MISCELLANEOUS STUDIES ON THE IODINE AND GOITRE PROBLEM IN NEW ZEALAND.

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(With 1 Figure and 3 Charts.)

CONTENTS.

I. Introduction.

The present paper represents a continuation of the researches into the natural sources of iodine in New Zealand which have been carried out in connection with goitre research during recent years at Otago University (*see* papers by Hercus, Benson and Carter, 1925; Hercus and Roberts, 1927; Hercus, Aitken, Thomson and Cox, 1931). Our recent studies on the occurrence of iodine have to do with the amount and nature of the iodine which is present in rather high concentration in some sea weeds and sea fish, and also with the question of the iodine content of pigs' thyroids, while those on iodine metabolism include an investigation of experimental goitre in rabbits and of the variations in the iodine content of the blood during anaesthesia. Miscellaneous studies related to the goitre problem and improvements in the technique of iodine estimation are also described.

Not all the work in the present paper has been carried out by the authors in collaboration; the purely chemical work in Sections II and III (2) is due to one of us (H. A. A. A.), while much of the work involved in Section V was also carried out by this author with the help of Dr W. S. Fogg, to whom we are greatly indebted for valuable assistance and advice.

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II. Analytical methods.

Throughout the present investigations the iodine analyses have been made by methods based on the procedure of Leitch and Henderson (1926). In cases where the analytical sample contains less than 2 microgrammes it was found necessary to modify the technique so as to permit of titration in very small volume in order to obtain a sharp end-point with the starch-iodide indicator (see Aitken, 1930). An alternative is to take a correspondingly larger sample for analysis and to perform the final titration in the smallest convenient volume which can be attained in a 20 c.c. Erlenmeyer or similar small flask. In order to eliminate the error involved in the use of the ordinary 0·1 c.c. pipette a convenient and accurate micro-burette was devised in which the standard solution floats on a thread of mercury, the position of the latter being adjusted by means of a fine screw projecting into a mercury reservoir (Aitken, 1931).

Some investigators have found large errors arising in methods based on V. Fellenberg's which they have ascribed to the alcoholic extraction of the potassium iodide being incomplete. Thus Andrew (1930) finds it necessary to multiply his experimental results by 3/2, while Adolph and Shen-Chao Ch'En (1930) use the factor 100/85. Andrew (*loc. cit.* p. 273) found that there was little or no loss involved in the first alcoholic extraction, while there was a considerable loss in the second. No attempt was made to explain this phenomenon, though experiments were conducted which seemed to show that the loss could not have been wholly due to over-heating.

Partly to seek an explanation of this curious anomaly and partly with a view to finding optimum conditions for the performance of the alcoholic extraction we have carried out some experiments on the partition of potassium iodide at low concentration between aqueous alcohol and potassium carbonate solution. The results showed that if the carbonate solution was saturated or nearly so the partition coefficient (concentration in alcohol/concentration in carbonate solution) was greater than 8 at all concentrations of iodine ranging from 79 microgrammes to 2·4 microgrammes of iodine per c.c. in the alcohol phase.

The partition coefficient in the case of alcohol in equilibrium with less concentrated potassium carbonate solution was found to decrease, but not

sufficiently to impair the alcoholic extraction unless the carbonate solution was very considerably diluted.

There remained the question of the distribution of the iodide in an aqueous paste of potassium carbonate under the conditions of extraction employed in actual practice. Andrew (*loc. cit.* p. 273) considered that some of the losses which he experienced may have resulted "from some of the iodide being retained by the potassium carbonate."

This point was investigated by dehydrating a paste containing a known amount of iodide almost instantaneously with an excess of absolute alcohol so that it fell to powder, and analysing the supernatant alcohol. Under these conditions the iodide in the aqueous phase would pass immediately into the alcohol. As was expected, practically all the iodide was found to be present in the alcohol; from which it follows that in a homogeneous potassium carbonate paste containing potassium iodide almost all the iodide is present in the aqueous phase.

None of these results would suggest any possibility of so incomplete an extraction as that observed by Andrew, and one is forced to conclude, despite his own experiments on the subject, that Andrew's losses were mainly due to over-heating in the presence of insufficient potassium carbonate to prevent volatilisation of the potassium iodide.

We consider that over-heating can best be avoided by placing the crucible inside a slightly larger one containing a few chips of pipe-clay to prevent actual contact; the large crucible which can be conveniently held in an iron ring is heated by a large non-luminous flame. A trial with the inner crucible empty and shaded from direct light will show how strong a flame can be used without bringing the inner crucible to a glow; or a blank experiment may be conducted and the temperature in the inner crucible read with a thermometer. The temperature should be below 500° C.

Using this method of combustion, it is convenient to employ small nickel crucibles (about 25 c.c. capacity) instead of platinum dishes for collecting the first alcoholic extract. The alcoholic extract in the crucible is evaporated to dryness with addition of a small amount of potassium carbonate solution, and the residue is combusted by placing the small crucible in a slightly larger one as just described. All new nickel crucibles are freed from iodine by fusing potassium carbonate in them for a period of about an hour.

As to the alcoholic extraction, we consider that the following is the most expeditious method of recovering the iodide quantitatively from the potassium carbonate: the potassium carbonate residue, weighing say 2 g., is treated with sufficient half-saturated potassium carbonate solution from a dropping bottle (1·25 c.c. will be required) to produce a smooth paste when rubbed with two glass rods with flattened ends. 1 c.c. of 90 per cent. alcohol is added to assist the formation of the paste. Successive small amounts of absolute alcohol are now added, the paste being kept well stirred, until the paste shows a tendency to crumble. The alcohol is immediately decanted, a small amount

of vaseline being smeared on the lip of the crucible. Another 2 c.c. of 90 per cent. alcohol is added to bring the paste back again to a thin consistency, and absolute alcohol added as before till the paste again commences to crumble, whereupon the alcohol is again decanted. Finally the process is repeated for a third time.

It will be seen that in the method described not only is advantage taken of the tendency of the iodide to distribute itself chiefly in the alcohol layer, but also much of the aqueous phase itself, including the iodide contained therein, is withdrawn at each extraction by means of the absolute alcohol.

Details of the methods used for typical cases are now given:

Blood. If sufficient of the sample is available 12 c.c. is taken in a 100 c.c. nickel crucible together with 8 c.c. of 50 per cent. potassium hydroxide solution. The contents are evaporated to dryness and heated below 500° C. till all fuming has ceased (about 16 min.). The residue is crushed, moistened, evaporated to dryness and heated again for a further 5 min. It is then taken up with about 50 c.c. of water, filtered hot, and the paper washed with hot water. The filtrate (which must be practically colourless, otherwise combustion has been inadequate) is transferred to a clean crucible and evaporated carefully to dryness. Any crusts are crushed down with a nickel rod, the residue is heated for about 5 min. and finally cooled.

The potassium iodide is then extracted from the residue as described above, the extract is decanted into a small Erlenmeyer or other suitable flask and evaporated to dryness after addition of a particle of purified pipe-clay. The residue is treated as described by Leitch and Henderson (1926) and the iodine estimated by titration in the smallest possible volume with $N/500$ thiosulphate. A 0·1 c.c. pipette drawn off to a fairly fine tip so as to make it deliver slowly is most convenient for this purpose. If in any case the colour of free iodine is discernible on adding the potassium iodide, this should be titrated till almost colourless before adding the starch.

Where only a 5 c.c. sample of blood is available, the first alcoholic extract is collected in a small nickel crucible (about 25 c.c.) and evaporated to dryness after adding 0·4 c.c. of saturated potassium carbonate solution. The residue is combusted in the usual way, and extracted with alcohol on a smaller scale, using first 1 c.c. of alcohol and then successive amounts of about 0·5 c.c. The extracts are decanted into a titration tube with the aid of a thin glass rod, a little water is added together with a tiny spicule of pumice and the contents are evaporated to dryness. To the residue of salt is added about 1 c.c. of water and the resulting solution is acidified by adding 3 drops of 1 per cent. sulphuric acid from a dropping bottle. The tube is replaced on the sand bath, another tiny spicule of pumice is dropped in, and when the contents begin to boil 3 drops of fresh saturated bromine water (kept in a brown glass dropping bottle) are added. The solution is evaporated to a volume of about 0·3 c.c. and cooled; a minute crystal of potassium iodide and a small drop of fresh

starch solution are added and the solution is titrated in good diffused light, the best conditions being obtained under the open sky.

