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Methods for the Micro-Determination of Iodine in Biological Material.

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ONE of the chief difficulties in iodine investigations in the past has been the lack of a method of analysis, sufficiently delicate, to determine accurately the very small amounts of iodine present in some biological substances. The literature relating to the methods proposed in the past has been reviewed so comprehensively by Scharrer (1928) that the older methods will not be discussed here. The modern methods can be arranged into three groups depending on the primary treatment of the material to be analysed. The material is decomposed so that iodine can be extracted in the form of a water-soluble compound. The methods can be classified as follows:—

(a) *Dry Oxidation in a Closed System.*—According to McClendon (1927), Schwaibold and Harder (1931), etc., the material is heated in a closed system in a current of oxygen, and the combustion products passed through an electrical precipitator or over a heated catalyst and finally through an alkaline solution which absorbs the iodine.

(b) *Nickel Dish Method.*—The method adopted by Van Fellenberg (1926) and modified by Scheffer (1930) and others, lays down that the material shall be mixed with an alkali in an open—usually nickel—dish and heated carefully until all the carbonaceous matter has been charred or totally oxidized. The iodine in the form of an alkali iodide is then extracted with \pm 95 per cent. alcohol, or with water and alcohol.

(c) *Wet or Acid Oxidation Method.*—Pfeiffer (1930) proposed the use of this method. The material is treated with perhydrol and concentrated sulphuric acid in a closed system and the iodine liberated is absorbed in an alkaline solution.

The numerous methods cited in the literature bear testimony to the fact that some essential detail is lacking in the procedure, and lately, workers have concentrated on perfecting the existing methods rather than proposing new ones, so that iodine can now be determined with a fair degree of accuracy by the experienced worker.

Although the greatest care should be taken in the determination of iodine when it has been converted into a soluble form free from organic matter, all possible precautions should be adopted in the primary treatment of the material to avoid loss of iodine; and, until such time as the carbonization in open crucibles has been perfected, the dry oxidation in a closed system should yield the best results,

although, unfortunately, it is very lengthy and therefore hardly suitable for routine analyses. A criticism against the use of the acid oxidation method in the analysis of plant material is the possible error which may be introduced by impurities, which, in the form of iodine, may be present in the large amount of reagents used. Consequently, it was decided to test the accuracy of the nickel dish method, the most rapid and hence the most adaptable for routine analysis, by comparing its efficiency with that of the dry oxidation method as a standard. The procedure adopted in the technique is as follows:—

CLOSED SYSTEM METHOD.

About 20 to 30 grams of dry material (grass, vegetables, etc.), are packed into a clear silica tube 28 inches long and one inch internal diameter. The tube is fitted with a rubber stopper at each end. The primary oxygen is led in at the one end and the secondary supply at the other end through a silica tube which passes through the catalyst consisting of silky fibrous asbestos. The material for analysis is kept in position by a plug made from Whatman No. 40 filter paper. The ashing is carried out as follows: The primary oxygen is turned on until the air in the tube and wash bottles is displaced. The catalyst is then heated by means of gas burners and when the temperature reaches $\pm 700^{\circ}$ C. the secondary oxygen is also turned on. The organic material furthest away from the catalyst is heated slowly until combustion starts. No more external heat is applied to the combustible material for some time and a combustion zone travels slowly along the length of the tube. Some carbon is deposited on the inside of the tube and this is finally burnt away by applying external heat.

The combustion products travel along the tube until they come into contact and mix with the secondary oxygen, and then pass through the heated catalyst where complete combustion takes place. In this way the tarry vapours are all destroyed and the oxidation products, which are now colourless, plus the excess secondary oxygen pass through wash bottles containing solutions of potassium carbonate. By including two of these absorption flasks in the circuit all the iodine contained in the combustion products is absorbed.

