60. The Estimation of Iodine in Thyroid Gland Material. An Examination of Kendall's Method of Combustion

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The preliminary step in the estimation of iodine in organic material is oxidation of the sample; either wet or dry combustion is used in open or closed systems, whether the sample varies in iodine content from that of blood (of the order of $10\mu\text{g./100 ml.}$), to that of dried thyroid gland powder (of the order of 400 mg./100 g.) [Fellenberg, 1924, 1930; Kendall, 1920; Kendall & Richardson, 1920; Leipert, 1933; McClendon, 1924; Middleton, 1932; Trevorrow & Fashena, 1935]. The open systems of combustion as used for analyses of thyroid gland powder require modifications and precautions in their application to estimations of such small amounts of iodine as occur in blood. While this paper refers to analyses of thyroid gland powder using Kendall's method of combustion, it may have significance for estimations of smaller amounts of iodine.

Kendall's method of combustion [1920] entails heating the sample to be analysed in a nickel crucible, with solid caustic soda moistened with water. The combustion proceeds with gentle heating at first and is completed by placing the nickel crucible inside a larger iron crucible and separated from it by a layer of sand. Heat is applied to the outer crucible supported in a specially designed furnace, the mixture fuses and organic matter is destroyed. Kendall recognized the possibility of loss of iodine by excessive or insufficient heating, but concluded that 'there is a wide range of temperature between the two limits and after a little experience no difficulty is encountered'. The solution of the alkaline mixture is oxidized with bromine and the estimation completed by titration with $N/200$ sodium thiosulphate.

The chief criticism to which this method has been subjected is the possibility of loss of iodine either as spray, as volatile substances, or through creeping of the sample up the sides of the crucible. Creeping up the sides of the crucible, never a difficulty in these laboratories, is avoided very simply by gentle controlled heating in the early stages of combustion. Experience with this method, nevertheless, has shown that there is a well-defined temperature of combustion above which loss of iodine does occur. It is the purpose of this report to show that this iodine is retained in the nickel crucible and is not lost by vaporization or spray, and further, that the retention of iodine in the crucible can be regulated by strict control of the maximum temperature of combustion.

An account of the circumstances which gave rise to this examination may be relevant to this report. Constant use of a method had been made employing a combustion process essentially that of Kendall, and a procedure for bromine oxidation and subsequent titration similar to that adopted by the British Pharmacopoeia 1932. This method had given general satisfaction for the determination of iodine in both thyroid gland powder and thyroid tablets, but an occasional high result within a series of estimations of iodine in thyroid gland tablets was a disturbing factor. On repetition of the analyses the high results did not recur, and the result was in accord with that calculated from the known dilution of the thyroid powder. No satisfactory explanation of these occasional high results had been forthcoming, until it was observed that they occurred within a series, only where the crucibles had previously been used for analyses of samples with considerably higher iodine content, namely, thyroid gland powder. It was finally shown that if iodine-free caustic soda was fused in the nickel crucibles immediately after they had been used for analyses of iodine in thyroid gland powder, and iodine estimations

carried out on the solution of the melt, titratable amounts of iodine were found to be present. Such 'blank fusions' were taken as evidence for the retention of iodine in the crucibles from the previous analyses. Subsequent fusions of caustic soda in the crucibles failed to show significant traces of iodine. Further, it was shown that significant amounts of iodine were retained in the crucible only if the maximum temperature of combustion was raised above 400°. Such retained iodine is not removed by boiling the crucibles in water but only by a further fusion of caustic soda.

Table 1 shows (a) the extent of this retention of iodine within the crucibles when a method essentially that of Kendall, without strict temperature regulation, is used (Table 1 A); (b) the increase in retained iodine when the maximum temperature of combustion is raised from 400° to 450° and 500° (Table I B, C, D); (c) the significance of retained iodine in analyses of smaller samples of thyroid gland powder both alone and together with lactose in approximately the proportions used in tablets, with a maximum temperature of combustion of 400° (Table 1 E, F).