It is also possible to eliminate the second alcoholic extraction and still perform the micro-titration. To do this, the first alcoholic extract (8–12 c.c.) is collected in a 20 c.c. Erlenmeyer flask and evaporated to dryness. 3 c.c. of distilled water is added and the flask is inclined and rotated so that all the dry residue goes into solution. 2 c.c. is then withdrawn with a pipette, transferred to the titration tube and the iodine estimated as described above.

Urine and milk. 50 c.c. of the specimen is taken for analysis and 2 c.c. of potash solution is added. In the case of milk the fat is saponified by gentle warming on the water bath. The rest of the analysis is as for blood.

Vegetable matter, e.g. cabbage. 40 g. of fresh, finely chopped sample is placed in a tared 500 c.c. Pyrex beaker with 8 c.c. of 50 per cent. potassium hydroxide solution and covered with distilled water. The contents are boiled down to low volume with occasional stirring, the beaker is weighed, most of the semi-solid contents are transferred to a 100 c.c. nickel crucible and the beaker is re-weighed. The material in the crucible is evaporated to dryness and combusted as for blood, the crucible being capped with an open, metal cone to prevent the escaping gases from catching fire or the charred residue commencing to glow.

Thyroids. 1 g. of fresh chopped gland is placed in a flask together with 2 c.c. of 50 per cent. potash solution, 10 c.c. of alcohol, and 15 c.c. of water. The mixture is allowed to stand for a day and is then warmed on the water bath till the tissue goes into solution. The volume is then made up to 100 c.c. and aliquots of 2–5 c.c. are taken and transferred to a 100 c.c. nickel crucible. 2 c.c. of potassium carbonate solution is added, the contents are evaporated to dryness, combusted, and the iodine extracted and estimated in the usual way.

Butter (see Aitken, 1932). 25 g. of butter is placed in an 8 in. nickel basin and 20 c.c. of 50 per cent. potash solution is added followed by 80 c.c. of alcohol. The butter is saponified by heating for an hour on the water bath and the contents of the basin are evaporated to dryness on the sand bath. The residue is combusted below red heat over a gas ring, the basin being set in a shallow iron dish about 5·5 in. in diameter and 1·5 in. deep so that an air space conducts the heat to the basin, eliminating the possibility of local over-heating. The melt is occasionally stirred and distributed with a nickel spatula set in a wooden handle. When evolution of gases has ceased combustion is continued for a further half-hour and the grey layer of potassium carbonate which remains is washed down with a small amount of water, evaporated to dryness, and again combusted for half an hour. The residue is taken up with 150 c.c. of water and filtered hot with gentle suction using a 4 in. Buchner funnel and a 250 c.c. filter flask. The filtrate and washings, which should be almost colourless, are now transferred to a clean basin, evaporated to dryness, and the residue combusted for a further quarter of an hour.

Half-saturated potassium carbonate solution (about 8 c.c.) is now added so as to produce a smooth paste when rubbed into the residue by means of two stout glass rods with flattened ends (addition of a little 90 per cent. alcohol assists formation of the paste). About 25 c.c. of 95 per cent. alcohol is added and stirred thoroughly into the paste. The extract is decanted into a 100 c.c. nickel crucible, and the extraction is repeated two or three times, a total volume of about 75 c.c. of alcohol being employed.

The alcoholic extract with addition of a little water is evaporated to dryness. The residue of salts is rinsed down with a few c.c. of water from a wash bottle and the resulting solution is decanted into a 25 c.c. nickel crucible. The process is repeated two or three times, a total of about 18 c.c. of water being used. The contents of the small crucible, after addition of about 10 drops of saturated potassium carbonate, are evaporated to dryness, combusted in the usual manner and the iodine estimated as described for blood.

In general a quantity of the material to be examined is brought into solution with aqueous or aqueous-alcoholic alkali, and aliquots sufficient to provide a reasonably accurate titration are taken for analysis. Further alkali is added, an excess such as would lead to free potassium hydroxide in the ash being avoided, and the estimation is carried out on the lines of those just described.

For other details as to procedure, purification of reagents, etc., the original papers should be consulted.

III. Iodine in New Zealand sea weeds.

(1) *The iodine content of some New Zealand sea weeds.*

Determinations of the iodine content of sea weeds are of interest both from their relation to oceanographical problems—for, according to Cameron (1915), the iodine content of a given species varies with the latitude, the prevailing ocean currents and the salinity—and also in connection with the position which sea weed occupies in the diet of edible marine animals. During the present investigation our attention was called to the latter aspect of the matter as a result of the observation that the stomach contents of a specimen of *Coridodox pullus*, the "Greenbone," a New Zealand edible fish, consisted for the most part of a macerated brown sea weed which on analysis was found to have an iodine content of 0·076 per cent. on the dry weight. This is about half the figure found by Cameron for some varieties of *Laminaria*.

As no analyses of local sea weeds were available for comparison and the question was one of general interest, samples of the commonest species growing on the Pacific coast between St Clair and Cape Saunders (Otago Peninsula) were collected and analysed.

Except in the case of *D'Urvillea* and *Macrocystis*, in which only the fronds were sampled, the whole plant was dried, and then analysed according to the method of Leitch and Henderson (1926). The water content of sea weeds varies from about 76 to about 93 per cent. Determinations for *Cystophora*

and *Macrocystis* gave values of 76 and 88 per cent. respectively. On analysis for iodine the following results were obtained:

	Iodine content (dry weight) %		Iodine content (dry weight) %
D'Urvillea antarctica	0·0023	*Gigartina angulata*	0·031
Macrocystis pyrifera	0·079	*Gigartina decipiens*	0·025
Cystophora retroflexa	0·103	*Gigartina clavifera*	0·020
Hormosira billardieri	0·046	*Pachyminia lusoria*	0·014
Adenocystis lessonii	0·028	*Rhodemela traversiana*	0·019
Bostrychia arbuscula	0·025		

In the above list, the low iodine content of the great kelp *D'Urvillea* is noteworthy by contrast with that of the next largest sea weed, *Macrocystis*, while *Cystophora*, which is a fairly small plant, has the highest iodine content of all. A diet of *Macrocystis*, young species of which are very tender, would account quite well for the iodine found in the stomach contents of the Greenbone.

For purposes of comparison, some results obtained by Cameron for similar varieties growing on the Pacific coast near Vancouver Island, British Columbia, may be quoted:

	Iodine content (dry weight) %		Iodine content (dry weight) %
Macrocystis pyrifera	0·17	*Rhodomela larix*	0·014
Gigartina radula	0·007	*Laminaria saccharina*	0·17
Gigartina mamillosa	0·016	*Laminaria bullata*	0·18

One observes that the local Gigartinas (edible sea weeds) have an iodine content more than double that of the species examined by Cameron, while the value for *Macrocystis pyrifera* is only about half Cameron's value for the same species. Such variations are common. In twelve samples of *Nereocystis lütkeana* from different localities Cameron found that the iodine content of the frond ranged from 0·08 to 0·29 per cent. Even plants growing in the same locality showed variations, from which Cameron concluded that "the amount of iodine present in an individual plant is within certain limits a variable quantity apparently determined by the plant cells themselves."

(2) *The nature of the iodine in sea weed.*

The question as to the mode of combination of the iodine in sea weed has not yet been definitely answered. Botanists have long known that most of the iodine is very loosely bound, and readily set free, probably by enzymes. Lunde and Closs (1930) find that in *Laminaria digitata* much iodine is extractable with water, and acidification causes precipitation of free iodine. The easily soluble iodine was thought to be present in iodide-like combination.