When all the carbonaceous matter is oxidized, the residue is cooled in a current of oxygen. The tube is then washed with distilled water and the combined washings, to which is added 0.5 c.c. saturated potassium carbonate, heated on the steam bath for some time and filtered. The contents of the wash bottles are poured into a platinum dish, the bottles washed with distilled water and these washings, together with the wash water from the silica tube, are added to the contents of the platinum dish. The solution is evaporated to dryness, the contents of the dish glowed over an open flame to remove the last traces of organic material, dissolved in a minimum quantity of water, and filtered. The dish is washed with small quantities of water which, after being filtered, are added to the main filtrate. The alkaline filtrate is neutralized with hydrochloric acid and a drop of excess acid added. After the addition of a few grains of pumice stone and about 1 c.c. freshly-prepared chlorine water the excess chlorine is boiled off. This is accomplished

after 10 minutes' brisk boiling. When the solution is cold a very small crystal of potassium iodide (about 0.001 gm.), and about 10 drops of a .25 per cent. starch solution are added and the iodine liberated is titrated against thiosulphate using a micro-pipette.

Chlorine water is prepared a few minutes before it is required by dropping concentrated hydrochloric acid on to potassium permanganate and passing the chlorine through distilled water contained in a conical flask fitted with an outlet tube. When the chlorine water is required, this tube is pushed down so that it reaches into the water. The positive pressure in the apparatus forces the chlorine water out drop by drop.

Blank determinations were carried out on the reagents employed. The same procedure as in the actual determination was adopted and also the same quantity of distilled water and reagents used. Iodine was not detected in any of these blanks. This showed definitely that after 10 minutes boiling all the chlorine had been driven off.

The asbestos used as catalyst is purified by heating it with concentrated sulphuric acid for twelve hours and washing it thoroughly, first with tap water and finally with distilled water. The asbestos is then dried and heated at $\pm 600^{\circ}$ C. to remove the last traces of the acid.

The closed system method is taken as a standard, i.e. one which gives as nearly absolute results as possible. Loss of iodine due to escape during the combustion process is eliminated, and after oxidation all the iodine is present either in the silica tube or else in the absorption flasks. In either case the final solution, i.e. washings from silica tube plus absorption liquid, contains all the iodine. The method gives excellent results on duplicate determinations and on recovering added iodine compounds as described in Tables I and II.

It was therefore decided to test out a simpler method suitable for routine analysis and to standardize it against the silica tube method. The manipulation of the apparatus employed in the acid oxidation (Pfeiffer, 1930) is too complicated and besides being unsuitable for bulky plant materials low in iodine, it offers no advantage over the dry oxidation in a closed system. The only alternative is the partial ashing of organic material in the presence of an alkali in open crucibles or dishes.

NICKEL DISH METHOD.

The method adopted is based on that described by Scheffer (1930) with slight modifications. A sample of finely ground dry plant material—about 20 to 60 gm., depending on the anticipated iodine content of the substance analysed—is weighed into a nickel dish and completely covered with water. To this 5 to 15 c.c. of a saturated potassium carbonate solution are added, and the contents stirred with a glass rod so that the alkali is mixed intimately with the individual particles of the organic material. The excess water is evaporated on the steam bath and the nickel dish placed in a large electric furnace the temperature of which can be regulated by means of a suitable resistance in the circuit. The current is switched on and the temperature raised to $\pm 150^{\circ}$ C. The resistance is then increased so that

the furnace is heated very gradually. On account of the swelling of starchy materials the procedure adopted in the case of grass and fibrous materials is slightly different to that for intumescent materials.

GRASS AND FIBROUS MATERIALS.

At approximately 250° to 270° C. the contents of the dish begin to glow and an ignition zone travels through the entire mass. The current is switched off as soon as spontaneous combustion takes place, and it is found that under these conditions the temperature of the furnace can be regulated not to exceed 290° C., although that of the glowing mass in the nickel dish may be slightly higher. This is important because the efficiency of the method depends largely on the temperature employed and at no stage during the carbonization should this exceed 300° C.

INTUMESCENT MATERIALS.

These substances swell to an enormous extent when heated above 200° C. To overcome this the dish is cooled from time to time and the contents broken up with a nickel spatula. This process is continued till intumescence ceases. The dish is then heated further to 290° C. and kept at this temperature until the material is totally charred.

When all the organic matter—fibrous and starchy—is carbonized, the dish is allowed to cool, the contents finely powdered and transferred to a conical flask. The dish is washed with small quantities of water and the washings added to the contents of the flask. The flask is heated on the steam bath, 50 c.c. absolute alcohol added, the flask shaken with a gyratory motion and the alcohol extract filtered. The residue is extracted with three successive portions of alcohol and finally it is transferred to the filter and washed with alcohol.