Table 1. *Control of temperature of combustion*

A 129 is defatted thyroid gland powder dried at 60°

	Sample g.	Max. temp. of furnace	No.	Iodine found		Blank fusion mg. iodine found	
				mg.	%		
A	A 129 approx. 0.2	Kendall's method. No temp. recorded	1	1.036	0.482	0.006	Mean weight of iodine retained = 0.024 mg.
			2	0.890	0.459	0.015	
			3	0.793	0.438	0.050	
			4	0.912	0.440	0.050	
			5	0.914	0.437	0.017	
			6	0.871	0.451	0.008	
				Av. 0.451			
B	A 129 approx. 0.2	500°	1	0.876	0.440	0.033	Mean weight of iodine retained = 0.020 mg.
			2	0.878	0.460	0.016	
			3	0.877	0.457	0.013	
			4	0.902	0.455	0.012	
			5	0.878	0.448	0.023	
			6	0.903	0.449	0.020	
				Av. 0.452			
C	A 129 approx. 0.2	450° (30 min.)	1	0.943	0.467	0.016	Mean weight of iodine retained = 0.020 mg.
			2	0.945	0.459	0.020	
			3	0.923	0.455	0.019	
			4	0.985	0.458	0.030	
			5	0.928	0.462	0.019	
			6	0.977	0.465	0.014	
				Av. 0.461			
D	A 129 approx. 0.2	400°	1	1.029	0.463	0.002	Mean weight of iodine retained = 0.002 mg.
			2	1.017	0.477	0.002	
			3	0.962	0.472	0.002	
			4	0.924	0.472	0.002	
			5	1.040	0.475	0.002	
			6	0.902	0.477	0.000	
				Av. 0.473			
E	A 129 approx. 0.036	400°	1	0.176	0.476	0.000	
			2	0.171	0.457	0.000	
			3	0.176	0.446	0.008	
			4	0.180	0.461	0.002	
			5	0.179	0.478	0.000	
				Av. 0.464			
F	A 129 approx. 0.036 + 0.16 of lactose	400°	1	0.165	0.462	0.000	
			2	0.166	0.464	0.000	
			3	0.168	0.457	0.000	
			4	0.182	0.477	0.001	
			5	0.190	0.474	0.000	
				Av. 0.467			

It is evident that by observing the procedure indicated by Kendall [1920] in an analysis of iodine in 0.2 g. of thyroid gland powder, the average retained iodine (recorded as the blank fusion) may be only of the order of the average variation of the determination (Table 1A). But if this retained iodine be superimposed on an analysis of iodine of the order of that found in thyroid gland tablets (Table 1 E, F), the apparent iodine content of the latter may be increased by as much as 30 %. The figures taken together illustrate the value of controlling the maximum temperature of combustion, and provide a solution of the difficulties experienced, which led to this investigation.

The effect of time for which combustion is maintained at 400°

The effect of the time for which the sample is maintained at 400° is demonstrated by the results of the following analyses of 0.2 g. of thyroid gland powder A131. Maintaining the final temperature of combustion at 400° for 15 min., the retained iodine amounted to 0.4 % of the iodine of the sample; after 30 min. it amounted to 0.6 %, while after 45 min. at 400° the retained iodine was still only 1.1 % of the iodine of the sample analysed. These results are the average of six analyses at each time interval of combustion at 400°.