In the present work *Cystophora retroflexa* was chosen on account of its high iodine content as a suitable material for the examination of the nature of the combined iodine. Tests were first made to see if crude proteins could be obtained at all readily. The fresh leaves were minced and extracted with sea

water yielding a viscous solution, but no well-marked precipitate could be obtained from this material on treatment with ammonium sulphate.

Several pounds of the leaves were then taken to the laboratory, rapidly dried in a current of warm air and ground to powder. The moisture content of the fresh leaves was 76 per cent.

A determination of water-soluble iodine was made by repeatedly extracting 1 g. of the powder with boiling water and analysing the extract. 83 per cent. of the total iodine was found to be water-soluble. This water-soluble iodine presumably represented both organic and inorganic forms of combination, and it was desirable to estimate the amount of each present. As the chemical methods of separation such as precipitation of iodide with silver nitrate, liberation of iodine with nitrite, or precipitation of the organic iodine with lead acetate offered little prospect of success in this instance, it was decided to use dialysis as a means of separating the inorganic iodine from the less diffusible organic iodine. 10 c.c. of the extract were then dialysed, whereupon 86 per cent. of the iodine was found in the dialysate. If this be reckoned as inorganic iodine, then 71 per cent. of the iodine of the original dried weed must be in inorganic form.

Another portion of the dried weed was extracted in a Soxhlet apparatus with 90 per cent. alcohol. Under these conditions 31 per cent. of the total solids and 77 per cent. of the total iodine was found to be alcohol-soluble.

From these results it was concluded that probably 70–80 per cent. of the total iodine was in inorganic or loosely bound combination.

It was now decided to investigate the nature of the remaining firmly bound iodine. It seemed not improbable that this would be present in the form of 3:5 di-iodotyrosine, an iodo-amino acid which has been isolated from iodised casein (Oswald, 1911), sponges (Wheeler and Mendel, 1909), corals (Sugimoto, 1925) and the thyroid gland (Harington and Randall, 1929). Hence it was decided first of all to carry out tests for this substance.

Wheeler and Mendel (*loc. cit.*) describe a fairly delicate, though not specific, test for di-iodotyrosine which consists in acidifying the solution containing the substance with nitric acid, adding silver nitrate and bringing the solution to the boil. Just as the solution reaches the boiling-point, a white turbidity of silver iodide appears.

This test was applied to the dried powder as follows: 10 g. of powder was hydrolysed with 30 c.c. of 40 per cent. baryta for 12 hours. The hydrolysate after filtration was acidified with nitric acid and 20 per cent. silver nitrate solution added till there was no further precipitate. The silver precipitate was filtered off and some of the filtrate was heated to boiling-point. There was an instant turbidity accompanied by some blackening due to organic matter. The presence of silver iodide was demonstrated by reducing the iodide in the precipitate with zinc and sulphuric acid, filtering it off from the reduced silver and adding silver nitrate. A white precipitate of silver iodide was produced.

A more specific test for the 3:5 di-iodophenolic group which could be used

to identify further the material is the reaction with sodium nitrite and ammonia, discovered by Kendall (1919) and employed by Harington and Randall (*loc. cit.*) as a rough quantitative estimation for di-iodotyrosine. For this test it is desirable to have the di-iodotyrosine partially isolated and in a concentration of about 0·5 mg. per c.c. It was therefore decided to hydrolyse some of the material on a slightly larger scale, following the procedure used by Foster (1929), and to attempt to separate the iodide by repeated extraction of the lead salts of the hydrolysed products with hot water, as an alternative to the use of silver nitrate and nitric acid. The lead salts could next be decomposed with sulphuric acid, and the filtrate neutralised and extracted with butyl alcohol. This programme was carried out as follows: 100 g. of the dried powder containing 103 mg. of iodine was hydrolysed with 350 c.c. of 40 per cent. baryta solution ($Ba(OH)_2$, $10H_2O$) for 18 hours, filtered hot through paper pulp and sand, and the filtrate chilled to 5° C. The baryta crystals were filtered off, the filtrate was acidified and to it was added 50 g. of lead acetate. The lead salts were precipitated by adding strong ammonia till the solution was just alkaline to phenolphthalein. The lead precipitate was filtered off, washed by suspension in 400 c.c. of warm water two or three times and then suspended in 200 c.c. of warm water and treated with 20 per cent. sulphuric acid till the solution was acid to congo red. The solution, when filtered from lead sulphate, gave Wheeler and Mendel's test, and on analysis was found to contain 15 mg. of iodine. It was concluded that a separation from iodide or loosely bound iodine had been effected.

The solution was neutralised with potassium carbonate solution, concentrated to 50 c.c. and just acidified with acetic acid. On shaking some of it with normal butyl alcohol it was found to yield a somewhat intractable emulsion necessitating centrifuging, and much of the brown colour of the solution passed into the butyl alcohol layer. The solution was therefore boiled with 4 g. of decolorising charcoal and filtered. There was then no emulsification and the butyl alcohol layer was quite colourless, but analysis showed that the solution now contained only 7 mg. of iodine. The solution was extracted with 20 c.c. of butyl alcohol (purified with bisulphate) at 70° C. The extract contained 0·4 mg. of iodine. After six further extractions the combined extracts contained 1·5 mg. of iodine. The butyl alcohol was removed *in vacuo* at 60° C. and the slight white residue was taken up with 5 c.c. of water, put in a test-tube, acidified with concentrated hydrochloric acid and the colour test carried out as follows:

Two drops of 3 per cent. sodium nitrite were added. After 2 min. 5 c.c. of butyl alcohol was added, shaken, allowed to stand and 3 c.c. of the butyl alcohol transferred to another tube. On adding a few drops of alcoholic ammonia to the latter an orange colour, faintly pink in the meniscus, developed. This is the colour which Kendall (*loc. cit.*) obtained when acetic or sulphuric acid was present; the test was therefore regarded as positive for di-iodotyrosine.

A review of the above procedure indicates that improvement could be effected at several points. Thus the use of decolorising charcoal is attended by a serious loss of iodine, and the extraction by means of butyl alcohol is not at all efficient. It was concluded from a study of the original paper on the subject by Dakin (1918) that the potassium sulphate present in the aqueous layer must have been responsible for the unsatisfactory extraction. Hence it would have been better to have neutralised the filtrate from the lead salts exactly with baryta instead of potassium carbonate. The use of charcoal might perhaps have been obviated by extracting the sea weed with ethyl alcohol before hydrolysis. This takes out a good deal of brown colouring matter, presumably the same as that which interferes with the butyl alcohol extraction.

(3) *Edible sea weeds.*

The high iodine content of sea weeds suggests that they might have a valuable dietetic application in augmenting iodine intake. The difficulty, however, is that the most abundant varieties of sea weed in general possess an unpleasant rank flavour which is very difficult to eliminate.

With a view to obtaining palatable iodine-containing food from sea weed we examined the nature of the pectin bodies in the very common weed *Macrocystis pyrifera*, which has an iodine content of 0·079 per cent. on the dry weight. 700 g. of fresh-minced *Macrocystis* was treated with 14 g. of citric acid. The slimy mass was heated for 3 hours on the water bath and filtered through a cloth. Alcohol was added in small quantities, about 8 per cent. of the volume of the filtrate being employed. The material was left for 24 hours, the supernatant liquid was decanted and the precipitate tipped on a Buchner filter, washed with alcohol and dried. The product was a fine, white amorphous powder which behaved like a pectic substance, giving a flocculent precipitate with calcium ion after treatment with alkali. It contained 0·17 mg. of iodine per g., probably adventitious. The yield was 7 g. (1 per cent. on the wet weight), which was too small for the process to have a practical application.