The alcohol extract plus 2 c.c. saturated potassium carbonate solution is slowly evaporated to dryness in a platinum dish without allowing the contents to boil. The dish is gently glowed over an open flame and the residue moistened with water and extracted with successive portions of alcohol until the alkali separates out. The residue is again moistened with water, extracted with alcohol as before and the filtered alcohol extract added to the main extract.

It is of the utmost importance in the extraction of potassium iodide to dissolve all the soluble material in a minimum quantity of water so that a fairly saturated solution is obtained, and then only to add the alcohol. In this way the alcohol is brought into direct contact with the iodide which cannot happen when a mixture of water and alcohol is used for extraction. In the latter case the iodine may be so occluded by the rest of the material present that extraction can only be complete when the material is ground to an impalpable powder. Scheffer (1930) illustrated this fact very clearly. Also Hercus and Aitken (1933) in studying the partition of potassium iodide at low concentrations between alcohol and potassium carbonate solution found that if the potassium carbonate was saturated or nearly

so and excess absolute alcohol added to dehydrate the paste, the partition coefficient was of such an order that under the conditions described in this paper for the extraction all the iodide should be present in the alcohol extract.

The filtered alcohol extract plus 0.5 c.c. potassium carbonate solution is transferred to a platinum dish, slowly evaporated to dryness and gently glowed. The residue is dissolved in a few c.c. water, the solution filtered, the dish washed three times with a few c.c. water, and the washings added to the main filtrate. The combined filtrate is neutralized with hydrochloric acid and a drop excess acid added. The rest of the procedure is similar to that described for the closed system method.

STANDARDIZING AND COMPARING THE EFFICIENCY OF THE METHODS.

The efficiency of the silica tube method was determined by adding 1 c.c. potassium iodate solution of known strength to filter paper (free from iodine) and also to grass and analysing the materials according to the closed system method. In some cases the iodine present in the two absorption flasks and in the washings of the silica tube was determined separately, while in others the various iodine containing fractions were all combined.

TABLE I.
The Efficiency of the Closed System Method.

Substance Analysed.	Weight of Material Analysed. Gram.	Iodine Present in Material. γ .	Iodine Added in the Form of KIO_3 . γ .	Total Iodine Present. γ .	Iodine in First Absorption Flask. γ .	Iodine in Second Absorption Flask. γ .	Iodine in Washings from Silica Tube. γ .	Total Iodine Recovered. γ .	Percentage Recovery.
Two filter papers.....	—	—	80	80	73.8	1.0	4.5	79.3	99
Two filter papers.....	—	—	80	80	74.2	0.9	3.6	78.7	98
Two filter papers.....	—	—	80	80	75.6	0.7	3.6	79.9	100
Mature Grass (18).....	9.3	*9.7	80	89.7	}		}	80	89
Green Grass (28).....	11.0	*7.3	80	87.3				78.6	78.6
Mature Grass (14).....	12.8	*8.0	80	88.0	}		}	79.3	90
Mature Grass (14).....	12.6	*7.9	80	78.9				78.7	78.7

These results need some explanation because the total iodine recovered is just equal to or less than that added to the grass. At first sight it may thus appear that none of the iodine originally present in the grass is recovered. This, however, is certainly not true and the explanation is offered in Table III. Here the same amount of iodine is added to samples of grass and where the iodine present in the original sample exceeds 12 γ the iodine recovered is always more than that added, even although the percentage recovery is less than that recorded in Table I. It is clear, therefore, that the iodine recovered is made up of that present in the material analysed as well as that added in the form of potassium iodate.

* The values used here are calculated from the results obtained for the closed system method *vide* Table II.

† The sign γ is used to denote a microgram or the millionth part of a gram.

The following table of results compares the efficiency of the nickel dish method with that of the dry combustion method:—

TABLE II.

