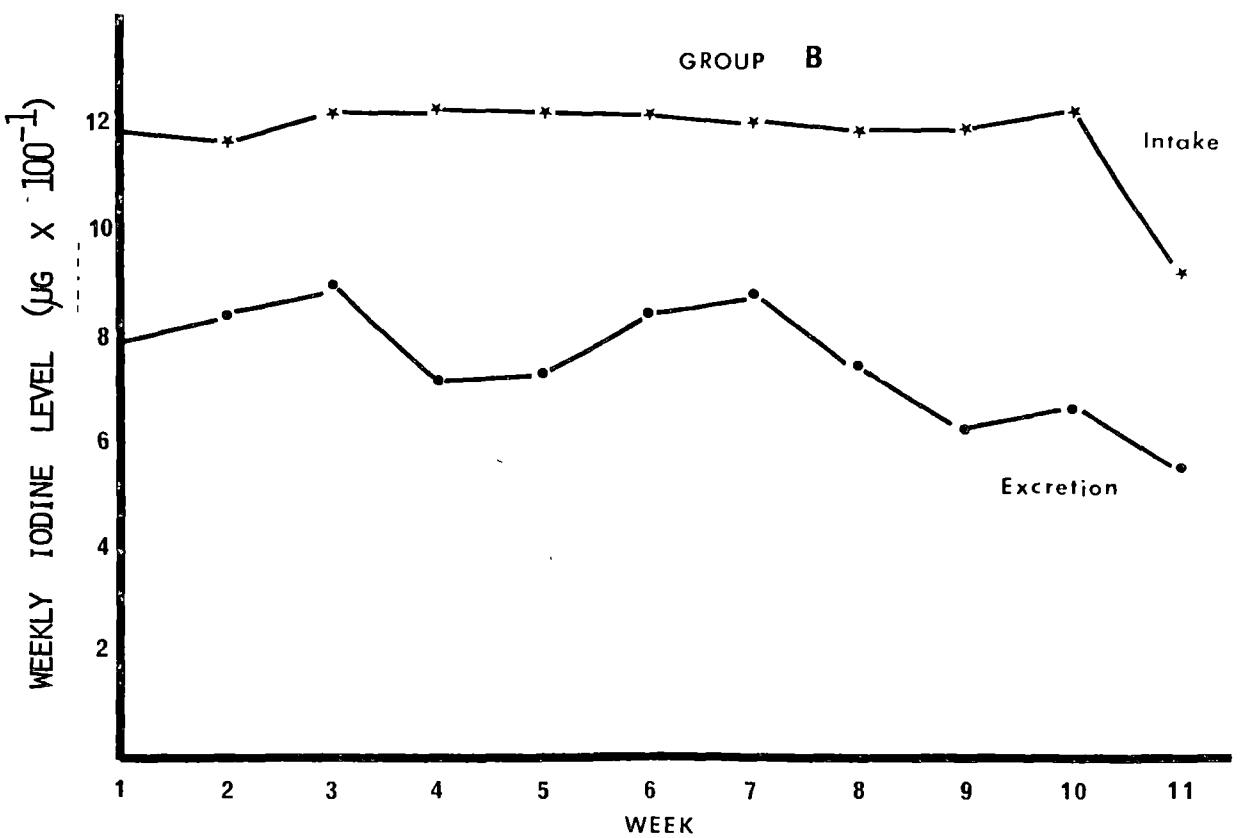


FIG. 2:16

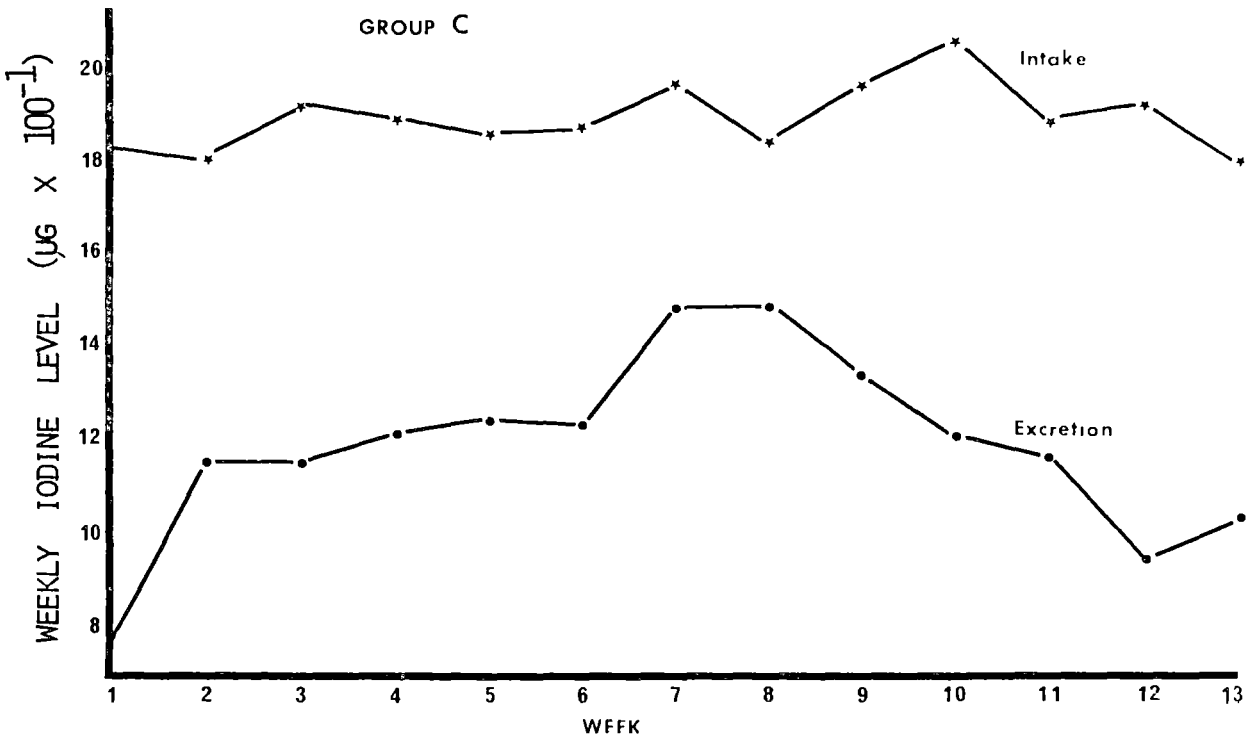
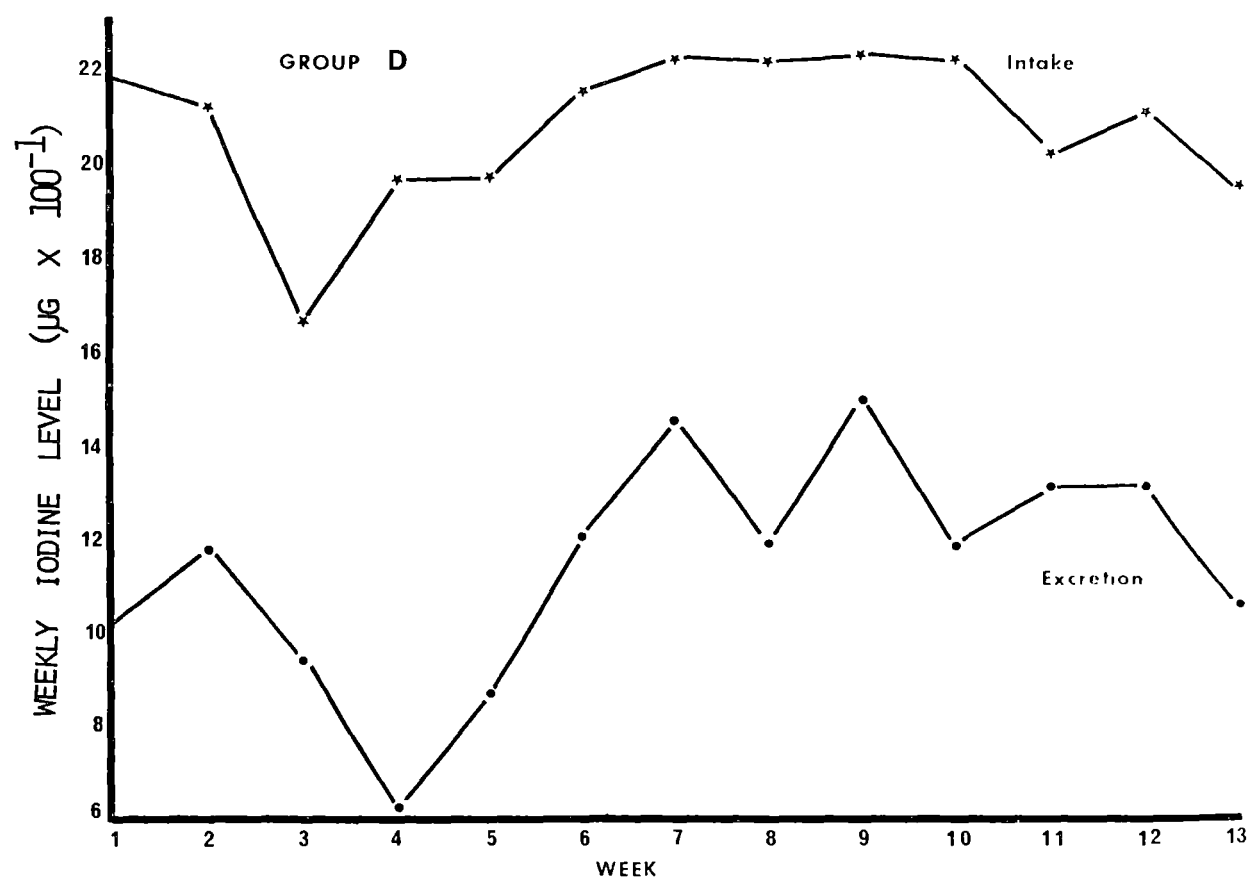


FIG. 2:17



also shown statistically by regression analysis (Table 2:24; Fig. 2:19). When all groups were considered together, there was a significant ($p < 0.01$) relationship between iodine intake and balance (Table 2:24; Fig. 2:18).