However, it appears that sea-weed jellies are not pectic in nature. Thus, a jelly made with the so-called "Irish Moss," which is found on New Zealand beaches, when diluted yields a solution which does not give a precipitate with calcium chloride after treatment with alkali. Also, there is a considerable variation in the "gelling" tendency of different sea weeds; in the case of small varieties such as Gigartinas, almost the whole of the plant consists of material which forms a jelly on cooking. *Gigartina clavifera*, when dried and powdered, was found to yield a jelly very readily, but the jelly was brown in colour and possessed a "fishy" odour. Extraction with alcohol removed much of the pigment, but also destroyed the gelling property.

When the weed has become bleached by natural weathering, the jellies obtained are practically colourless and odourless; but it is difficult to devise a rapid method of treating the dried weed to replace the natural process of slow weathering.

IV. FURTHER OBSERVATIONS ON THE IODINE CONTENT OF NEW ZEALAND FISHES.

The value of a fish diet in goitre prophylaxis has been stressed by many authors. Thus Lunde (1927) finds from a comparison of various districts in Norway that goitre is in general much less prevalent in those parts where fish is eaten plentifully. There is, however, a considerable variation in the iodine content of different species of fish. For example, in New Zealand, the iodine content of groper muscle was found to be 72 microgrammes per kg., whereas that of blue cod was 812 microgrammes per kg. Norwegian fish appear to be considerably richer in iodine than New Zealand fish, as the following extract from Lunde's table shows.

Klippfisch:	aus Lofoten	1,210 microgrammes per kg.
	aus Sunnmöre	2,080 ,,
Stockfisch:	"Dorsch"	10,800 ,,
	"Köhler"	2,420 ,,

On the assumption that kelp-feeding fish have a higher iodine content than other fish, arrangements were made for the Marine Biological Station at Portobello, Otago Harbour, to supply us with specimens of any edible kelp-feeding fish which might be exploited in goitre prophylaxis. We were informed that in addition to blue cod, two other species of kelp-feeders were plentiful on the coast, the greenbone, which appears to live entirely on kelp, and the yellow-eyed mullet, which feeds on kelp during the summer and autumn months.

The stomach contents of a greenbone which we examined were found to consist of a slimy mass of macerated brown sea weed which, when dried, had an iodine content of 0·76 microgrammes per g.—roughly the iodine content of the kelp *Macrocystis pyrifera*.

The fresh fish had an iodine content of 1870 microgrammes per kg. Samples of the fried and boiled fish were found to have iodine contents of 2240 microgrammes per kg. and 1780 microgrammes per kg. respectively. The greenbone-liver oil was also analysed and found to contain 7400 microgrammes per kg., which is much the same as the iodine content of cod-liver oil. From these results it is clear that the greenbone, which incidentally is a very palatable fish, has much to recommend it from the point of view of goitre prophylaxis.

The intestinal tract of a yellow-eyed mullet, caught in late autumn, was found to contain not sea weed but a reddish gritty material containing chitinous particles suggestive of macerated shrimps. This material when dried had an iodine content of 2180 microgrammes per kg., much lower than the iodine content of the stomach contents of the greenbone.

The fresh fish muscle had an iodine content of 670 microgrammes per kg. After baking and frying the iodine content was 420 microgrammes per kg.

and 430 microgrammes per kg. respectively, while the boiled fish had an iodine content of 380 microgrammes per kg. These values are of the same order as the corresponding figures for blue cod.

A popular shell-fish in New Zealand, generally used for making soup, is the toheroa, a large bivalve which makes its habitat in wet beach sand. This was found to have an iodine content of 760 microgrammes per kg. and contained about 30 microgrammes per fish.

Two species of mussel, *Mytilus canaliculus* and *Mytilus planulatus*, which were found in the same locality, had iodine contents of 1850 microgrammes per kg. and 7360 microgrammes per kg. respectively. *Mytilus canaliculus* is four or five times as large as *planulatus*, so that here the iodine content happens to be proportionately larger. Studies of the distribution of the iodine in these and other molluscs would probably yield interesting results from the point of view of comparative physiology.

V. Variations in the iodine content of the blood during anaesthesia.

The fact that administration of ether or chloroform to animals produces immediate changes in the iodine content of the blood was first observed by Leitch (1927). In general, the result obtained was a progressive decrease in blood iodine, though in other cases using the same anaesthetic a marked rise was observed.

It seemed obvious that such an effect, apart from having some clinical importance, should prove useful as a means of investigating the rôle of the thyroid gland in controlling iodine circulation; and a series of experiments was therefore carried out in which various anaesthetics were administered to normal rabbits and also to the same animals subsequent to thyroidectomy. Our results showed, however, that the presence of the thyroid gland was not necessary to evoke the effect, though its removal leads to a marked fall in the general level of blood iodine. It was concluded that the change in concentration of blood iodine under anaesthesia was due to a process affecting the iodine content of tissues in general, the mechanism being perhaps connected with the alterations in cell permeability produced by anaesthetics.

This latter view would raise the question as to whether the iodine concerned in the change of concentration is in the organic or ionised form, and in this connection it is interesting to note that the largest relative changes recorded by Leitch refer to animals in which the blood iodine determined prior to anaesthesia was quite abnormally high, an effect which can almost always be ascribed to the presence of iodides in the diet. Ionised iodine may also be excreted by the thyroid according to recent investigations by Sturm (1930).

The rabbits used in our experiments were the ordinary grey variety. The blood was sampled from an incision made with a sharp-edged needle in the marginal vein of the ear, the ear being kept warm before a heater to dilate

the blood vessels. The volatile anaesthetics were in each case carefully administered so as to excite the animal as little as possible. The fixed anaesthetics were injected subcutaneously with the exception of the avertin, which was given rectally as in ordinary clinical practice.

A period of at least a fortnight was allowed to elapse after thyroidectomy before the experiments were repeated. We are indebted to Dr W. S. Fogg for administering the anaesthetics and performing the thyroidectomies.

The following tables show the results obtained:

		Iodine content of blood
Rabbit A.	*Chloroform.*	
	Before administration	49 microgrammes per kg.
	After 20 min.	144 ,,
	After 60 min., death heart blood	32 ,,
Rabbit B.	*Ether.*	
	Before administration	48 ,,
	After 60 min.	85 ,,
Rabbit C.	*Amytal* (0·11 g. per kg. body wt. 10 % solution).	
	Before administration	63 microgrammes per kg.
	After 30 min.	64 ,,
	After 50 min.	77 ,,
Rabbit D.	*Avertin* (0·25 c.c. rectally; 1 g. per c.c. of solution).	
	Before administration	129 microgrammes per kg.
	After 20 min.	137 ,,
Rabbit E.	*Chloroform.*	
	Before administration	107 ,,
	After 20 min.	119 ,,
	Three weeks after thyroidectomy:	
	Before administration	34 ,,
	After 20 min.	51 ,,
	After 40 min.	55 ,,
Rabbit F.	*Ether.*	
	Before administration	60 ,,
	After 20 min.	29 ,,
	Two weeks after thyroidectomy. (The rabbit was showing symptoms of tetany):	
	Before administration	68 microgrammes per kg.
	After 20 min.	36 ,,
Rabbit G.	*Chloral* (0·6 g. per kg. body wt.)	
	Before administration	60 ,,
	After 20 min.	58 ,,
	After 55 min.	48 ,,
	Four weeks after thyroidectomy:	
	Before administration	20 ,,
	After 20 min.	Less than 20 ,,
	After 60 min.	Less than 20 ,,
Rabbit H.	*Urethane.*	
	Before administration	99 ,,
	After 15 min.	66 ,,
	After 40 min.	49 ,,
	Four weeks after thyroidectomy:	
	Before administration	Less than 20 ,,
	After 20 min.	Trace
	After 50 min.	Trace

It will be seen that a normal value for blood iodine in the rabbits used for the experiment would be about 60–80 microgrammes per kg. The value

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obtained by Leitch at the Rowett Institute was considerably higher, being 100–150 microgrammes per kg.; the difference is probably due to the well-known effect of environment on iodine intake. The diet of the rabbits consisted mainly of local-grown cabbage and turnips.