Method finally adopted

The method finally adopted for the estimation of iodine will be described in detail. Approximately 0.2 g. of the powdered sample (thyroid gland powder or tablets), accurately weighed, is placed in a nickel crucible 6 cm. in diameter and 5.5 cm. high, together with 10 g. of caustic soda broken into small pieces, and the mixture moistened with water, drop by drop. The crucible is placed on a sand bath, the temperature of which is sufficient to drive off all moisture but never sufficient to melt it completely. Care is taken from the start to heat the sample gradually, and assist its even dispersion by gentle rotation from time to time. The crucible is then transferred to a second sand bath, the temperature of which is sufficient to melt the sample gradually but which never rises to 400°. Cautious heating and gentle rotation allow the sample to melt and finally become clear with a short initial frothing, which subsides as the sample melts, without rising up more than one-fifth of the side of the crucible. When the melt completely clears, the combustion is continued by the addition of a few mg. of potassium nitrate, the crucible being placed in an electric furnace pre-heated to 400°. After a slight fall in temperature the furnace is brought back to 400° in about 5 min., maintained at that temperature for 15 min., then the crucible is removed. Throughout the combustion a very small air space is left between the lid and the rim of the crucible. At no stage is the lid allowed to fit completely over the rim, thus closing this gap. The estimation is completed by the oxidation of the iodine to sodium iodate. The melt, after cooling slightly, is poured on to the lid of the crucible and dissolved with hot water, the crucible and lid are washed carefully free from alkali and the solution, approximately 200–250 ml., is transferred to a 500 ml. Erlenmeyer flask. To the cooled solution are added 2 drops of 0.2 % methyl orange, 1 ml. of 10 % sodium metabisulphite solution and concentrated phosphoric acid till the solution is acid to methyl orange. The addition of a small amount of powdered talc promotes smooth boiling. Bromine vapour is blown on to the surface of the solution, which is gently rotated, until it is definitely yellow, and, after boiling for 10 min. the solution, cooled to room temperature, is ready for titration. Titration is carried out with *N*/200 sodium thiosulphate after the addition of approximately 0.3 g. of powdered potassium iodide and 5 ml. of 0.5 % starch solution containing 0.1 % salicylic acid. *N*/200 sodium thiosulphate containing 0.4 % amyl alcohol [Hercus, 1931] retains its strength for several months but is checked from time to time with a standard potassium iodate solution. When blank fusions are carried out, 10 g. of caustic soda, moistened with a few drops of water, are placed in the crucible which has been washed free from alkali from the previous analysis. The combustion and bromine oxidation are then

continued as in the method just outlined. The total time for combustion may amount to 2-3 hr. when six samples may be run simultaneously. Where samples are solutions of alkaline hydrolysates, they are evaporated slowly to a small volume in the nickel crucibles before the 10 g. of solid caustic soda are added for combustion.

Recovery of iodine

Using the above method of estimation of iodine in the caustic soda obtained from the blank fusion, it is possible that any extraneous material of an oxidizing nature might react with the added potassium iodide and be recorded as iodine. To check this possibility, known amounts of iodine in the form of potassium iodide were taken and subjected to the combustion process at 400 and 500° respectively, and estimated as in the method described for thyroid analyses. Each recovery was followed by a blank fusion of caustic soda as before.

The procedure in these experiments was as follows. Solutions of potassium iodide were made up to contain approximately 1 and 0.18 mg. of iodine/ml. respectively. 1 ml. of the freshly prepared solution of potassium iodide, previously analysed, was added to 10 g. of solid caustic soda in a nickel crucible and subjected to controlled combustion. The estimation of iodine in the solution of the melt was concluded as in the method outlined. The solutions of potassium iodide were previously analysed for iodine by placing 1 ml. of these solutions in 200 ml. of water containing 10 g. of caustic soda and 1 ml. of 10 % sodium metabisulphite, then acidifying with phosphoric acid using methyl orange as indicator. The bromine oxidation and subsequent titration with *N*/200 sodium thiosulphate were completed as already described in the routine procedure.