Some of the variation in iodine excretion between weeks may have been due to the analytical technique, as subsamples of only 0.2 g were taken for chemical analysis and may not have been representative of the whole sample. To reduce sampling error to a minimum, two independent subsamples were taken for analysis and, if there was more than 5% variation between the analytical results, two more subsamples were analysed and the mean of all four taken.

Thyroidal Radioiodine Uptakes

Thyroidal radioiodine levels were measured on all ewes on 3.6.72 and 22.8.72 to ascertain the effects of differing iodine intake on the avidity of the gland for iodine. The mean uptake figures for each group are shown in Table 2:25 (complete figures Appendix 1:12; statistical analysis Appendix 2:10).

There were no significant differences between any of the groups within an uptake time period in the June sampling. This of course would be expected as the animals were randomly assigned to each group and no treatments had been applied.

In August, at 6.0 and 24 hours the overall uptake values were significantly lower (0.01 level) than in June, with the exception of Group A which was similar to the June uptake level. This indicates that all except the lowest intake group had a drop in rate of iodine uptake by the thyroid during the experimental period, presumably due to an increase in iodine intake. Another factor which may have been operating to an unknown extent in all groups was a reduction in thyroxine needs in housed sheep through the removal of cold stress. Cold stress has been shown to slightly increase thyroxine secretion rate (Brooks, Pipes and Ross, 1962), and rate of thyroxine degradation (Freinkel and Lewis, 1957) in sheep.

TABLE 2:24

Regression equations for weekly iodine balance against iodine intake, and excretion in iodine balance experiment.

A. Regression of iodine balance against intake for all animals

$Y = 0.56\ X - 285$ Y = I balance

X = I intake

Confidence limits of slope ($p < 0.01$) 0.45 to 0.68

B. Regression of iodine balance against excretion for each group

Group A	
$Y = -0.75X + 554$	Confidence limits of slope ($p < 0.01$) -0.93 and -0.53
Group B	
$Y = -0.62X + 904$	-0.85 and -0.37
Group C	
$Y = -0.62X + 1426$	-0.85 and -0.37
Group D	
$Y = -0.58X + 1622$	-0.82 and -0.35

Y = I balance

X = I excretion

Figure 2:18

Regression analysis of iodine balance on iodine intake, on a weekly basis for all animals in the first 9 weeks of the iodine balance experiment.

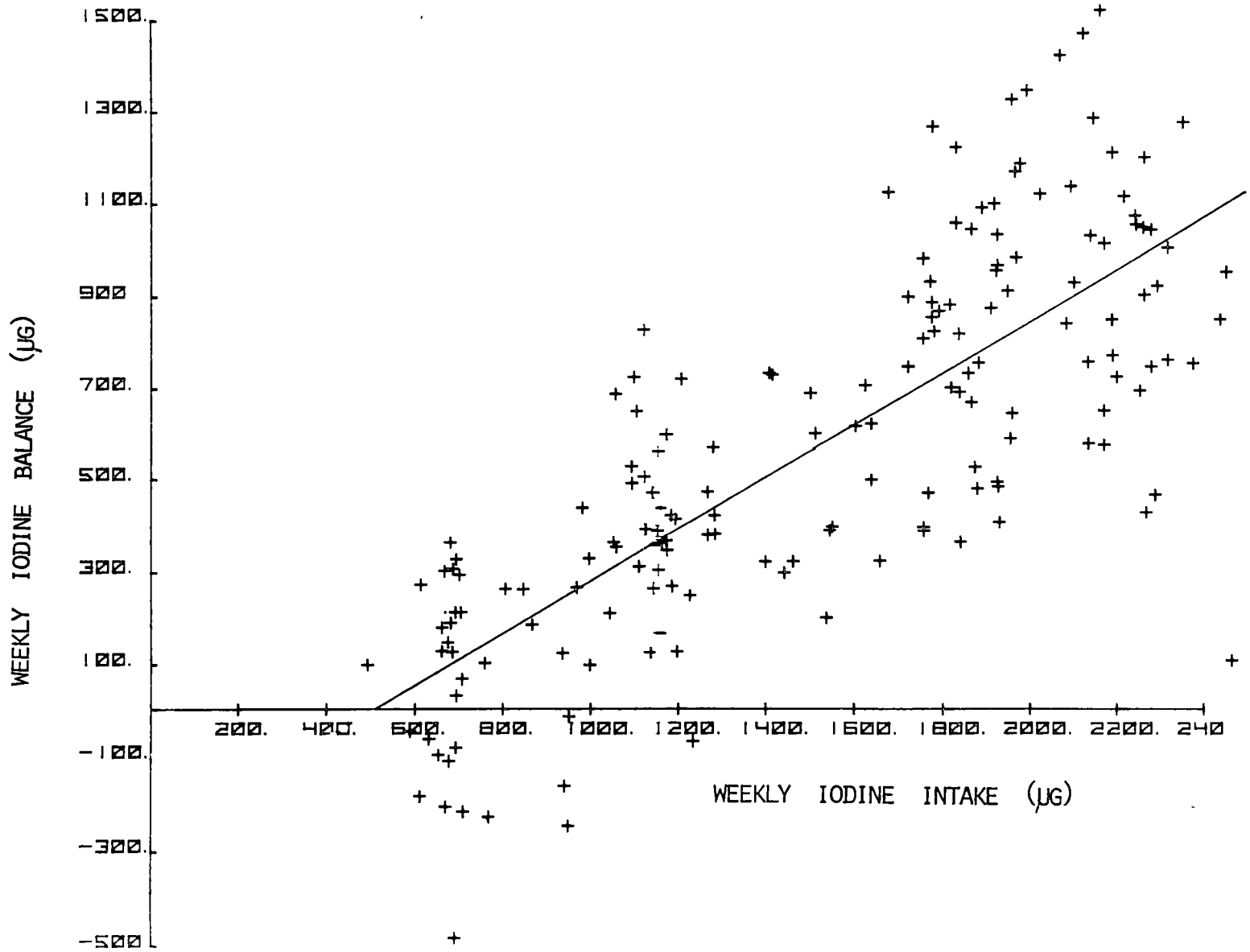

1500.
1300.
1100.
900
700.
500.
300.
100.
-100.
-300.
-500
WEEKLY IODINE BALANCE (µG)
200.
400.
600.
800.
1000.
1200.
1400.
1600.
1800.
2000.
2200.
240
WEEKLY IODINE INTAKE (µG)

Figure 2:19

Regression analysis of iodine balance on iodine excretion for each group during the first 9 weeks of the iodine balance experiment.

Legend

+	Group A
□	Group B
X	Group C
V	Group D

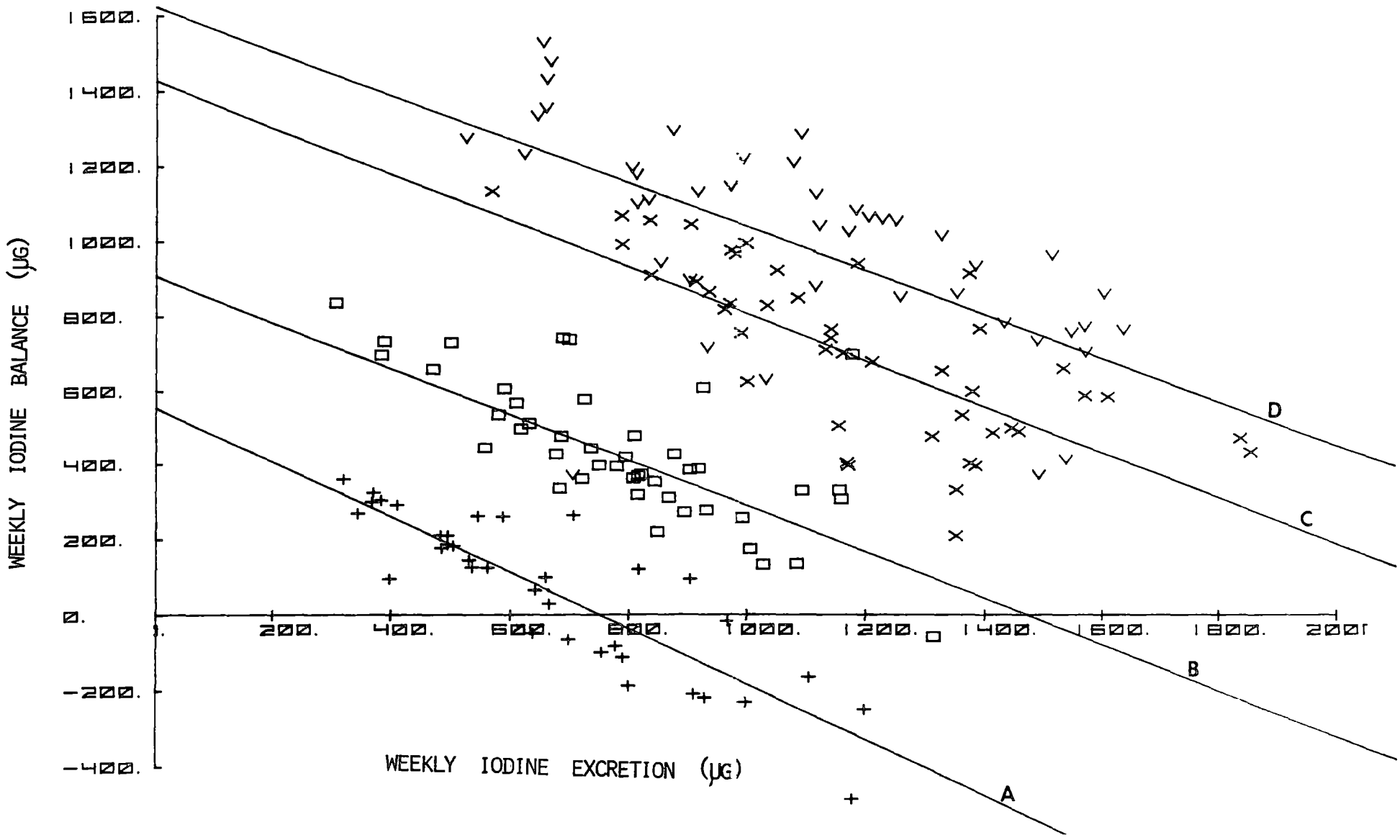

1600.
1400.
1200.
1000.
800.
600.
400.
200.
0.
-200.
-400.
WEEKLY IODINE BALANCE (µG)
200.
400.
600.
800.
1000.
1200.
1400.
1600.
1800.
2001
WEEKLY IODINE EXCRETION (µG)
A
B
C
D

TABLE 2:25

Mean thyroidal radioiodine uptake (%) of ewes in iodine balance study as measured in June and August, 1972.