The effects of anaesthesia shown above are quite similar to those observed by Leitch. The results display very little uniformity, the same anaesthetic sometimes producing a fall in blood iodine and sometimes a rise. The low iodine content of blood from the heart in the case of the rabbit killed with chloroform is noteworthy; a similar result is recorded by Leitch.

Except in the case of one rabbit where the blood had been sampled only a fortnight after the operation, thyroidectomy is seen to result in a marked fall in blood iodine. But the effect of anaesthetics still persists after removal of the gland.

The effect is thus presumably due to a direct or indirect reaction of the tissues in general to anaesthetics. If the reaction is a direct one, it should be possible to produce it in the case of isolated tissues or cells. From this point of view it was decided to test *in vitro* the effect of ether and chloroform on the permeability of blood erythrocytes to potassium iodide, thyroxine and saline thyroid extract, these being considered typical of the forms of iodine likely to be involved in the actual process which takes place in the living animal. Erythrocytes were chosen because, though not a typical tissue, they have the great advantage of being susceptible of easy quantitative sampling. In two of the experiments chicken blood (in which the corpuscles are nucleated and therefore more akin to typical tissue cells than are mammalian corpuscles) was employed, and pigs' blood in a third experiment. A further experiment on the effect of ether on isolated thyroid tissue was also carried out.

The procedure was as follows:

(1) *Effect of ether and chloroform on the distribution of iodine between corpuscles and serum in the case of a red cell suspension in serum iodised with potassium iodide.*

30 c.c. of freshly sampled defibrinated chicken blood was centrifuged and 10 c.c. of the serum was rejected. The residue was shaken to resuspend the red cells and iodised by addition of 0·4 c.c. of a KI solution (0·1–0·2 per cent.). 4 c.c. quantities of the suspension were taken in three centrifuge tubes, treated with 0·4 c.c. of saline, 0·4 c.c. of 1·4 per cent. ether saline and 0·4 c.c. of saline saturated with chloroform respectively and incubated at 37° C. for 30 min. The amount of ether used gives a concentration corresponding to that obtaining in blood during ether anaesthesia (Sollmann, *Manual of Pharmacology*, p. 712).

After centrifuging, 1 c.c. quantities of the supernatant serum were analysed with the following results:

	Serum : iodine content
Tube (1) (0·4 c.c. normal saline)	0·48 × 42 micrommes per c.c.
Tube (2) (0·4 c.c. ether saline)	0·38 × 42 ,,
Tube (3) (0·4 c.c. chloroform saline)	0·41 × 42 ,,

It is clear that 21 and 14 per cent. of the iodine has passed from the serum into the red cells under the influence of ether and chloroform respectively.

(2) *Effect of ether and chloroform on a red cell suspension iodised with thyroxin.*

15 c.c. of defibrinated chicken blood was centrifuged and 5 c.c. of the serum was rejected. 6 c.c. of a solution of thyroxin in saline (containing about 220 microgrammes of iodine) was then added.

5 c.c. lots were taken in centrifuge tubes, treated with 0·6 c.c. quantities of normal saline, ether saline and chloroform saline and incubated for 35 min. After centrifuging again, 2 c.c. quantities of the supernatant serum were analysed with the following results:

	Serum : iodine content
Tube (1) (normal saline)	0·58 × 21 microgrammes per c.c.
Tube (2) (ether saline)	0·58 × 21 ,,
Tube (3) (chloroform saline)	0·59 × 21 ,,

These results indicate that anaesthetics produce no well-marked change in the permeability of red cells for thyroxine.

(3) *Effect of ether and chloroform on a red cell suspension iodised with freshly prepared saline thyroid extract.*

40 c.c. of defibrinated pigs' blood was centrifuged, 20 c.c. of serum was rejected and 12 c.c. of a thyroid extract (made by grinding up 5 g. of fresh pigs' thyroid with 20 c.c. of normal saline and filtering) was added and well mixed in.

10 c.c. lots were taken and treated with 0·4 c.c. quantities of normal saline, ether saline and chloroform saline. The tubes were incubated for 1 hour, centrifuged, and 5 c.c. quantities of the serum were analysed with the following results:

	Serum : iodine content
Tube (1) (normal saline)	2·05 × 8·4 microgrammes per c.c.
Tube (2) (ether saline)	2·10 × 8·4 ,,
Tube (3) (chloroform saline)	2·10 × 8·4 ,,

From the results it is clear that anaesthetics produce no well-marked change in the permeability of red cells for the iodine compounds of saline thyroid extract.

(4) *The effect of ether on the permeability of thyroid tissue.*

3 g. of pig thyroid (the animal was killed less than half an hour previously) was sliced into pieces 0·5 mm. thick under serum saline (50 per cent. pig serum in saline) and gently washed by decantation with serum saline in a 50 c.c. Erlenmeyer flask. 25 c.c. of pig serum was then added and the system was incubated for 20 min. 5 c.c. of supernatant serum was then removed and analysed. After incubation for a further 20 min. another 5 c.c. sample was analysed and 0·1 c.c. of 8 per cent. (by volume) ether water was then added. Finally after another 20 min. incubation a third sample of 5 c.c. was analysed.

	Serum : iodine content
Sample (1) (after 20 min. incubation)	0·40 × 8·4 microgrammes per c.c.
Sample (2) (after a further 20 min.)	0·45 × 8·4 ,,
Sample (3) (addition of ether and incubation for further 20 min.)	0·53 × 8·4 ,,

Conclusions cannot be drawn with certainty here as iodine equilibrium may not have been attained between thyroid and serum before addition of the ether. However, the result suggests that the ether has led to a slight increase in the permeability of the gland for its own secretion.

From the findings in the above experiments one may infer that only in the case of inorganic iodine of the body could a redistribution between tissues and serum be brought about by the direct action of anaesthetics. Apropos of the possibility of an indirect action, it is interesting to note that Sturm (1930) finds that increase of secretion from the thyroid gland is related to stimulation of the sympathetic. Injection of adrenalin into a dog produces a 10 times increase in the iodine content of the thyroid-venous blood, which, if distributed through the whole blood volume, would produce an increase of 210 micrograms per kg. On the other hand, perfusion of the isolated, nerveless thyroid gland with blood containing adrenalin gives rise only to capillary contraction without increased secretion. Apropos of this effect of the sympathetic it may be mentioned that in an earlier paper (Hercus, Aitken *et al.* 1931, p. 517) we referred to the possible effect of fear and exhaustion in exciting thyroid secretion in hares which had been coursed over long distances. We found recently that the iodine content of the thyroids of three hares excised from the animals immediately after they had been coursed distances of about 500, 1000 and 1500 yards in a hare drive on the Taieri Plain were respectively 0·84, 1·05 and 0·44 mg. per g. on the fresh weight. There were no accompanying differences in histological appearance, however.

Sturm also observes that stimulation of the parasympathetic system by injection of choline has no influence on the secretion of the thyroid gland. In earlier experiments on human subjects he observed that the iodine level of the blood sank when the vagus was stimulated. These changes must therefore be independent of the thyroid gland, and probably arise from an alteration in the systemic mechanism regulating iodine metabolism, in which the body tissues must play a certain part.

These conclusions are in agreement with our observations on the occurrence of changes in the iodine level of the blood after thyroidectomy.

VI. Experimental goitre and iodine balance in rabbits.

The fact that rabbits on a diet of cabbages gradually develop goitre was established by Chesney, Clawson and Webster (1928). 486 rabbits kept on such a diet and autopsied at various times between July 30th, 1926 and February 29th, 1928 had an average thyroid weight of 2·93 g., the corresponding value for normal rabbits being 0·23 g. The enlargement was characterised by the production of new thyroid acinar cells and was associated with a slightly reduced metabolic rate and a tendency to enlargement of the suprarenals.

