I.—ESTIMATION OF IODINE IN SOILS.

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Recent work has tended to emphasise the importance of iodine both in the animal and vegetable worlds. This element is an essential constituent of thyroxin the active principle of the thyroid secretion; and the almost universal occurrence of a thyroid mechanism amongst the vertebrates points to the conclusion that there is a definite iodine metabolism in these forms which is an essential part of their vital processes (cf. Swingle, *J. Gen. Physiol.*, 1920, **2**, 161).

A deficiency of iodine in the thyroid has been considered by many workers to be at any rate a predisposing cause of goitre and this deficiency has been attributed to a lack of iodine in the soils from which the food and water supplies of persons so affected have been derived (Bruce, *Pres. Address, British Association*, 1924).

In the vegetable kingdom also it is believed that iodine plays an important part in the synthetic processes of the living cell (Stoklasa, *Z. Angew. Chemie*, 1927, **40**, 20). The detection and estimation of small quantities of iodine in biological materials, foodstuffs, soils, etc., is therefore a process of much importance and various methods have been suggested in recent years. Of these the more important are those described by Kendall (*J. Biol. Chem.*, 1914, **19**, 251), Kelly and Husband (*Biochem. J.*, 1924, **18**, 951), Pickworth (*Biochem. J.*, 1925, **19**, 769), McClendon (*J. Biol. Chem.*, 1924, **60**, 289), Hercus, Benson and Carter (*J. Hygiene*, 1924, **24**, 321 and Leitch and Henderson *Biochem. J.*, 1926, **20**, 1003).

Kendall's method as modified by Kelly and Husband consists in fusing the material with sodium hydroxide, the destruction of the organic matter being assisted by the addition of potassium nitrate. The iodine is then extracted with water, oxidised to iodate by means of bromine water and the iodine liberated on addition of potassium iodide titrated with *N*/200 sodium thiosulphate. In Pickworth's method the iodide extracted after fusion of the material is oxidised by means of potassium permanganate, the excess of this reagent being removed by animal charcoal. The iodate is treated with potassium iodide and the liberated iodine titrated as above with thiosulphate.

McClendon mixes the material with lime and carries out a combustion in a stream of oxygen, any iodine volatilised being absorbed in alkali. The absorption liquid, as also the extract of the combustion residue, is acidified, any iodate present reduced to iodide by the addition of a trace of arsenious acid and the iodine liberated by means of sodium nitrite. The free iodine is then taken up in carbon tetrachloride and estimated colorimetrically.

Fellenberg, who at the suggestion of the Swiss Goitre Commission has made an extended study of the methods of iodine estimation, ignited the material with potassium hydroxide and extracted the ash with alcohol. The iodine liberated by the addition of sulphuric acid and potassium nitrite was taken up in chloroform and estimated colorimetrically. Fellenberg's method has been modified by Leitch and Henderson, who prefer the titration method to the colorimetric method ; these authors also utilise the iodate-potassium iodide reaction by which the amount of iodine to be titrated is increased six times. The methods outlined above have been utilised chiefly for biological materials and foodstuffs and but little work is on record relating to the estimation of iodine in soils. In the latter case the difficulties are much increased as, owing to the large amount of inert material present, it is by no means easy to bring about a satisfactory destruction of the organic matter without loss of iodine. The largest series of soil analyses is that carried out by Hercus, Benson and Carter in New Zealand (*J. Hygiene*, 1924, **24**, 321). These authors give the results obtained with some 400 different soil samples, the iodine content of which varied between nil and 700 parts per 10 million parts of soil.

The method employed was to soak the soil with 8 per cent. alkali and ignite it at a low red heat for about 15 minutes ; the ignited material was extracted with boiling water, the extract neutralised, the iodide oxidised to free iodine by nitro-sulphuric acid and the free iodine taken up in carbon disulphide and titrated with $N/1270$ thiosulphate. Figures for control soils with known amounts of added iodine are not quoted. The authors however realised that the extraction of the soluble iodine from the soil by this method was not always complete and state (*loc. cit.*, 331) 'the method here described yields figures approximately *proportional* to the amount of soluble iodine in by far the greater number of the soils analysed.'