	JUNE			AUGUST		
Group	2.5 Hr	6 Hr	24 Hr	2.5 Hr	6 Hr	24 Hr
A	6.7	13.4	24.7	10.1	19.7	26.6
B	6.2	11.2	25.6	5.3	8.7	14.6
C	5.5	11.1	22.7	4.6	8.5	17.1
D	7.8	14.3	28.7	3.2	6.5	12.7

Lambing results

The lamb weights, thyroid weights, thyroid to body weight ratio and histology of the glands are presented in Table 2:26, with statistical analyses in Appendix 2:11.

There was no significant statistical difference between groups in lamb weight, thyroid size or mean diameter of thyroid follicles. There was a difference in thyroid to body weight ratios in that results for groups A and B were significantly ($p < 0.05$) higher than groups C and D.

Histological examination is a more accurate means of determining the actual state of the thyroid (Kossila *et al.* 1970), and the data presented in Table 2:26 reveal that in group A, the lowest iodine intake group, there was some degree of abnormality in all lamb thyroids, including two with invaginated follicles. In group B, there were two normal lambs, and four with colloid depletion and cell hypertrophy. In both of the above groups follicular cells were enlarged. In Group C, one individual had general colloid depletion, one had partial depletion and one had some cell hypertrophy, but all still had follicular cells somewhat larger than normal. In group D, all lambs had histologically normal thyroids.

It seems evident that the changes in thyroid structure as iodine deficiency increases are firstly, some cell enlargement, secondly, cell proliferation and colloid depletion, thirdly, invagination of follicles due to the excess cells present and finally follicles either collapse or become filled with the excess cells. In the field, a number of lambs with very large thyroids have been found with extremely large follicles, up to 0.5 mm in diameter, filled with a lightly staining colloid.

It is interesting to note from the results of this experiment that histologically normal lamb thyroids occurred at mean iodine intakes of the dam of over 300 µg/day during the latter part of pregnancy. This was an artificial situation in that the animals were not exposed to extremes of cold and movement was restricted, therefore maternal thyroxine needs would have been

TABLE 2:26

Data for all lambs born in the animal house in an iodine balance experiment, 1972.

Group	Ewe No.	Mean Iodine Intake (µg/day)	Mean Balance µg/day	Lamb Wt (kg)	Thyroid Wt (g)	T/B * Ratio	Mean Follicle Diam (µ)	Histology
A	1	97	14	4.3	5.7	1.3	142	Follicles invaginated and colloid depleted, excess cell division, cells H = 3W
	5	127	9	3.3	1.7	0.51	87	Generally normal, some excess cell division, cells cuboidal.
	16A	98	10	2.8	1.3	0.46	73	Colloid depleted some excess cell division, cells cuboidal.
	B			2.4	0.9	0.37	70	As 16A.
	17A	94	18	2.8	0.7	0.23	80	Some follicular invagination, colloid depleted, some follicles filled with excess cells, cells H = 1-2W.
	B			2.5	0.8	0.32	75	As 17A.
	Mean			3.0	1.9	0.54	88	
B	3A	155	49	2.2	1.2	0.55	72	Colloid depleted excess cell division, cells H=2W.
	B			2.3	1.2	0.53	74	As 3A.
	7A	192	76	2.7	0.9	0.34	75	Normal cells cuboidal.
	B			2.8	0.6	0.21	63	As 7A.
	12	168	40	2.4	0.8	0.33	79	Generally normal some colloid depletion, cells 2H=W.
	14A	167	68	2.6	2.4	0.91	101	Colloid depleted, some follicular invagination and excess cell division, cells cuboidal.
	B			1.6	1.1	0.68	81	As 14A.
	18	157	74	3.9	2.8	0.71	114	Normal, some cell division, cells 2H = W
	Mean			2.6	1.4	0.53	82	

Cont.

C	2	257	109	3.9	1.1	0.28	81	Normal cells cuboidal.
	6	261	105	4.2	1.1	0.26	79	As 2.
	8	278	115	4.8	1.3	0.27	80	Colloid depleted, cells 1-1.5H=W.
	10	236	85	2.4	0.7	0.30	68	Some follicles, colloid depleted, cells cuboidal.
	13A	302	99	2.5	0.6	0.24	58	Normal, some excess cell division, cells cuboidal.
	B			4.0	0.6	0.15	53	As 13A.
	Mean			3.6	0.9	0.25	70	
D	4	307	152	4.0	0.5	0.13	61	Normal cells 2H=W
	9	308	158	7.1	1.1	0.16	114	" " "
	11	314	135	3.8	0.5	0.15	78	" " "
	15	293	119		LAMB DEAD AT BIRTH			
	20	264	112	3.8	1.0	0.27	99	" " "
	Mean			4.7	0.8	0.18	88	

* T/B Ratio = $\frac{\text{Thyroid wt. (g)}}{\text{Body wt. (kg)}}$

reduced. Despite this, the level is similar to that noted under field conditions in New Zealand by Butler and Johnson (1957) as being the minimum necessary to prevent goitre occurring. This result suggests that the sheep used in the field trial, and in this experiment were not genetically different, in terms of iodine requirements, from those used by Butler and Johnson. Below this intake, reaction to iodine deficiency appears to depend on the individual animal.

DISCUSSION

An outbreak of congenital goitre in sheep in 1968 appeared to be typical of the nature of the disease in Tasmania. The outbreak followed an autumn of above average rainfall. If affected lambs survived for the first few days of life they usually recovered, the enlarged thyroid receding to normal by about 3 months of age. This is similar to New Zealand experience (Andrews and Sinclair 1962), and this recovery is probably due to the high concentration of iodine in the ewes' milk (Falconer 1963b).

The sporadic nature of goitre in Tasmanian sheep, and the rapid recovery of affected lambs would exclude the possibility of genetic origin of the disease as occured in South Australia (Falconer 1966a and b; Rac *et al.* 1968; Mayo and Mulhearn 1969) and the response to iodine therapy, which is normally used, excludes the thiouracil -type goitrogens from being involved.

Results from the grazing trial established at Bothwell indicated that in each year, ewes grazing pastures on a light sandy soil had more goitrous lambs than those grazing clay soil pastures. The presence of thiocyanate-type goitrogens was initially suspected as a factor because other workers (e.g. Sinclair and Andrews 1958 and Wallach 1965) gave the impression that iodine deficiency goitre is uncommon, as few authors report feed iodine levels or estimates of iodine intake by animals. A second reason for considering thiocyanate-type goitrogens was the sporadic nature of the disease. This observation corresponded to a suggestion by Cunningham (1955) that sporadic outbreaks of goitre could occur if the production of cyanogenic glucosides by clover differed between years, and was acting in a low iodine environment.

Analysis of serum from ewes in the field trial indicated that the ewes in the plots with a greater goitre incidence had higher blood thiocyanate levels. Although these levels were low, it was possible that thiocyanate was a contributing factor to goitre production, as there are no published reports of the effects of low thiocyanate intake on marginal iodine intakes.

A feeding experiment designed to accentuate the thiocyanate effect, with higher thiocyanate levels, and a lower iodine intake than under field conditions, showed that thiocyanate, at the levels found in the field, had no effect on the incidence or severity of goitre.

The other common thiocyanate-type goitrogen, nitrate, was found in higher levels in herbage from the sandy soil plots than from that grown on the clay. However, the nitrate content of both ewe and lamb serum was constant at low levels, irrespective of the herbage grazed and the degree of goitre found in the lambs. Presumably therefore, the levels of nitrate in the herbage were low enough to allow ruminal degradation of the ingested nitrate.

When goitrogens were excluded from involvement in goitre production under conditions existing in the field trial, it seemed evident that the problem was due to iodine deficiency, and there may have been a differential iodine intake between ewes on the two soil types. Because of the two methods of supplying water to the plots, it was possible that the difference in iodine intake could have been a result of higher iodine supply from either the herbage, or water, or both, in the clay plots.

In 1973, however, it was shown conclusively that the type of water supplied to the animals throughout a summer and following breeding season had no effect on subsequent goitre incidence in the lambs.

Plant samples collected from each of the field trial plots at regular intervals showed a high degree of variability in iodine content between collections. This variability, taking ryegrass as an example, was less than that found by Butler and Glenday (1962) in randomly selected plants, and probably resulted from natural variation between plants in iodine content. In general, however, there was a significantly higher iodine content in plants from the clay plots.

Although herbage samples were collected from all species present in each plot, visual examinations of the pasture revealed that not all spec-

ies were being grazed. During most of the year, ryegrass *(Lolium perenne* L*)* and subterranean clover *(Trifolium subterraneum* L.*)* were grazed almost exclusively. Other species appeared to be grazed only when these two were limited in availability. The major proportion of the herbage iodine, therefore, would have been supplied via these two species, with some contribution from other species in late summer (January-February) and late winter (September-October).