Webster and Chesney (1930) described the thyroid enlargement obtained

in this way as a diffuse parenchymatous goitre. They found that rabbits kept in standard cages and fed with cabbage for 345 days had an average thyroid weight of 2·5 g., whereas another batch kept on cabbage for 420 days but receiving in addition 7·5 mg. of iodine per week had glands with an average weight of 0·27 g.

Webster, Marine and Cipra (1931), continuing this work, found that white winter cabbage was much more goitrigenous than summer cabbage, while Baumann, Cipra and Marine (1931) found that the fats extracted from cabbage with ether when fed to rabbits led to the appearance of definite thyroid hyperplasia in 30 days.

Since these findings appeared to provide a means of producing a reliable type of experimental goitre for research purposes, we decided to test the goitrigenous properties of New Zealand cabbage. Experimental goitre has for years been an unachieved desideratum in our research. Iodine deficiency experiments which were carried out here by Drennan, Malcolm and Cox (1931) gave rise to a histologically active type of gland but not to macroscopic changes.

A batch of six grey rabbits, separately caged, were put on a diet of 250 g. of cabbage per day at the end of February, 1931. On March 10th, three other rabbits commenced a diet of the same quantity of steamed green cabbage, while another three received only white cabbage heart. Experiments were also commenced with rats and mice, but these did not survive on a cabbage diet.

The rabbits were palpated from time to time with negative results; at the beginning of October, after 205 days, several were killed by asphyxiation with coal gas and the thyroids weighed. The average weight was 0·55 g. (about $2\frac{1}{2}$ times normal) and the iodine content 0·10 microgrammes per g. The different diets employed did not appear to lead to different results.

Chesney, Clawson and Webster (*loc. cit.*) found that rabbits kept on a cabbage diet for 200 days had an average thyroid weight of 2·25 g., the enlargement being palpable. We surmised that the small enlargements we obtained in the same time might have been related to the iodine content of New Zealand cabbage, and in order to clear up this question an iodine balance experiment was commenced with one of the animals.

The arrangement shown in Fig. 1 was devised for the purpose of collecting the rabbit's excreta. The cage was placed on a wire stage above an inclined fan-shaped trough so that the urine and faeces dropped into the trough. A false bottom of wire netting in the trough effectively separated the faeces from the urine. Faeces and urine were collected over 4-day periods and analysed. The cabbage not eaten by the rabbit was also collected each day and weighed; the diet was 250 g. as before.

The results are shown in Table I.

The daily excretion of urine ranged from 150 to 240 c.c., while the faeces generally averaged about 3–5 g. per day. Table I shows that the balance is a fairly definite negative one. The high concentration of iodine in

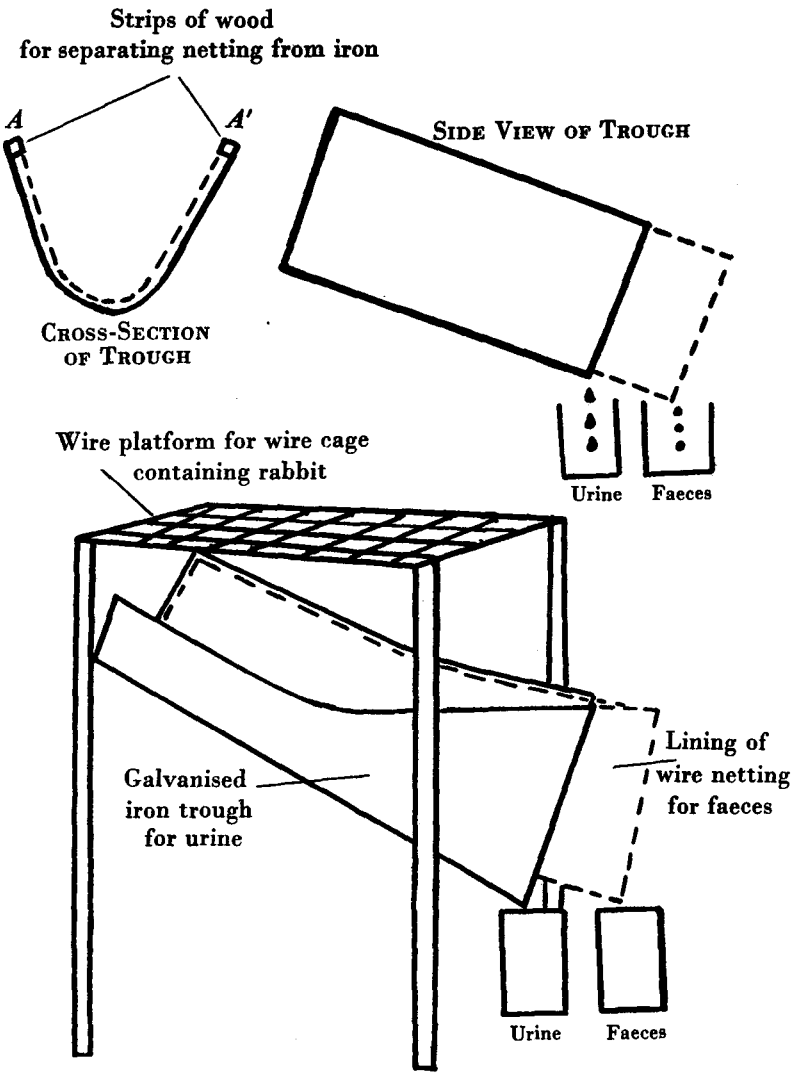

Fig. 1. Arrangement for collecting rabbit urine and faeces.

Table I. *Iodine balance of rabbit on cabbage diet.*

Date	Iodine content in γ/kg. of			Daily iodine intake or excretion (γ) in			Iodine balance (γ)
	Cabbage	Urine	Faeces	Cabbage	Urine	Faeces	
Sept. 19	19	24	110	4·65	4·06	0·43	+0·16
,, 26	17	19	131	4·25	4·64	0·67	−1·06
Oct. 3	17	29	310	4·25	4·86	1·55	−2·16
,, 10	12	16	72	2·94	3·20	0·32	−0·58
,, 17	12	21	50	2·94	3·48	0·41	−0·95

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the faeces is noteworthy. The iodine content of the cabbage is of the same order as that of the cabbage used by Webster, Marine and Cipra, so that one may conclude that the weaker goitrigenous power of the cabbage is due to lower concentration of the goitrigenous agent rather than to the presence of iodine.

While this work was in progress we became acquainted with the investigations of Baumann, Cipra and Marine (1931), which showed that the lipoids extracted from cabbage with ether contained the goitrigenous agent. As recent work on the lipoid matter of spinach and cabbage by Collison and Smedley-MacLean (1931) had shown that these lipoids are characterised by the presence of oleic, linolenic and linoleic acids, we thought that the effect of administration of linseed oil which contains these unsaturated substances might be worth investigating from an empirical point of view.

From October 28th the rabbit was therefore given 1 c.c. of raw linseed oil per day by mouth with a pipette. This administration of oil was not found to cause any scouring; indeed, during the period when the rabbit was receiving oil it was twice observed not to defecate for several days in succession. The results are shown in Table II.

Table II. *Iodine balance of rabbit on cabbage diet together with linseed oil.*

	Iodine content in γ/kg. of			Daily iodine intake or excretion (γ) in			Iodine balance
Date	Cabbage	Urine	Faeces	Cabbage	Urine	Faeces	(γ)
Nov. 5	17	34	132	3·95	2·16	0·73	+ 1·06
,, 19	20	21	270	4·77	3·15	1·08	+ 0·54
,, 26	13	16	120	2·10	1·00	0·45	+ 0·55
Mar. 16	16	18	380	3·76	1·80	1·04	+ 0·92
Apr. 14	17	17	520	3·62	4·68	3·12	− 5·82
,, 21	70	15	85	14·7	3·45	0·21	+11·04
May 5	20	21	280	4·9	4·83	0·56	− 0·49

The balance is seen to have become slightly positive for several weeks. The results for April 14th and April 21st must be regarded as anomalous.