Table 2, which follows, records the results of these recoveries, and shows that when potassium iodide is subjected to the combustion process adopted for the estimation of

Table 2

	Max. temp. of furnace	mg. of iodine taken as KI	No.	Iodine found mg.	Blank fusion mg. iodine found	Total mg. iodine found in estimation and blank fusion
A	500°	0.985	1	0.951	0.017	0.968
			2	0.966	0.013	0.979
			3	0.951	0.025	0.976
			4	0.956	0.010	0.966
			5	0.984	0.011	0.995
			6	0.945	0.031	0.976
			Av.	0.959		Av. 0.977
B	500°	0.181	1	0.182	0.002	0.184
			2	0.176	0.007	0.183
			3	0.180	0.001	0.181
			4	0.181	0.001	0.182
			5	0.179	0.002	0.181
			6	0.181	0.001	0.182
			Av.	0.180		Av. 0.182
C	400°	1.007	1	1.006	0.003	1.009
			2	0.997	0.008	1.005
			3	1.003	0.006	1.009
			4	0.995	0.008	1.003
			5	1.003	0.007	1.010
			Av.	1.001		Av. 1.007
D	400°	0.184	1	0.180	0.003	0.183
			2	0.184	0.001	0.185
			3	0.185	0.001	0.186
			4	0.181	0.004	0.185
			5	0.183	0.002	0.185
			6	0.184	0.004	0.188
			Av.	0.183		Av. 0.185

iodine in an organic substance, more than 99% of the iodine can be accounted for by the sum of that estimated by the method outlined, together with that retained in the crucible. These results support the view that blank fusions record the retained iodine and not extraneous material of an oxidizing nature. Furthermore, the fact that more than 99% of the iodine can be accounted for even when the maximum temperature of combustion is raised to 500° is an answer to the criticism that iodine is lost as spray or by volatilization in Kendall's method of combustion. In addition this means that in an estimation of approximately 1 mg. of iodine, with the maximum temperature of combustion of 500°, the average retained iodine may amount to 2% of that present just as was observed with the samples of thyroid in Table 1B.

The foregoing recoveries of iodine were, by design, carried out in the absence of organic matter. To check the recovery of inorganic iodine in the presence of organic matter, iodine in the form of potassium iodide was added to 0.2 g. samples of thyroid gland powder, previously analysed by the routine method adopted. The total iodine was estimated, and each estimation was followed by a blank fusion as before in the same crucible (Table 3). The average of the iodine estimated in the six analyses was 99% of

Table 3. *Recovery of iodine when added to 0.2 g. samples of thyroid powder of 0.410% I using controlled combustion at 400°*

No.	mg. iodine in thyroid sample I	mg. iodine added as KI II	Total mg. iodine taken I + II	mg. iodine found III	Blank fusion mg. iodine found IV	Total mg. iodine found + mg. iodine in blank fusion III + IV	Total recovery as % of total iodine taken
1	0.841	1.011	1.852	1.845	0.009	1.854	100.1
2	0.816	1.011	1.827	1.830	0.013	1.843	100.9
3	0.841	1.011	1.852	1.833	0.007	1.840	99.3
4	0.832	1.011	1.843	1.753	0.033	1.786	96.9
5	0.828	1.011	1.839	1.827	0.017	1.844	100.3
6	0.865	1.011	1.876	1.887	0.002	1.889	100.8
							Av. 99.7%

the total iodine taken, while the sum of the iodine estimated, together with that found in the blank fusion, gave an average total recovery of 99.7%. Table 3, together with Table 1 D, E, F, also show the limits of accuracy of estimations of iodine varying from 1.8 to 0.18 mg. In the former case a loss of 1% may be expected, while in the latter the loss may be between 1 and 2%, or approximately 0.003 mg. This is very close to the limit of approximately 0.001 mg. of iodine that can be detected by this procedure in a volume of 200 ml.