There was not a significant difference in iodine content of ryegrass and subterranean clover between years, or between seasons within a year, but there was a tendency for ryegrass to have higher iodine contents in autumn and winter than summer and spring, and the reverse in subterranean clover. This pattern was also reported by Johnson and Butler (1957) under New Zealand conditions.

Although there was no difference in iodine content of herbage between years, there did appear to be a difference in goitre incidence. The increase in goitre severity on the sand soil between 1970 and 1971 could be explained by continuous iodine depletion of the ewes during the two years they were confined to this area. Similar observations of goitre increase were made by Hopkirk *et al.* (1930) with ewes maintained for one or two years on alluvial soil in New Zealand. In 1971 and 1973, when ewes had been set stocked on the plots for at least 10 months the goitre incidence was above 90% of all lambs born on the sandy soil, while in 1972 it was only 56%, although the numbers in this group were small. This drop in goitre incidence in 1972, compared to other years, is correlated with the amount of autumn rainfall, a higher goitre incidence being associated with higher rainfall, and tends to confirm observations by Dept. of Agriculture personnel (Pillinger 1969 pers. comm.) that goitre occurs in years with a good autumn growing season.

It was initially suspected that the influence of rainfall on goitre incidence was through dilution of available iodine in the soil water, with

a consequent reduction in the amount taken up by plants. This factor could have been intensified by dilution of iodine already within the plant due to stimulation of plant growth by the increased rainfall. Subsequent pasture iodine analysis, and an experiment with plants grown under controlled water regimes showed that this dilution did not occur, despite dry matter production being much higher with frequent watering than with less frequent watering. The drop in goitre incidence in 1972 probably reflects an increased iodine intake by ewes during that year. This increase could not have come from plant or water sources, as these did not differ significantly in iodine content from other years. The only apparent difference between the field plots in 1972 and other years was in pasture height (see Fig. 2:9). Following a poor autumn growing season the pasture height was less than 3 cm by August 1972, and it was under conditions similar to this that Healy *et al.* (1972) showed that ingestion of soil by grazing ewes reduced the incidence of congenital goitre in lambs.

The pastures in the Bothwell grazing trial had well developed mats of decaying organic matter on the soil surface (Fig. 2:11, 2:12), and the surface soil was much higher in iodine content than the remainder of the horizon, and the plant material growing on it, particularly in the sandy soil. For example, taking values from Tables 2:8, 2:9 and 2:10, the following values in µg/kg can be derived for the mean iodine contents of pasture plants and soils in the grazing trial at Bothwell.

	Plot	
	Sand	Clay
Ryegrass iodine content	176	289
Clover " "	156	244
Top 2 cm of soil	1080	1180
Remainder of A horizon	254	885

Healy (1967) has suggested that, under conditions of pasture shortage, sheep can ingest up to 400 g of soil daily, and under the conditions found

at Bothwell an intake approaching this would be more than adequate to supply the grazing ewe's iodine requirements.

It would seem possible therefore, that in years of poor autumn rainfall and consequently depressed pasture growth, the iodine supply of grazing ewes could be supplemented by intake of soil material.

In years with an adequate autumn pasture growth the intake of soil would not occur to a large extent and, therefore, would not contribute greatly to the animal's iodine nutrition. However, Healy *et al.* (1972) found that even when pasture was between 8 and 10 cm in height there was still a low percentage of soil in the faeces, and this could be part of the reason for the differences in goitre found between plots in the field trial. It is obvious from a consideration of the data summarized previously that goitre should occur in lambs born to ewes grazing the sandy soil herbage, as this supplies less than the approximately 300 µg iodine/kg that was found to be necessary to prevent goitre in sheep in the iodine balance study. Also there should have been some goitre in the lambs from ewes on the clay plots, if the only iodine intake occurred from the plants and water. A low intake of organic matter from the soil surface could occur, as pasture heights were only 5-8 cm in September following a good autumn growing season. A soil intake of 8% of the feed dry weight intake could increase the iodine intake of ewes in the clay plots to a level which prevented goitre, but a much larger intake (approximately 20% of the feed dry weight intake) would be necessary for the ewes in the sand plots to achieve the same result.

Another factor which could be affecting the goitre incidence between years would be the leaching of iodine from the surface organic matter in years of high autumn rainfall. This leaching was not monitored throughout the period of the trial, and there appears to be no literature available with regard to rate of leaching of iodine from soil under natural conditions. It is possible, however, that leaching of iodine does occur, and this would

reduce the soil iodine available to animals in years of adequate pasture growth. If leaching did occur it would tend to be more obvious in the sandy soil than the clay, because of their relative differences in permeability.

Ingestion of soil material high in organic matter could also explain the observations made by Blood and Henderson (1968) and Dawbarn and Farr (1932). Blood and Henderson reported that goitre in lambs may occur when permanent pasture is ploughed, but the disease may not reappear in subsequent years. This could be related to the destruction and subsequent reformation of an organic matter mat, higher in iodine content than the soil, on the soil surface. Dawbarn and Farr found that in drought conditions the iodine content of sheep thyroids approximately doubled, although there was no variation in the level of plant and water iodine.

Even though the ewes used in the field trial had been bred in a closed flock for at least 20 years, there is no indication that natural selection has resulted in animals with a reduced iodine requirement. An iodine balance study showed that thyroid abnormality occurred in lambs if the ewes had an iodine intake of less than 300 µg iodine/day, which was similar to the minimum level for normal lamb development suggested by Butler and Johnson (1957).

This iodine balance study also showed that the iodine balance of ewes, even when on very low iodine intakes, does not change during the last three months of pregnancy. This suggests that iodine excretion is constant, and is not affected by the increased demands for iodine within the ewe's body when the foetal thyroid also begins accumulating iodine. At this stage, presumably, the foetal and maternal thyroids compete for circulating iodine.

The minimum daily faecal and urinary iodine loss of pregnant ewes projected from the regression diagram Fig. 2:18 (Table 2:24) in the iodine balance study was approximately 73 µg iodine/day when the animals were in zero

iodine balance. At intakes above this level, both the iodine balance and iodine excretion increase, with the percentage of iodine retained in the body increasing from 12.5% at an intake of 104 µg iodine/day, to 36.3% at an intake of 168 µg iodine/day; 38.6% at an intake of 267 µg iodine/day and 45.4% at an intake of 297 µg iodine/day. These figures indicate that under iodine deficiency conditions the greatest proportion of ingested iodine is excreted and this proportion increases as iodine deficiency increases.

It should be noted that in the data presented in Table 2:26 there ~~is~~ *is* a lack of correlation between lamb thyroid weight and histology of the gland. Lambs 17 A and 17 B for example had thyroids in the low normal weight range, but these were histologically abnormal, whereas lamb 18 had a thyroid which would be classed as goitrous in regard to size, but histologically was almost completely normal. This observation has also been recorded in lambs from ewes grazing in the field (Table 2;6a) on pasture on both sand and clay soils. A similar observation was made in goats in Japan by Takamori (1957), who reported that there was no definite and constant relationship between the size of thyroids, which were examined by palpation, and the degree of the histological changes. In dairy cattle however, Kossila *et al.* (1970) found that thyroid weight was closely correlated with epithelial cell height and general histological activity of the gland. This suggests there may be a species difference in the response of the thyroid gland to iodine deficiency.

In sheep and goats therefore it is possible that the incidence of congenital goitre may be underestimated if palpation is the sole criterion. As a consequence deaths due to thyroid deficiency may also be underestimated.

Throughout this project thyroidal radioiodine uptakes were measured, but interpretation of the data obtained was difficult. In 1972, radioiodine uptakes were measured in the third and fifth months of pregnancy in both animal house and field ewes. In both cases the latter uptakes were lower than the former, indicating either a possible competition for

radioiodine between the foetal and maternal thyroids within the ewe's body, or changes in iodine nutrition of the ewe. In the field, iodine nutrition could have been increased by the ewe's ingesting soil material of high iodine content. In the animal house trial, the previous iodine nutrition of the ewes was unknown, and the differences in radioiodine uptakes may have been a reflection of two different levels of iodine nutrition. Under these conditions where the actual iodine intake and previous iodine nutritional history of animals is not accurately known, radioiodine uptake values are probably only valid in a comparison of ewes at the same stage of pregnancy. This would give an estimate of relative iodine intake between the animals.

Although this study was not designed specifically to find a means of preventing goitre in sheep, several points of interest have arisen. From the field trial, it is obvious that ewes grazed on clay soil will have a very low percentage of lambs with goitre. The results obtained in the grazing trial also indicate that ewes will be relatively free from goitrous lambs if they are grazed on pasture on sandy soil throughout the year, but are transferred to pasture on clay soil after mating. It is probable, therefore, that congenital goitre in sheep can be prevented by flock management on properties in which it is a problem, providing that the various soil types necessary exist on the property.