On May 5th the balance was again negative. The rabbit was then killed and examined. The thyroid was found to weigh 1·2 g. and had an iodine content of 0·074 microgrammes per g. The gland was thus definitely goitrous, but not excessively so, and one was therefore inclined to attribute the enlargement to the fact that this rabbit had been on a cabbage diet for 420 days rather than to goitrigenous properties in the oil.

The general conclusion was that our local cabbage has so weak a goitrigenous power that a period of about 1000 days would be required to produce palpable goitres in rabbits; and that the goitrigenous property was not associated with the presence of unsaturated fatty acids in cabbage lipoids.

VII. Miscellaneous.

(1) *The urinary iodine excretion of Samoans compared with that of inhabitants of a New Zealand centre of endemicity.*

The determination of iodine excretion in the urine affords a ready means of gaining a knowledge of the level of iodine intake without having recourse to the time consuming investigation of the iodine content of separate articles of diet. Lunde (1927) determined the urinary iodine excretion of 65 persons from 10 districts around Oslo, and tabulated the values against the goitre incidence in school children as follows:

γ iodine per 24 hours	40	48	29	39	64	48	56	65	87	61
Goitre %	60	55	54	54	43	40	39	37	36	30

In the goitre-free district of Vik i Sogn the mean iodine excretion for 24 hours was found to be 173 microgrammes. It is clear that, apart from some anomalies, a high iodine excretion is associated with low goitre index and *vice versa*.

We have recently completed a similar comparison between the Christchurch endemic centre in New Zealand and the goitre-free island of Samoa.

The urine of twenty patients of Christchurch Mental Hospital, eleven of whom had goitre, was examined. The mean excretion per 24 hours was 1600 c.c. of urine containing 26 microgrammes of iodine. The goitre index for the Canterbury area, of which Christchurch is the capital, is 64 per cent.

The urine of twelve inhabitants of Samoa (goitre-free) was found to have a mean iodine content of 189 microgrammes per litre or 302 microgrammes in a 24-hour specimen of 1600 c.c. The mean iodine content of six samples of Samoan blood was also determined and found to be 155 microgrammes per kg. The high urinary iodine excretion in Samoa is of course related to the high iodine content of Samoan foodstuffs to which we have made reference elsewhere (Hercus, Aitken, Thomson and Cox, 1931).

As regards the relation between thyroid enlargement and iodine excretion, McCarrison and Madhava (1932) consider that "unless the size of the thyroid can be accurately measured...and unless the subjects can be kept under uniform conditions as to season, diet, etc....no data of sufficient accuracy can be accumulated which would enable a judgment to be given for or against the association in question." Even if it is conceded, however, that such perfect conditions are requisite in statistical studies, it may be pointed out that it is not the degree of thyroid enlargement, but the incidence of palpable goitre which has been correlated with urinary iodine excretion, so that measurements of the thyroid gland are not involved.

The incidence of goitre in Christchurch Mental Hospital from which the urine samples were obtained is discussed in the next section.

(2) *The incidence of goitre in Christchurch Mental Hospital.*

During 1930, 391 male patients at Christchurch Mental Hospital were examined and 122 or 31·2 per cent. were found to have enlarged thyroids. The patients were grouped according to "chronological" or actual age and also according to "institutional" age, *i.e.* according to the time of residence in the hospital. The latter classification was adopted in view of the fact that the Christchurch Mental Hospital is a notable centre of goitre endemicity and has been for many years (see Hacon, 1888). There is also a high incidence in the city of Christchurch; one of us (C. E. H.) found in 1927 that of 338 pupils in a girls' school 77 per cent. had visible goitres and of 121 female factory hands 68 per cent. had visible goitres.

The information relating to Christchurch Mental Hospital is contained in Charts I and II and Table III which shows the size classification of the 122 cases of goitre.

Table III.

(i)

	Institutional age										
	1	1–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	Total
Large nodular	.	.	1	7	6	4	1	2	1	.	22
Medium nodular	.	.	2	1	3	1	.	.	.	.	7
Small nodular	.	3	.	.	2	.	1	.	2	.	8
Total nodular	.	3	3	8	11	5	2	2	3	.	37

(ii)

Large diffuse	2	4	4	6	4	.	.	.	.	2	22
Medium diffuse	1	2	2	.	.	2	1	.	.	.	8
Small diffuse	7	15	11	7	6	5	3	1	.	.	55
Total diffuse	10	21	17	13	10	7	4	1	.	2	85
Totals	10	24	20	21	21	12	6	3	3	2	122

In Chart I the percentage incidence of diffuse nodular and total goitres is shown plotted against institutional age. The ages 25–45 years have been grouped together because of the small number of patients in 5-year groups over 25 years' institutional age. The apparent decrease in incidence after 25 years is not significant. It is noteworthy that there are no nodular goitres in the group 0–1 year's and only a small incidence up to 10 years' institutional age. From 10 to 20 years' there is a very rapid increase in the incidence of nodular goitres, the incidence of diffuse goitres only increasing slightly over the same period. Thereafter the incidence is apparently unchanged within the limits of error.

Chart II shows nodular goitre to be a disease of later life, the incidence being low up to ages of 40 years. After the age of 40 years the diffuse goitres fall rapidly and are replaced by nodular goitres, the percentage of total goitres increasing only slightly. This illustrates the formation of nodular goitres from diffuse goitres of long standing. The fall of the incidence in the last group (70–90 years) is due to the presence in this group of large numbers of senile

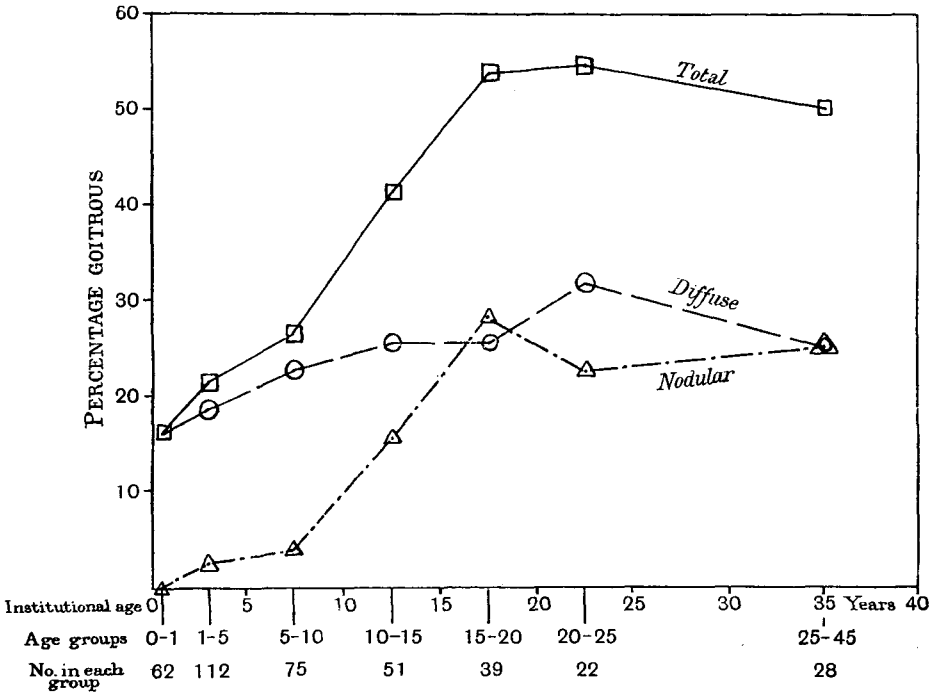

Chart I.