EXPERIMENTAL.

The Carter method was first tested on a sample of local soil. It was anticipated that difficulties might arise owing to the considerable amount of iron present in this soil as evidence has previously been brought forward indicating that loss of iodine may occur in such circumstances. This point therefore was examined first. To eliminate any difficulties that might arise from the presence of organic matter the soils used in this experiment were first ignited with ammonium nitrate. A known weight of the soil was then treated with a dilute solution of potassium iodide of such strength that the sample when dried contained 1,000 parts iodine per 10 millions parts of soil. A second sample was prepared in the same way containing half this quantity of iodine. A third sample of soil without added iodine was used as a control. The iodine in all three samples was then estimated by Carter's method with the following results:—

...	A	B	C
Parts iodine added per 10 million parts soil	1,000	500	0
Iodine titration	10·0	5·5	0
	8·0	3·9	0
	6·5	4·0	0
Theoretical titration figure	25·0	12·5	0

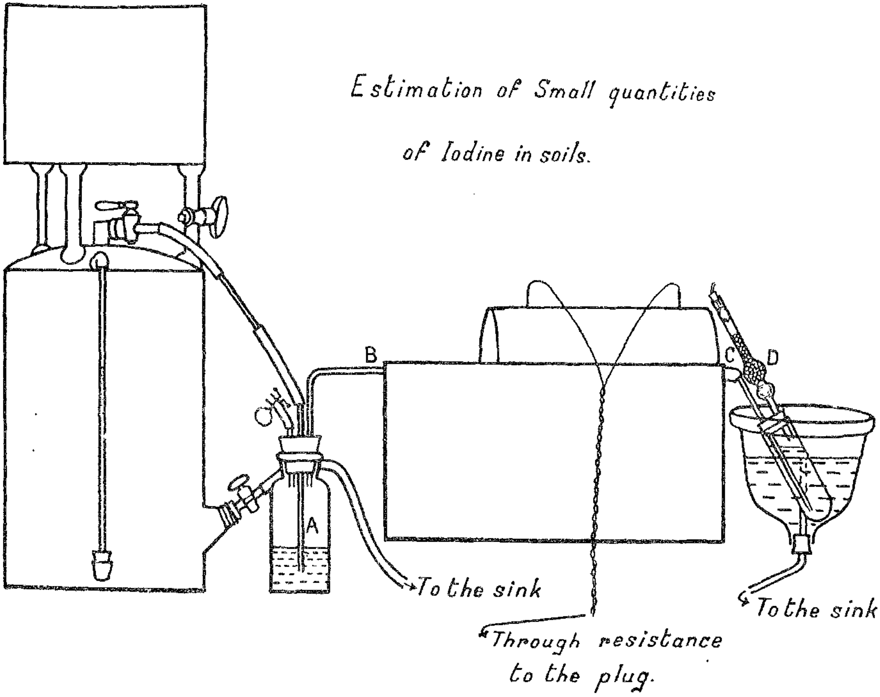
That the unsatisfactory results obtained were probably due to the iron content of the soil is indicated by the following experiments carried out with pure quartz sand and similar sand impregnated with oxide of iron.