DISCUSSION

Long-continued use of nickel crucibles for fusion of caustic soda leads to irregular pitting of the surface and finally to leaking of the caustic soda. The increasing amount of iodine retained in the crucibles as the temperature of combustion is increased, and the presence of a fine precipitate of nickel oxide in the solution of the melt, are factors that suggest that the caustic soda seeps into the surface of the crucible carrying with it the iodine. Thus a demonstration is provided of the gradual and irregular attack on the crucibles resulting finally in holes. The amount of iodine retained in the crucibles depends both on the concentration of the iodine in the caustic soda and the temperature to which it is raised. This report draws attention to the possibility of controlling the amount of iodine retained, by controlling the maximum temperature of combustion. Nevertheless, where large amounts of iodine have been determined even with a maximum temperature of combustion of 400°, or where the previous history of the crucibles is unknown, a blank fusion of caustic soda is a wise precaution before further use of the crucibles.

That such a source of loss has not been emphasized before is understood, when it is seen that where the same crucibles are used continuously for analyses of approximately uniform amounts of iodine, such losses cancel one another out in a series of estimations. Crucibles from three different sources have shown the same behaviour.

Control of temperature by degree of redness of the crucible is never reliable, as it is so largely influenced by the variable daylight in which the crucible is viewed. Elmer's discussion [1938] of this point further emphasizes its importance. Temperature control of combustion has been given careful attention in estimations of iodine in blood [Kendall & Richardson, 1920; Elmer, 1938; Harvey, 1935; Stimmel & McCullagh, 1936] and possible loss through volatilization has been discussed. In estimating larger quantities of iodine, Sigurjonsson [1938] refers to the risk of loss by volatilization unless the temperature of ashing is controlled. This method is similar in principle to that of Middleton [1932], and to the procedure adopted by the 1936 Addendum to the British Pharmacopoeia. Experience here with the latter method shows that one of the precautions necessary to make this method reliable, is rigid control of the temperature of combustion at 450–550° rather than reliance on 'dull red heat'. It should be mentioned that Foster & Gutman [1930] and Leland & Foster [1932] modify Kendall's method of combustion without discussing their reasons for so doing. They judge the conclusion of combustion by the appearance of a coating of nickel oxide on the bottom of the crucible. As this coating may take place below 400° they probably avoid the retention of iodine within the crucible, but temperature control should provide a more reliable guide. Nevertheless, indications are to hand that in analyses of thyroid powder containing approximately 0.13 mg. of iodine, maximum recoveries can only be obtained if combustion is continued for 15 min. at 400°, after the addition of potassium nitrate and the coating over of the bottom of the crucible. Thus, while temperature control of combustion has been used in certain methods of iodine estimations, this paper draws attention to the need for its wider application and to an additional source of loss which it may influence.

With temperature control of the caustic soda fusion, Kendall's method of combustion, as a preliminary to the determination of iodine in thyroid gland preparations, can be handled confidently. The combustion would be simplified by using a suitable furnace fitted with temperature control throughout, rather than sand baths in the initial stages.

A commercial nickel was stated to contain 99.3% nickel with maximum limits of impurities of iron 0.4%, copper 0.1%, silicon 0.05%, carbon 0.1%, sulphur 0.02% and manganese (trace). While there are no indications that these elements are responsible for the retention of iodine in the method under discussion, the practical considerations are unaltered, should this be due to impurities in the nickel.

When this systematic examination was begun, the crucibles were new. The analyses recorded in Table 2D represent the 100th fusion in each crucible. It is quite possible that as crucibles age, this source of loss may increase, although crucibles which have had constant use for several years, recently gave negligible amounts of retained iodine after controlled combustion of thyroid samples at 400°.

The observations made in connexion with estimations of iodine are of possible significance wherever nickel crucibles are used for fusions of caustic soda in analytical work.

SUMMARY

1. Possible sources of loss of iodine during the combustion of organic matter are discussed in relation to the estimation of iodine in thyroid gland material.
2. Kendall's method of combustion is reviewed and shown to be reliable when subjected to temperature control.
3. A possible limiting factor is suggested in the use of nickel crucibles for caustic soda fusions.

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