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APPENDICES

APPENDIX 1:1

Pasture Species Composition (%)

Plot	*Lolium perenne*	*Trifolium subterraneum*	*Vulpia bromoides*	*Holcus lanatus*	*Hordeum leporinum* *Bromus unioloides*	*Poa australis*	*Erodium* Sp.	B.L.W.
	18.6.70							
A	37	27	-	16	13	3	3	1
B	36	24	-	17	18	-	4	1
C	25	12	22	20	20	-	-	1
D	41	17	6	17	15	2	2	T
	13.9.70							
A	33	41	-	10	10	3	2	1
B	27	41	-	15	12	-	3	2
C	37	12	17	17	15	-	T	2
D	34	40	3	11	9	2	1	T
	4.6.71							
A	41	29	1	6	18	4		1
B	39	28	3	11	18	-	-	1
C	45	17	23	-	10	-	3	2
D	31	32	8	6	20	2	1	T
	26.8.71							
A	39	25	4	1	27	4	-	T
B	32	28	8	2	26	-	2	2
C	46	17	23	-	10	-	3	2
D	36	27	8	-	26	2	1	T

APPENDIX 1:2

Iodine Content of all Species in Pasture (µg/kg)

Date	Species	Plot A Sand	B Sand	C Clay	D Clay
10.6.70	*Trifolium*	120	185	207	243
	Lolium	285	170	198	275
	*Hordeum**	246	213		202
	Vulpia			430	
	Holcus		129		
21.8.70	*Trifolium*	204	160	220	155
	Lolium	172	280	276	246
	Hordeum	154	194		220
	Vulpia		178	352	271
	Holcus		250		
18.10.70	*Trifolium*	160	66	274	295
	Lolium	52	63	290	140
	Hordeum	145	101		196
	Vulpia		90	120	
	Holcus		93		
	Bromus	67	72		180
12.1.71	*Lolium*	170	170	186	350
	Hordeum	205	280	224	324
	Vulpia		392	253	
	Holcus		168		
	Bromus	298	340		342
5.3.71	*Trifolium*	156	102	149	111
	Lolium	125	160	173	135
	Hordeum	152	160		285
	Holcus	170			

* In vegetative stages Hordeum and Bromus were difficult to separate in the field, and they were therefore collected and analysed together.

Cont.

Date	Species	Plot A Sand	Plot B Sand	Plot C Clay	Plot D Clay
13.5.71	Trifolium	460	315	584	205
	Lolium	453	327	657	251
	Hordeum	930	1032		
	Holcus		552		
	Vulpia			1732	
25.6.71	Trifolium	95	167	242	205
	Lolium	262	120	168	251
	Hordeum	493	194		
	Vulpia			490	
	Holcus		152		
	Poa +	296			
2.8.71	Trifolium	136	141	282	146
	Lolium	192	230	257	223
	Hordeum	128	162		223
	Vulpia		207	304	238
	Holcus		236		
	Poa	240			142
24.9.71	Trifolium	158	106	212	137
	Lolium	93	167	450	189
	Hordeum	108	158		262
	Vulpia		147	324	165
	Holcus		146		
	Poa	254			243
22.11.71	Trifolium	133	88	250	585
	Lolium	58	42	286	127
	Hordeum	256	94		226
	Bromus	40	23		263
	Vulpia		77	56	101
	Holcus		71		
	Poa	224			238

+ Up till this stage none of the Poa tussocks had shown any sign of grazing.

Cont.

Date	Species	Plot A Sand	B Sand	C Clay	D Clay
20.12.71	Trifolium	143	247	272	601
	Lolium	44	52	260	469
	Hordeum	105	99		167
	Bromus	114	74		119
	Vulpia		111	156	
	Holcus		105		
	Poa	151			324
9.2.72	Lolium	157	146	171	323
	Hordeum	174	152		313
	Bromus	376	315		319
	Vulpia		356	216	
	Holcus		204		
	Poa	344			667
14.4.72	Trifolium	202	135	149	245
	Lolium	247	227	587	125
	Hordeum	319	359		382
	Vulpia		346	405	
	Holcus		226		
	Poa	920			924
18.5.72	Trifolium	192	85	141	56
	Lolium	205	286	243	180
	Hordeum	202	330		274
	Vulpia		322	371	
	Holcus		341		
	Poa	783			509
30.6.72	Trifolium	90	40	56	103
	Lolium	177	155	318	335
	Hordeum	242	241	374	187
	Vulpia		258	323	
	Holcus		308		
	Poa	824			185

Cont.

Date	Species	Plot A Sand	B Sand	C Clay	D Clay
10.8.72	Trifolium	163	119	197	242
	Lolium	169	153	312	339
	Hordeum	116	230	286	155
	Vulpia		316	456	
	Holcus		255		
	Poa	525			392
22.2.73	Trifolium	219	237	224	240
	Lolium	176	158	202	328
	Hordeum	91	123		295
	Vulpia		275	290	
	Holcus		173		
	Poa	376			540
12.4.73	Trifolium	200	148	230	264
	Lolium	273	214	294	179
	Hordeum	285	315		326
	Vulpia		321	387	
	Holcus		158		
	Poa	556			140
12.6.73	Trifolium	84	76	102	115
	Lolium	199	170	330	344
	Hordeum	250	273		186
	Vulpia		221	285	
	Holcus		310		
	Poa	621			596
27.7.73	Trifolium	148	106	192	134
	Lolium	189	154	321	388
	Hordeum	182	222		176
	Vulpia		245	356	
	Holcus		241		
	Poa	540			589

Cont.

Date	Species	Plot A Sand	B Sand	C Clay	D Clay
16.9.73	Trifolium	158	121	232	134
	Lolium	135	147	355	307
	Hordeum	115	148		285
	Vulpia		176	320	
	Holcus		121		
	Poa	321			450

APPENDIX 1:3

Radioiodine Uptake 11.9.71

(% of initial dose)

Plot B

	T2	T7.0	T24
	30.7	54.0	46.6
	34.1	45.7	51.6
	20.0	47.3	54.0
	22.2	56.1	65.8
	20.1	63.9	45.7
	33.8	60.7	45.4
	20.0	49.1	55.5
	18.7	48.4	61.4
$\overline{X}$	25	53.2	53.3

Plot C

	13.2	24.6	46.9
	10.4	39.1	55.1
	10.0	28.6	55.1
	10.1	18.4	33.4
	19.3	45.1	71.5
	11.3	31.4	42.0
	8.5	20.3	43.2
	11.4	32.0	34.8
$\overline{X}$	11.8	29.9	47.8

APPENDIX 1:4

Radioiodine Uptake 1972

Plot A

	1.6.72			24.8.72		
SHEEP NO.	T2	T6	T24	T2	T6	T24
5	9.8	15.8	62.8	-	27.8	42.0
7	12.2	13.7	31.9	-	23.0	30.8
8	7.8	13.1	38.1	8.9	17.7	26.3
16	14.0	32.1	68.1	-	39.7	58.5
17	8.9	11.9	39.8	-	30.3	63.3
X	10.5	17.3	48.1	8.9	27.7	44.1
4*	3.9	12.4	29.1	-	11.4	32.1
12*	17.5	32.5	53.4	16.1	33.2	69.6
18*	9.6	22.4	49.0	17.4	26.7	54.7
21*	5.2	11.5	28.5	-	22.7	30.3
22*	9.7	23.4	40.7	7.1	18.2	51.7
X	9.2	20.4	40.1	13.5	22.4	47.7

Plot B

Maintained on Plot C Previously

5	7.1	11.9	30.9	7.9	20.9	38.0
10	9.7	18.3	43.6	13.9	32.0	35.3
11	13.7	20.9	39.1	7.2	18.2	35.3
12	13.2	20.3	46.1	6.8	19.0	38.5
19	9.3	13.6	39.6	12.0	23.6	34.9
X	10.6	17.0	39.9	9.6	22.7	36.4

Maintained on Plot B previously

4	8.8	22.4	46.9	14.5	24.7	28.6
5	14.6	29.6	70.8	9.1	21.5	27.3
6	13.4	31.4	63.8	10.3	17.9	34.9
9	13.2	25.2	52.0	12.1	26.3	40.2
17	16.3	28.8	61.0	17.4	32.6	58.7
X	13.3	27.5	58.9	12.7	24.6	37.9

* Drenched with 250mg KI on 15.3.72

Cont.

Plot C

Maintained on Plot C previously

1	5.3	9.3	22.5	5.6	9.2	21.0
3	10.8	17.0	55.0	6.7	11.4	17.1
7	6.6	11.1	31.7	5.7	11.6	34.5
13	7.7	10.4	31.7	5.1	5.6	23.2
14	7.9	16.3	39.6	3.3	5.6	17.3
X	7.7	12.8	36.1	5.3	8.7	23.5

Maintained on Plot B previously

8	8.8	18.3	47.5	8.0	13.9	32.6
12	10.9	39.6	76.7	8.6	15.8	41.8
19	13.6	26.7	47.2	8.8	14.8	37.3
22	8.5	23.5	45.6	4.6	5.7	22.0
24	9.9	24.6	63.8	10.0	20.1	22.4
X	10.3	26.5	56.2	8.0	14.1	31.2

Plot D

4	10.9	24.7	44.6	12.5	19.6	32.2
8	9.4	19.3	44.8	13.9	27.9	51.7
10	5.3	9.8	22.8	5.0	9.0	22.5
14	8.8	19.3	37.6	9.0	18.0	44.0
20	5.0	10.0	30.6	4.0	10.5	21.6
X	7.9	16.6	36.1	8.9	17.0	34.4

APPENDIX 1:5

Ewe Serum Thiocyanate Levels (µg/ml)

July

N - Ewes which had normal lambs
G - Ewes which had goitrous lambs

Plot A		Plot B		Plot C		Plot D	
N	G	N	G	N	G	N	G
0.7	1.4	0.6	2.5	0.2		0.2	0.3
1.1	1.2	1.3	1.0	0.3		0.2	
2.4	2.3	1.2	1.5	0.2		0.3	
2.6	0.8	1.0	2.9	0.2		0.5	
2.5	3.3	0.6	1.6	0.2		0.8	
2.6	2.1	1.5	1.0	0.2		0.6	
3.1	2.2	0.9	1.4	0.8		0.3	
2.6	3.2	1.5	1.1	0.3		0.3	
2.0	3.3	1.7		0.3		0.6	
1.6	2.5	1.8		0.3		0.6	
1.5		1.0		0.4		0.9	
		0.9		0.3		0.4	
		1.3		0.3		0.7	
		1.0		0.2		0.5	
				0.3		0.4	
				0.2		0.2	
				0.2		0.2	
				0.2		0.7	
				0.3		1.0	
				0.2			

August

Plot A		Plot B		Plot C		Plot D	
N	G	N	G	N	G	N	G
2.2	2.2	2.4	1.4	0.3		0.3	0.2
2.9	3.3	1.0	1.4	0.3		0.3	
3.7	3.1	2.0	2.3	0.2		0.3	
2.4	2.1	1.7	2.3	0.2		0.4	
5.1	3.6	2.3	1.9	0.3		0.7	
3.3	2.3	1.4	1.9	0.4		0.2	
4.3	2.8	2.5	1.9	0.3		0.6	
2.6	3.4	2.1	1.5	0.3		0.7	
2.6	4.4	1.8		0.3		0.4	
2.4	3.3	1.3		0.3		1.0	
3.2	3.2	1.2		0.3		0.2	
		2.3		0.2		0.7	
		1.5		0.3		0.4	
				0.2		0.3	
				0.3		0.4	
				0.1		1.0	
				0.2		1.3	
				0.2		0.8	
				0.1			
				0.3			

One ewe in each of plots A and B which had 1 normal and 1 goitrous lamb was not included.