The sand used was first boiled with commercial hydrochloric acid for 15 minutes, well washed and dried. Potassium iodide solution was then added in such quantity as to give samples with an iodine content of 1,000 and 500 parts per 10 million of sand respectively. A second portion of the sand was treated with a solution of iron pernitrate, dried and strongly ignited. In this way the sand was impregnated with iron oxide, the content of iron being 1 per cent. and 3 per cent. respectively in the two samples prepared. Potassium iodide was then added so as to give a sample containing 1,000 parts iodine per 10 million of sand. All the samples were then analysed by Carter's method with the following results:—

...	Samples free from iron		Samples containing oxide of iron	
	A	B	C. 1 per cent. iron	D. 3 per cent. iron
Parts iodine added per 10 million sand	1,000	500	1000	1000
Titration figures	10·0	4·9	5·55	5·50
	9·9	4·9	5·65	5·55
Theoretical titration value ...	10·0	5·0	10·0	10·0

It will be seen that while the sand alone has given correct results, in the presence of iron the result is only 56 per cent. of the correct figure. The loss may be due to either volatilisation of free iodine in the ignition or to a failure in the extraction process in the one case. To facilitate the extraction it was considered advisable to raise the temperature of ignition; to guard against loss of iodine by volatilisation the ignition was carried out in a combustion tube and any volatile products collected in caustic alkali. In this respect the process resembled McClendon's method. It was soon realised however that the extraction process could be dispensed with altogether if the soil were ignited at a sufficiently high temperature without the addition of alkali; extraction being a troublesome and slow process this procedure has obvious advantages. The whole of the iodine will now be in the absorption liquid and can be estimated in this solution by means of any of the methods already described. Theoretically the oxidation of the iodine to iodate and treatment of this with excess of potassium iodide seems advantageous as by this method six times as much iodine is obtained for titration as is actually present in the soil. In practice however we have found this procedure unsatisfactory. During combustion of the soil other compounds capable of liberating iodine from potassium iodide appear to be formed and the results are consequently high. We have found it preferable to estimate the iodine by titration with *N*/1270 thiosulphate after extraction with carbon tetrachloride. This solvent has been found to give more reliable results than carbon disulphide which was used by Carter. The method finally adopted is as follows.

The combustion of the air-dried soil is carried out in a nickel boat about 10 inches long in a silica combustion tube in a stream of oxygen. For soils rich in iodine 5 gms. can be used, the quantity being increased if the figure is likely to be low. The combustion tube is heated to about 850° in a simple type of electric furnace. One end of the tube is drawn out to a fine point which is bent over and passes directly into the absorption vessel containing a small quantity of 10 per cent. sodium hydroxide. We have not found it necessary to use any cooling coil as suggested by McClendon. The combustion is continued for one hour by which time the whole of the iodine present in the sample will have been driven over into the alkaline solution. The latter, together with the washings of the absorption vessel, is then transferred to a 100 c.c. stoppered bottle and neutralised by the careful addition of sulphuric acid; 2 c.c. of carbon tetrachloride are then added and the iodine liberated from the iodide by the addition of 3–5 drops of nitro-sulphuric acid, prepared by heating concentrated nitric acid with starch and absorbing the nitric oxide fumes in sulphuric acid. After vigorous shaking the carbon tetrachloride containing the iodine is separated, washed twice with a very small quantity of water and freed from acid by adding 2 c.c. of sodium acetate solution. The iodine is then



Estimation of Small quantities of Iodine in soils.

titrated with $N/1270$ sodium thiosulphate. If necessary a second extraction with carbon tetrachloride may be made. The thiosulphate solution is kept in the dark and standardised at frequent intervals.

We have found this method to be simple and reliable. The coloration produced by the presence of 0·005 mgm. iodine in 2 c.c. of carbon tetrachloride is perfectly perceptible. In 25 gms. of soil this would correspond to 2 parts of iodine per 10 million of soil. This quantity of iodine, however, only requires one drop of $N/1270$ thiosulphate and cannot with certainty be detected in a soil analysis. Using the above quantity of soil, however, 5 parts of iodine per 10 million can be detected and greater amounts estimated.

The results obtained with a number of Indian soils examined varied from nil to 400 parts per 10 million. Details will be found in another publication (McCarrison, Newcomb, Viswanath and Norris, *Ind. Jour. Med. Res.*, 1927, **15**, 207).

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