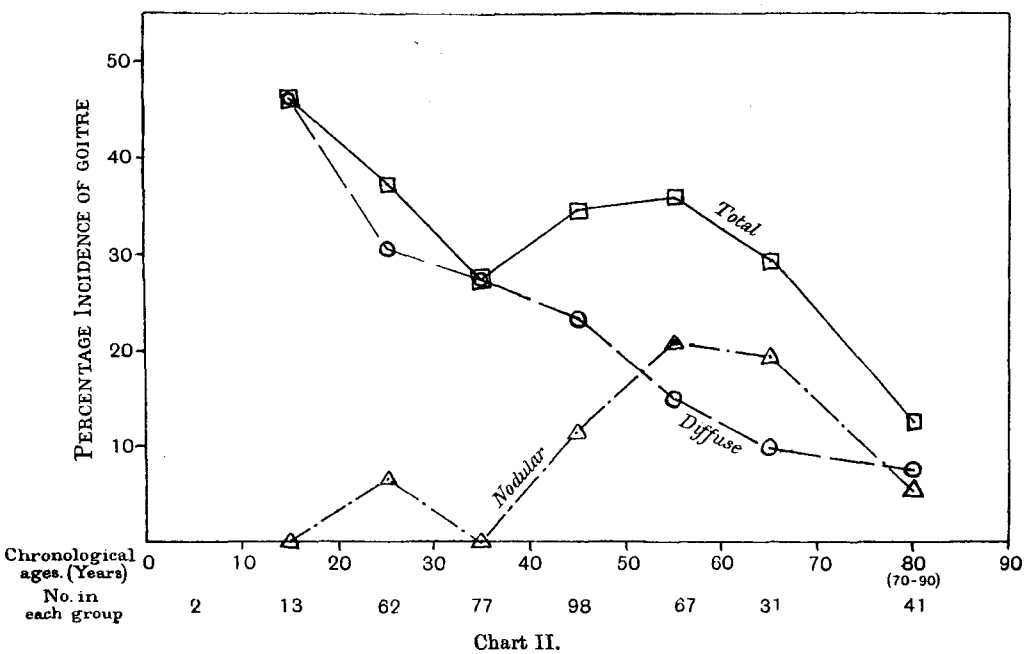

Chart II.

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patients recently admitted and coming from districts of lower endemicity. This accounts for the incidence of diffuse goitres again becoming greater than that of the nodular types.

The size classification given in Table III is interesting in that it shows a preponderance of small diffuse goitres while small nodular goitres are relatively infrequent. Apart from the possibility of a more rapid growth of the nodular type, this would seem to be due to the direct production of medium and large nodular goitres from the diffuse type of corresponding size.

To summarise, in this centre of high endemicity, the incidence of goitre increases to about 54 per cent. with 20 years' residence. Exposed to these highly goitrigenous conditions, diffuse goitres develop into nodular goitres, particularly in patients over 40 years of age.

(3) *The iodine content of Otago pigs' thyroids.*

Seidell and Fenger (1913) found a well-marked, seasonal variation in the iodine content of pigs' thyroids, there being on an average more than twice as much iodine present in the glands in summer and autumn as in winter and spring. As pig's thyroid represents the raw material of the thyroid extract and thyroxin industries this seasonal variation in iodine content, affecting the quality of the therapeutic preparations, has had to be taken into consideration in America. In order to get information as to the iodine content of our local thyroids arrangements were made to have half a dozen glands regularly supplied us from Dunedin City Abattoirs, Burnside, during 1930 and 1931. The glands (one lobe only being sampled) were placed in alcohol and brought to the laboratory where they were freed from connective tissue and the iodine in each gland determined. The values obtained for percentage iodine in the fresh gland multiplied by four were taken as representing the iodine content of the dried gland.

The average values for the batches of glands are plotted in Chart III. The chart does not indicate the existence of a well-marked seasonal variation such as has been observed elsewhere, though there appears to be an increase in the iodine content of the gland from September to December. On the other hand the highest values were obtained from samples collected in May. The batches of glands were generally from different localities and this might be expected to lead to anomalies. For lamb thyroids (Simpson, 1930) the maximum iodine content is said to occur in autumn or early winter.

The mean iodine content of all glands examined was 0·14 per cent., which is considerably less than the yearly average for desiccated, fat-free glands in North Dakota (0·32 per cent.) and Texas (0·60 per cent.) (Fenger, Andrew and Vollertsen, 1931). Some allowance may be made for the fact that the glands were not freed from fat, but the general conclusion is that a certain grade of iodine deficiency prevails among Otago pigs. As to the weight of the glands, the average fresh weight per lobe was 10·3 g., individual lobes ranging in weight from 5 to 27 g. Kendall and Simonsen (1928) state that about 5000

animals were required to supply 45 kg. of glands. This works out at 4·5 g. per lobe. We were informed that markedly goitrous glands were met with from time to time at the abattoirs. One gland which was sent to us was almost the size of a hen's egg and had an iodine content of 0·87 microgrammes per g.

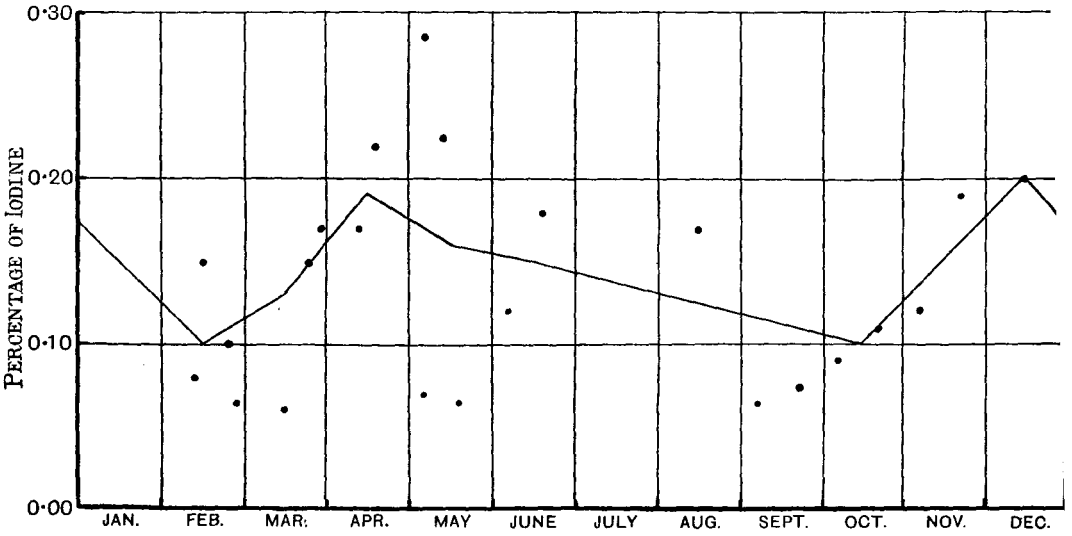

Chart III.

VIII. Summary.

1. Methods for the estimation of iodine in natural products are described.

2. Common sea weeds on the Pacific coast of Otago have an iodine content of the same order as similar species on the Pacific coast of British Columbia.

3. In *Cystophora retroflexa*, which contains over 0·1 per cent. of iodine, 70–80 per cent. of the iodine appears to be in inorganic or loosely bound combination. Precipitation and colour tests indicate that di-iodotyrosine is also present.

4. Observations are recorded of the iodine content of kelp-feeding and other fish.

5. Administration of anaesthetics produces fluctuations in the concentration of blood iodine in rabbits, the effect persisting after removal of the thyroid gland. Experiments *in vitro* suggest that only in the case of inorganic iodine could a redistribution of iodine between tissues and serum be brought about by the direct action of anaesthetics.

6. New Zealand cabbage possesses goitrigenous properties for rabbits but in a much smaller degree than American cabbage.

7. The urinary excretion of Samoans is compared with that of inhabitants of Christchurch Mental Hospital.

8. The incidence and course of development of goitre at Christchurch Mental Hospital is discussed.

9. Values are given for the iodine content of Otago pigs' thyroids.

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