APPENDIX 1:6

Nitrate Content of Pasture Species (ppm)

	A	B	C	D
5.3.71				
Lolium	1756	1430	613	1165
Trifolium	1347	2349	1153	947
Hordeum	1745	4831	-	665
Holcus	-	1124	-	-
13.5.71				
Lolium	1135	933	680	1049
Trifolium	1052	1139	181	696
Hordeum	-	2021	-	1210
Holcus		929	-	-
Vulpia	-	-	179	-
25.6.71				
Lolium	2551	1248	805	1058
Trifolium	573	625	691	965
Hordeum	3523	990	-	1097
Holcus	-	928	-	-
Vulpia	-	-	1068	-
Poa	104	-	-	
2.8.71				
Lolium	752	385	314	455
Trifolium	467	364	135	493
Hordeum	1636	1665	-	232
Holcus	-	534	-	-
Vulpia	-	790	136	287
Poa	92	-		547
24.9.71				
Lolium	723	411	360	514
Trifolium	461	325	158	406
Hordeum	1890	1563	-	320
Holcus	-	620	-	-
Vulpia	-	476	206	273
Poa	108	-	-	421

APPENDIX 1:7

Ewe Serum Nitrate 20.8.71 (ppm)

(All Ewes)

Plot A	B	C	D
2.4	3.4	2.1	3.5
3.1	3.8	2.1	2.6
3.1	2.8	3.2	2.9
3.6	2.9	2.6	3.8
2.2	3.1	2.8	3.8
2.3	2.1	3.2	2.3
2.9	2.0	2.4	2.0
3.0	2.0	2.5	2.6
2.0	2.4	2.0	2.8
2.1	2.9	3.0	3.1
2.0	3.3	2.9	2.2
2.6	2.4	3.2	3.0
2.5	2.4	2.9	2.4
3.8	3.6	2.7	2.5
2.2	3.2	3.7	2.1
3.1	2.6	2.1	2.0
2.3	2.2	2.0	2.0
3.0	2.0	2.4	2.8
2.1	2.9	2.9	2.3
2.0	3.3	2.1	2.6
2.4	2.9	2.6	2.7
2.9	3.8		
3.3	2.7		
2.0	2.7		
3.6	2.1		
2.7	2.8		

Cont.

Ewe Serum Nitrate 4.6.71

(Random Sample of Ewes)

A	B	C	D
2.0	2.7	3.1	2.6
2.6	3.3	3.8	2.0
2.5	3.3	3.1	2.4
3.6	2.5	2.0	3.0
2.3	3.1	2.6	2.0
3.2	2.2	2.4	3.8
3.4	2.1	2.3	2.8
2.1	2.4	2.7	3.2
2.1	2.3	2.0	2.0
2.4	2.6	2.1	2.2
2.6	2.7	2.6	2.6

Ewe Serum Nitrate 13.8.71

(Random Sample of Ewes)

A	B	C	D
2.9	3.2	2.7	2.7
2.5	2.8	2.2	3.2
3.4	3.6	2.0	3.6
3.8	2.2	2.0	2.6
2.8	2.3	2.6	2.9
3.0	2.1	2.4	3.2
2.5	2.0	2.3	2.1
2.0	2.9	3.8	2.0
2.0	2.7	2.7	3.3
2.3	2.5	3.0	2.0
2.7	2.6	2.6	2.8

APPENDIX 1:8

Blood Thiocyanate Content of Ewe Dosed with 0.5g KCNS as a single dose.

TIME (HR)	0	0.5	1	2	3	4	8	12	24
CNS^- (mg%)	0.8	2.8	4.0	5.2	5.4	4.9	2.3	0.85	0.8

APPENDIX 1:9

Ewe Radioiodine Uptakes 1971 (Animal House)

TREATMENT	SHEEP	2 HR	6.5 HR	24 HR
Basal	4	37.3	52.9	66.9
	5	10.1	26.8	39.7
	11	10.8	24.1	47.2
	$\bar{X}$	19.4	34.5	51.2
Basal + KCNS	2	11.1	23.8	29.6
	8	5.3	9.6	33.9
	10	17.9	36.3	59.3
	X	11.4	23.2	40.6
Basal + KCNS + KI	3	2.1	5.4	9.1
	6	4.5	5.1	9.9
	7	2.4	3.4	7.3
	9	3.5	4.8	10.3
	X	3.1	4.7	9.2

APPENDIX 1:10

Animal House 1972. Ewe Metabolic Body Weights (Kg 0.75)

Treatment	Ewe Number	DATE					
		12.6.72	26.6	10.7	24.7	7.8	21.8
A	16	12.8	15.6	15.6	16.1	16.9	17.2
	19	13.3	15.5	14.6	15.9	16.2	16.5
	5	16.4	19.4	19.2	20.2	21.2	20.2
	1	15.2	16.3	16.6	17.4	17.7	17.8
	17	14.2	15.9	15.9	16.6	17.4	18.5
B	18	13.3	15.6	15.6	16.5	16.8	17.2
	14	13.4	16.3	16.8	16.5	17.4	17.5
	12	14.2	16.5	16.2	16.8	17.2	16.4
	3	15.2	16.8	16.6	17.4	18.1	18.4
	7	14.6	17.4	17.7	18.8	20.4	20.6
C	6	14.2	16.9	17.1	16.9	18.7	19.4
	2	13.8	15.6	16.6	17.5	18.0	19.0
	8	14.4	16.5	16.2	16.8	18.0	19.1
	13	15.3	18.0	18.1	19.4	20.0	20.1
	10	14.1	15.6	16.1	16.8	18.1	16.1
D	15	13.4	15.3	14.4	15.0	16.2	16.7
	9	14.5	16.6	16.2	16.9	17.7	18.8
	20	14.4	16.3	15.6	16.1	17.1	16.8
	4	15.2	17.4	16.8	17.5	18.1	19.1
	11	14.6	16.9	16.6	17.2	18.1	18.7

APPENDIX 1:11

Ewe Weekly Iodine Balance (µg I)

SHEEP	I	E^1	B	I	E^2	B	I	E^3	B	I	E^4	B
GROUP A												
1	685	562	123	589	638	-49	709	929	-220	704	495	209
5	806	546	260	847	588	259	951	968	-17	969	707	262
16	661	536	125	661	485	176	767	998	-231	759	660	99
17	701	411	290	632	698	-66	689	1176	-487	686	384	302
$\bar{X}$	713	514	199	682	602	80	779	1018	-191	780	562	218
GROUP B												
3	1143	676	467	997	673	324	1145	885	260	1174	812	362
7	1269	894	375	1269	800	469	1401	1084	317	1417	690	727
12	1198	1075	123	1234	1304	-70	1286	909	377	1285	867	418
14	1107	460	647	1164	806	358	1159	996	163	1209	491	718
18	1157	858	299	1157	600	557	1096	608	488	1060	711	349
$\bar{X}$	1175	793	382	1164	837	327	1217	896	321	1229	714	514
GROUP C												
2	1834	778	1056	1823	1124	699	1784	962	822	1779	926	853
6	1928	896	1032	1928	1437	491	1958	1371	587	1914	1077	837
8	1759	779	980	1759	953	806	1926	972	954	1877	1353	524
10	1680	559	1121	1606	992	614	1725	828	897	1642	1146	496
13	1869	826	1043	1869	1202	667	2174	1601	573	2174	1525	649
$\bar{X}$	1814	768	1046	1797	1142	649	1913	1147	766	1877	1205	671
GROUP D												
4	2321	1562	759	2256	1564	692	1795	1107	688	2072	652	1420
9	2125	658	1467	2149	866	1283	1628	924	704	1833	614	1219
11	2175	1163	1012	2248	1196	1052	2193	985	1208	2164	646	1518
15	2026	908	1118	1775	845	930	1054	695	359	1960	636	1324
20	2267	1070	1197	2194	1425	769	1642	1023	619	1780	516	1264
$\bar{X}$	2183	1072	1110	2124	1179	945	1662	947	715	1962	613	1355

I = Weekly Intake
E = Weekly Excretion
B = Weekly Balance

Cont.