Substance Analysed.	Dry Combustion Method.					Nickel Dish Method.	
	Iodine in First Absorption Flask.	Iodine in Second Absorption Flask.	Iodine in Washings from silica tube.	Total Iodine.	Average.	Total Iodine in Material.	Average.
	γ%	γ%	γ%	γ%	γ%	γ%	γ%
Mature Grass (18).....	94.8	2.8	7.0	106.6	104.6	102	102
Mature Grass (18).....	84.1	4.1	15.7	103.9			
Mature Grass (18).....	88.8	4.9	11.6	105.3	62.8	60	60
Mature Grass (14).....	54.3	3.8	4.7	62.8			
Green Grass (28).....	43.3	3.9	17.6	65.8	65.6	62.3	62.3
Green Grass (28).....	40.8	3.9	18.5	63.2			
Green Grass (28).....	34.1	4.0	29.6	67.7			
Mature Grass (20).....		161.			161	152, 164	158
Mature Grass (40B).....		111.5			111.5	114.1	112
						110.7	
						110.8	
Potatoes (S. 990).....		79.0			79.0	112.4	
						83.0	82.4
						79.5	
						82.5	
						84.6	
Potatoes (S. 991).....		92.2			92.2	110.8	112
						113.2	
Wheat (S. 9891).....		10.1			10.1	10.5	10.2
						10.0	
Wheat (S. 989 II).....		16.3			16.3	14.8	14.5
						14.7	
						14.0	

The results obtained by the nickel dish method agree fairly well with those of the standard method and lie within the limits of experimental error. The efficiency of the nickel dish method was also determined in a more direct manner. One cubic centimeter potassium iodate solution of known strength was again added to the organic material and the analysis carried out as already described. The results given in Table III show that the percentage recovery is not much lower than that obtained with the closed system, *vide* Table I.

TABLE III.
Nickel Dish Method.

Material.	Weight : gram.	Iodine	Iodine	Total Iodine.	Iodine	% Re- covery.
		Present.	Added.		Re- covered.	
		γ	γ	γ	γ	
Mature Grass (14).....	12.1	7.6	80	87.6	73.7	84
Mature Grass (14).....	10.8	6.8	80	86.8	74.4	86
Mature Grass (20).....	13.8	21.8	80	101.8	84.5	83
Mature Grass (20).....	15.0	23.7	80	103.7	83.6	81
Mature Grass (40B).....	14.2	15.9	80	95.9	87.0	90
Mature Grass (40B).....	17.1	19.2	80	99.2	94.1	95
Mature Grass (40B).....	11.3	12.7	80	92.7	82.8	89
Mature Grass (40B).....	10.3	11.5	80	91.5	80.3	88

Emphasis has been laid on the fact that the maximum temperature employed during the carbonization stage in the nickel dish method should not exceed 300° C. Widmann (1932) has shown that when a temperature range of 300° to 350° C. is employed, the values for blood average only 36 per cent. of those obtained with an absolute method, while in the case of a range 200° to 250° C. the average figure is 88 per cent. This figure agrees very well with the average percentage recovery of Table III, which is 87 per cent.

It was found that, if the temperature is allowed to exceed 300° C. for any length of time, as much as 60 per cent. of the iodine present is lost. This is borne out by the results tabulated below:—

TABLE IV.

Material.	Silica Tube Method. $\gamma\%$	Nickel Dish Method.	
		250° to 300° C.	± 400° C.
		$\gamma\%$	$\gamma\%$
Wheat I.....	10.1	10.2	7.5, 8.2
Wheat II.....	16.3	14.5	13.2
Mature Grass (20).....	161.0	158.0	87.3, 97.5
Mature Grass (14).....	62.8	60.0	51.0
Mature Grass (28).....	65.6	62.3	48.1

Tables II and III illustrate the efficiency of the nickel dish method as long as the specified temperature range is adopted and the alcohol extraction carried to completion. For this reason as well as the suitability of the method for routine work, this method was adopted throughout the present investigation and the same procedure followed with slight modifications to suit the requirements of the different types of materials analysed.

SUMMARY.

Micro-analytical methods for the determination of iodine in biological substances are discussed and the efficiency of the nickel dish method compared with that of the dry oxidation in a closed system. It is shown that under the conditions specified the error in duplicate analyses does not exceed 20 per cent., while over 80 per cent. of the total iodine present, i.e. iodine in the original material plus the amount added, can be recovered.

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