Ewe Weekly Iodine Balance (μg I)

SHEEP	I	E^5	B	I	E^6	B	I	E^7	B	I	E^8	B
GROUP A												
1	677	790	-113	611	799	-188	693	777	-84	692	483	209
5	940	1105	-165	948	1198	-250	999	905	94	937	817	120
16	707	642	65	653	753	-100	694	665	29	680	321	359
17	667	369	298	669	909	-210	675	531	144	613	345	268
$\bar{X}$	748	727	21	720	915	-187	765	720	45	731	492	239
GROUP B												
3	1160	726	434	1045	838	207	1150	798	352	1112	805	307
7	1410	679	731	1464	1147	317	1504	1169	687	1444	1150	294
12	1176	834	342	1187	922	265	1139	1018	121	1124	622	502
14	1228	983	245	1282	715	567	1127	740	387	1195	785	410
18	1101	379	722	1095	570	525	1085	667	418	1059	374	685
$\bar{X}$	1215	720	494	1215	838	376	1201	878	322	1187	747	439
GROUP C												
2	1820	940	880	1862	1132	730	1882	1405	477	1539	1342	197
6	1886	1133	753	1770	1303	467	1930	1449	481	1841	1025	816
8	1760	1376	384	1842	1153	689	1962	1319	643	1952	1042	910
10	1660	1343	317	1725	981	744	1759	1367	392	1547	1162	385
13	2138	1383	755	2138	1562	576	2270	1845	425	2291	1828	463
$\bar{X}$	1853	1235	617	1867	1226	641	1961	1477	483	1834	1280	554
GROUP D												
4	1995	651	1344	2098	964	1134	2143	1114	1029	2203	1481	722
9	1980	796	1184	2265	1219	1046	2379	1628	751	2356	1083	1273
11	2088	1249	839	2442	1595	847	2456	1506	950	2297	1377	920
15	1893	805	1088	2192	1345	847	2283	1539	744	2283	1242	1041
20	1921	824	1097	1779	894	885	1843	1483	360	1969	803	1166
$\bar{X}$	1975	865	1092	2155	1203	951	2221	1454	766	2222	1197	1024

I = Weekly Intake
E = Weekly Excretion
B = Weekly Balance

Cont.

Ewe Weekly Iodine Balance (µg I)

SHEEP	I	E^{9}	B	I	E^{10}	B	I	E^{11}	B	I	E^{12}	B
GROUP A												
1	493	398	95	684	857	-173	734	402	332	769	225	544
5	867	505	182	630	760	-130						
16	681	495	186	566	584	-18						
17	694	371	323	680	582	98	554	496	58	663	323	340
$\bar{X}$	684	442	196	640	696	-55			195			442
GROUP B												
3	1154	770	384	1236	685	551	621	493	128			
7	1515	917	598	1430	750	680	937	424	513	1162	512	650
12	982	547	435									
14	1123	298	825	1084	657	427						
18	1175	580	595	1130	564	566	1176	737	439	1087	530	557
$\bar{X}$	1190	622	567	1220	664	556	911	551	360			603
GROUP C												
2	1929	964	965	1940	1055	855	1873	1015	858	1852	733	1119
6	1972	990	982	1942	1053	889	1649	1156	493	1553	634	919
8	2106	1178	928	2070	1236	834	2103	1321	782	2096	1104	992
10	1553	1160	393									
13	2267	1366	901	2273	1458	815	1895	1181	714	2173	1307	866
$\bar{X}$	1965	1132	833	2056	1200	855	1880	1168	711	1918	945	974
GROUP D												
4	2246	1175	1071	2327	982	1345	2163	1019	1144	1910	737	1173
9	2220	1108	1112	2326	1064	1262	2348	1467	881	2463	1186	1277
11	2321	1319	1002	2153	1114	1039	1793	1354	439	2254	1512	742
15	2466	2362	104	2466	1572	894	2146	1429	717			
20	1932	1529	403	1845	1200	645	1658	1301	357	1790	1021	769
$\bar{X}$	2237	1500	738	2223	1186	1037	2021	1314	707	2104	1114	990

I = Weekly Intake
E = Weekly Excretion
B = Weekly Balance

Cont.

Ewe Weekly Iodine Balance (µg I)

SHEEP	I	E^{13}	B	I	E^{14}	B
GROUP A						
1	729	346	383	781	482	299
5						
16						
17	630	362	268			
$\bar{X}$			325			
GROUP B						
3						
7	1259	682	577			
12						
14						
18	929	393	536			
$\bar{X}$			556			
GROUP C						
2	1655	919	736	1613	1151	462
6	1516	746	770			
8	2051	1043	1008			
10						
13	1976	1419	557			
$\bar{X}$	1799	1032	767			
GROUP D						
4	2430	1062	1368			
9	1997	1102	895			
11	2040	1336	704			
15						
20	1408	740	668			
$\bar{X}$	1968	1060	908			

I = Weekly Intake
E = Weekly Excretion
B = Weekly Balance

APPENDIX 1:12

Ewe Radioiodine Uptakes. (Iodine Balance Expt., 1972)

	3.6.72			22.8.72		
SHEEP NO.	T2.5	T6.0	T24	T2.5	T6.0	T24
A						
1	5.5	10.4	16.6	18.0	33.0	36.6
5	4.5	9.3	17.3	2.3	4.3	7.9
16	8.0	16.9	33.6	12.7	29.4	39.6
17	8.8	17.0	31.4	7.4	11.9	22.4
$\bar{X}$	6.7	13.4	24.7	10.1	19.7	26.6
B						
3	5.0	12.0	22.7	13.4	18.2	29.8
7	7.8	12.2	30.1	3.0	5.3	12.8
12	4.8	9.2	26.4	0.7	1.1	2.4
14	4.6	8.4	20.6	7.4	15.2	20.4
18	8.6	14.4	28.0	1.9	3.9	7.5
$\bar{X}$	6.2	11.2	25.6	5.3	8.7	14.6
C						
2	5.1	9.9	20.4	6.4	12.4	24.2
6	4.6	8.0	16.3	3.5	6.8	16.8
8	6.7	14.3	27.3	3.8	7.9	16.2
10	5.1	12.8	20.2	4.7	9.0	16.3
13	6.2	10.7	29.3	4.8	6.6	12.3
$\bar{X}$	5.5	11.1	22.7	4.6	8.5	17.1
D						
4	6.1	12.7	23.6	5.5	11.6	19.1
9	11.1	20.1	35.0	4.6	7.8	14.8
11	8.5	16.1	34.8	2.0	3.3	5.4
15	7.0	10.4	25.4	0.8	1.4	3.0
20	6.5	12.2	24.7	3.2	8.3	21.1
$\bar{X}$	7.8	14.3	28.7	3.2	6.5	12.7

APPENDIX 2.

STATISTICAL ANALYSES

In the following tables the normal indications of level of significance have been used. These are:-

Symbol	Level of significance
*	5%
**	1%

Where Duncan's Multiple Range Test has been used figures underlined do not differ significantly from one another.

APPENDIX 2:1

Iodine Content of Ryegrass in Field Trial

Source of Variation	d.f.	s.s.	m.s.	f.
Sand v Clay Soil	1	278300.3	278300.3	42.1**
Harvest Time	20	466409.6	23320.5	3.5**
Reps. within soils	2	5197.3	2598.6	< 1
Error	60	396671.7	6611.2	
Total	83	1116578.9		

Duncan's multiple range test (0.01 level) for time of harvest

$\bar{I}$ Date	22.11.71	18.10.70	5.3.71	9.2.72	25.6.71
$\bar{I}$ content	128.25	136.25	148.25	199.25	200.25
$\bar{I}$ Date	20.12.71	22.2.73	12.1.71	24.9.71	2.8.71
$\bar{I}$ content	206.25	216	219	224.75	225.5
$\bar{I}$ Date	18.5.72	10.6.70	16.9.73	12.4.73	10.8.72
$\bar{I}$ content	228.5	232	236	240	243.25

$\bar{I}$ Date	21.8.70	30.6.72	12.6.73	27.7.73	14.4.72	13.5.71
$\bar{I}$ content	243.5	246.25	260.75	263	333.25	504

Iodine Content of Subterranean Clover in Field Trial

Source of Variation	d.f.	s.s.	m.s.	f.
Sand Clay Soil	1	98352	98352.0	15.4**
Harvest Time	18	578533.7	32140.8	5.0**
Reps. within Soils	2	13965.0	6982.5	1.1n.s.
Error	54	343936.0	6369.2	
Total	75	1034787.7		

Duncan's multiple range test (0.01 level) for time of harvest

$\bar{I}$	Date	12.6.73	30.6.72	18.5.72	5.3.71	27.7.73
	content	94.25	97.25	118.5	129.5	145
$\bar{I}$	Date	14.4.72	24.9.71	16.9.73	2.8.71	25.6.71
	content	152.75	153.25	161.25	176.25	177.25
$\bar{I}$	Date	10.8.72	21.8.70	10.6.70	18.1070	12.4.73
	content	180.25	184.75	188.75	198.75	210.5
$\bar{I}$	Date	22.2.73	22.11.71	20.12.71	13.5.71	
	content	230	264	315.75	488	

APPENDIX 2:2

I Content of A Horizon of Soil in Bothwell Field Trial

Source of var.	d.f.	s.s.	m.s.	f.
Between soils	3	1969476	656492	425.5**
Error	16	24682	1543	
Total	19	1994158		

Duncan's Multiple Range Test (0.01 level)

Plot	A	B	D	C
$\bar{X}$	253	256	873	890

I content of top 2cm of soil

Source of var.	d.f.	s.s.	m.s.	f.
Between soils	3	571483.6	190494.5	1.83n.s.
Error	16	1660668.4	103791.8	
Total	19	2232152		

Soil loss on ignition (top 2cm)

Source of var.	d.f.	s.s.	m.s.	f.
Between Soil Types	3	10.25	3.4	< 1 n.s.
Error	12	13507	1125.6	
Total	15	13517.25		

APPENDIX 2:3

Radioiodine Uptake 1971. (Field Trial)

24 Hours

Source of Var.	d.f.	s.s.	m.s.	f.
Plot Effect	1	123.2	123.2	1.15 n.s.
Error	14	1502.0	107.2	
Total	15	1625.2		

7.0 Hours

Source of Var.	d.f.	s.s.	m.s.	f.
Plot Effect	1	2155.3	2155.3	19.8**
Error	14	887.2	63.37	
Total	15	3042.5		

2.1 Hours

Source of Var.	d.f.	s.s.	m.s.	f.
Plot Effect	1	694.3	694.3	24.8**
Error	14	391.9	28.0	
Total	15	1086.2		

APPENDIX 2:4

Radioiodine Uptake (Field Trial, 1972)

Plots B and C.

24 Hour.

Source of var.	d.f.	s.s.	m.s.	f.
Plots	3	2011.8	670.6	6.38**
Periods	1	2468.0	2468.0	23.48**
Error	35	3679.1	105.1	
Total	39	8158.9		

Plots X Periods interaction was added to the error factor to increase the precision of the estimate.

Duncan's Multiple Range Test (0.01 level)

JUNE

Plot	C (c previously)	B (c previously)	C (b prev.)	B (b prev.)
$\bar{X}$	36.1	39.9	56.2	58.9

AUGUST

Plot	Cc	Cb	Bc	Bb
$\bar{X}$	22.6	31.2	36.4	37.9

6 Hour.

Source of var.	d.f.	s.s.	m.s.	f.
Plots	3	1198.4	399.5	11.22**
Periods	1	98.3	98.3	2.76n.s.
Error	35	1248.3	35.6	
Total	39	2545		

Duncan's Multiple Range Test (0.01 level)

JUNE

Plot	C (c prev.)	B (c prev.)	C (b prev.)	B (b prev)
$\bar{X}$	12.8	17.0	26.5	27.5

Cont.

AUGUST

Plot	C (c prev.)	C (b prev.)	B (c prev.)	B (b prev)
$\bar{X}$	8.6	14.1	22.7	24.6

2 Hour.

Source of var.	d.f.	s.s.	m.s.	f.
Plots	3	215.5	71.8	11.99**
Periods	1	25.1	25.1	4.19*
Error	35	209.8	5.99	
Total	39	450.4		

Duncan's Multiple Range Test (0.01 level)

JUNE

Plot	C (c prev.)	C (b prev.)	B (c prev.)	B (b prev.)
$\bar{X}$	7.7	10.3	10.6	13.3

AUGUST

Plot	C(c)	C(b)	B(c)	B(b)
$\bar{X}$	5.3	8.0	9.6	12.7

Plot A. Untreated ewes - not pregnant
2 Hour uptake excluded.

Source of var.	d.f.	s.s.	m.s.	f.
Periods	1	0.1	0.1	n.s.
Time of Uptake	1	3618.1	3618.1	22.14**
T x P	1	76.0	76.0	<1 n.s.
Error	16	2814.5	163.4	
Total	19	6508.7		

Plot D. Non pregnant ewes

Source of var.	d.f.	s.s.	m.s.	f.
Periods	1	0.1	0.1	<1 n.s.
Time of Uptake	2	3734.8	1867.4	26.7**
T x P	2	10.5	5.25	<1 n.s.
Error	23	1604.8	69.8	
Total	29	5350.2		

APPENDIX 2:5

Nitrate Content of Subterranean Clover (Field Trial, 1971)

Source of var.	d.f.	s.s.	m.s.	f.
Sand v. Clay	1	413856.9	413856.9	7.09*
Time of Harvest	4	3379898	844974.5	14.47**
Soil v Time	4	797860	199465	3.42n.s.
Error	10	583794.1	58379.4	
Total	19	5175409		

Duncan's Multiple Range Test (0.01 level)
Time of Harvest

Date	24.9	2.8	25.6	13.5	5.3
$\bar{X}$	338	365	714	792	1449

Nitrate Content of Ryegrass (Field Trial, 1971)

Source of var.	d.f.	s.s.	m.s.	f.
Sand v. Clay	1	929236.5	929236.5	7.08*
Time of Harvest	4	2883134.3	720783.6	5.49*
Soil v. Time	4	582889.4	145722.4	1.11
Error	10	1312696.8	131269.7	
Total	19	5707957		

Duncan's Multiple Range Test Time of Harvest (0.01 level)

Date	2.8	24.9	13.5	5.3	25.6
Mean	477	502	949	1241	1415

APPENDIX 2:6

Glasshouse Trial - Plant I Content

Source of Var.	d.f.	s.s.	m.s.	f.
Harvest	4	6528	1632	2.56 n.s.
Species	2	2859	1430	2.24 n.s.
Soil	1	792	792	1.24 n.s.
Water Level	1	1421	1421	2.23 n.s.
Harvest x Species	8	11514	1439	2.25 n.s.
Harvest x Soil	4	2215	554	< 1
Harvest x Water	4	1047	262	< 1
Species x Soil	2	1524	762	1.20 n.s.
Species x Water	2	2072	1036	1.62 n.s.
Soil x Water	1	416	416	< 1
Error	30	19122	638	
Total	59	49510		

Glasshouse Trial

Total D.M. Production - Final Harvest

Source of Var.	d.f.	s.s.	m.s.	f.
Species	2	518.6	259.3	89.4**
Soil	1	90.8	90.8	31.3**
Water	1	19.2	19.2	6.6*
Error	7	20.3	2.9	
Total	11	648.9		

Total Iodine Content of Final Harvest

Source of var.	d.f.	s.s.	m.s.	f.
Species	2	4337511	2168756	38.2**
Soil	1	1122408	1122408	19.8**
Water	1	170408	170408	3.0n.s.
Error	7	397137	56734	
Total	11	6027464		

APPENDIX 2:7

Radioiodine Uptake - Animal House 1971

2 Hr uptake

Source of Var.	d.f.	s.s.	m.s.	f.
Diff. Between Groups	2	457.8	152.6	2.8 n.s.
Error	7	564	80.6	
Total	9	1021.8		

6.5 Hr uptake

Source of Var.	d.f.	s.s.	m.s.	f.
Diff. Between Groups	2	1598	799	6.5*
Error	7	858	122.6	
Total	9	2456		

t test

Between Control	and +KCNS	1.25 n.s.
" "	" +KCNS + KI	3.52**
" +KCNS	" " "	2.2 n.s.

24 Hr uptake

Source of Var.	d.f.	s.s.	m.s.	f.
Diff. Between Groups	2	3536.8	1718.4	13.1**
Error	7	915.4	130.7	
Total	9	4352.2		

t test

Between Control	and + KCNS	1.14 n.s.
" "	" + KCNS + KI	4.8**
" + KCNS	" " "	3.6**

APPENDIX 2:8

Lambing. Animal House 1971

Lamb Weight

Source of var.	d.f.	s.s.	m.s.	f.
Treatment	2	1.7	0.85	2.8n.s.
Error	13	4.6	0.3	
Total	15	6.3		

Thyroid Weight

Source of Var.	d.f.	s.s.	m.s.	f.
Treatment	2	497.9	248.9	3.63 n.s.
Error	13	890.3	68.4	
Total	15	1388.2		

Mean Follicle Diameter

Source of Var.	d.f.	s.s.	m.s.	f.
Treatment	2	0.032	0.16	6.4*
Error	12	0.03	0.0025	
Total	14	0.062		

t test	T value
Basal v KCNS treated	2.8*
Basal v KI treated	3.8*
KI v KCNS	1.0 n.s.

Thyroid/Body Weight Ratio

Source of Var.	d.f.	s.s.	m.s.	f.
Treatment	2	39.2	19.6	3.4 n.s.
Error	13	73.5	5.7	
Total	15	112.7		

APPENDIX 2:9

Iodine Balance

Source of Var.	d.f.	s.s.	m.s.	f.
Time	7	1238967	176995	15.9**
Linear Effect of Time	1	47665	47665	4.2*
Group	3	17776188	5925396	531.7**
Group x Time	24	2883430	120143	10.7**
Error	136	1515526	11144	
Total	170			

t test on means

S.E. for A compared with B, C, D = 17.6

t for closest comparison = 16.4 (sig at 0.01)

S.E. for B compared with C and D or C compared with D = 15.7

t for comparison of B with C = 21.8 (sig at 0.01)

C with D = 17.2 (" " ")

APPENDIX 2:10

Iodine Balance

Analysis of Variance of Radioiodine Uptakes

2 Hour

Source of Var.	d.f.	s.s.	m.s.	f.
Period	1	8.9	8.9	< 1 n.s.
Group	3	57.2	19.1	1.8 n.s.
Period x Group	3	71.5	23.8	2.3 n.s.
Error	30	311.2	10.4	
Total	37	448.8		

6 Hour

Source of Var.	d.f.	s.s.	m.s.	f.
Period	1	319.4	319.4	14.1**
Group	3	264.5	88.2	3.9*
Period x Group	3	308.8	102.9	4.5*
Error	30	676.8	22.6	
Total	37	1569.5		

In Period 2, Group A was significantly greater than the others ($t = 3.5^{**}$)

In Period 2, Group D was significantly lower than in Period 1 ($t = 2.6^{*}$)

Overall Period 2 is lower than Period 1.

24 Hour

Source of Var.	d.f.	s.s.	m.s.	f.
Period	1	633.0	633.0	9.3**
Group	3	190.5	63.5	< 1 n.s.
Group x Period	3	394.0	131.3	1.9 n.s.
Error	30	2038.3	67.9	
Total	37	3210.8		

Period 2 is significantly lower than Period 1.

APPENDIX 2:11

I Balance-Lambing

Thyroid Weight

Source of Var.	d.f.	s.s.	m.s.	f.
Groups	3	3.91	1.30	1.1 n.s.
Error	20	23.62	1.18	
Total	23	27.53		

Lamb Weight

Source of Var.	d.f.	s.s.	m.s.	f.
Groups	3	13.1	4.3	2.4 n.s.
Error	20	36.1	18	
Total	23	49.2		

Thyroid/Body Weight Ratio

Source of Var.	d.f.	s.s.	m.s.	f.
Groups	3	0.577	0.192	3.31*
Error	20	1.162	0.058	
Total	23	1.739		

t values

Between Groups

A & B	0.07	A & C	2.09*	B & D	2.37*
B & C	2.15*	A & D	2.31*	C & D	0.45

Mean Follicle Diameter

Source of Var.	d.f.	s.s.	m.s.	f.
Group	3	1232.4	410.8	1.02 n.s.
Error	20	8031.5	401.6	
Total	23	9263.9		