DETERMINATION OF IODINE IN CONNECTION WITH STUDIES IN THYROID ACTIVITY.

THIRD PAPER.

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In 1912 I published a method for the determination of iodine in the presence of the other halogens and organic matter (1). In 1914 the method was modified in part (2), and during the past 6 years has been in constant use. Recently it has become necessary to determine the iodine content of animal tissues, and, as no method yet published was suitable for this work, attempts were made to refine the method published in 1914, in order to make it applicable for the determination of the iodine in blood and tissue.

The faults of the iodine method, previously described, apply entirely to the solution obtained after fusion with sodium hydride. The fusion originally described is still used without change.

In trying to improve the accuracy of the method, it was first necessary to secure a perfect blank in the absence of iodide. It was pointed out in a previous paper (2) that the presence of salts assists rather than interferes with the liberation of iodine from potassium iodide by an oxidizing agent. Blanks were therefore prepared, using sodium phosphate made by neutralizing the amount of sodium hydroxide required by the method with phosphoric acid. The neutralization was carried to the turning point of methyl orange. When such a solution was oxidized with bromine, then cooled, and phosphoric acid, potassium iodide, and starch were added, a perfect blank sometimes was obtained, but the blue color rapidly developed if the solution was allowed to stand.
In the determination it is essential to control the hydrogen ion concentration during the boiling with bromine. This is done by neutralizing the alkaline solution with phosphoric acid until methyl orange is just pink. This acidity, however, is not great enough for the final reaction between potassium iodide and the iodic acid resulting from the oxidation with bromine. It is necessary to add about 5 cc. of 20 per cent phosphoric acid at the time the potassium iodide is added. As we failed consistently to secure a perfect blank after oxidation with bromine, it appeared probable that the phosphoric acid which was used to acidify the solution at the time the potassium iodide was added was the cause for the liberation of iodine, and attempts were therefore made to obtain a perfect blank with nothing present except water and phosphoric acid. The test as finally adopted is as follows: 1 gm. of potassium iodide is placed in a dry flask. 15 cc. of phosphoric acid, 85 per cent, syrupy, are added to the potassium iodide. This produces a yellow color which, however, is not free iodine. The flask is allowed to stand for 15 minutes. 100 cc. of water are added. This will cause a partial or complete disappearance of the yellow color. After starch is added the solution should remain colorless. With the phosphoric acid available on the market it is rarely possible to obtain a perfect blank after such a procedure.

Since within the phosphoric acid itself there is a source of error causing high results, it is necessary to treat the acid so that this oxidizing action may be removed. This may be accomplished by diluting the phosphoric acid with four equal volumes of water, placing in a large beaker, and boiling the solution after the addition of a suitable amount of aluminum in the form of strips. It was found that the reduction proceeds slowly and requires prolonged boiling. It is possible to reduce all oxidizing agents so that when 25 cc. of the reduced acid are added to 1 gm. of potassium iodide, the solution is allowed to stand 15 minutes, and water and starch are added, a perfect blank will be obtained which will remain colorless for at least 15 minutes. When phosphoric acid which has been so reduced is used, the method is found to give very much more consistent results, and the solutions will remain colorless after titration for as long as from 30 to 45 minutes. It is unnecessary to reduce the phosphoric acid used to
neutralize the sodium hydroxide. Treatment of the solution with bromine destroys the oxidizing action of the impurities in the phosphoric acid.

In the original method talcum was recommended for the rapid removal of bromine from the solution. It was found, however, that when the talcum settled to the bottom of the titration flask the solution turned blue, suggesting that the talcum, although it caused a rapid expulsion of bromine from the solution, had retained a small amount which was absorbed in some manner and was not reduced by boiling with salicylic acid. Another disadvantage was the cloudiness of the solution. A solution turbid with talcum is very much more difficult to titrate than a water-clear solution. Some substitute for talcum was urgently needed. Many different substances were tried among which may be mentioned sand, brick, granite, pumice stone, capillary glass tubing, paper fibers, asbestos fibers, powdered charcoal, powdered glass, powdered porcelain, powdered hard coal, coke, and eventually hard coal in lumps of about 0.5 cm. in diameter. It was found that some of these agents assisted slightly in producing rapid boiling, but they all, except the paper fibers, retained traces of bromine which liberated iodine, giving high results. The paper fibers caused a slight reduction of the iodic acid.

Hard coal in lumps possesses a unique power to induce rapid boiling, but it also absorbs small amounts of bromine, so that after the titration is finished the solution in the immediate vicinity of the lump of coal turns blue, even in a flask which should be a perfect blank. In order to prevent high results from the oxidation by the bromine held in the coal, it was decided to remove the piece of coal after the solution had been boiled, and before titration. In order to accomplish this, two pieces of coal were tied together with a piece of thread. This thread could be picked up easily with a glass rod terminating in a hook. When pieces of coal tied together with a thread are used a number of times, added iodine cannot be quantitatively recovered, and it was found that the explanation for the low results is the reduction of iodic acid by the thread. It was a simple matter to change the thread to a glass hook which was passed through a hole in the lump of coal made by a dental drill. For some time coal which was removed by means of a glass hook was used, the coal
being washed between determinations and used a large number of times. This, however, was found impracticable, since there is a slow oxidation of the coal by the bromine, resulting in a softening and disintegration which eventually has the effect of reducing iodic acid and causing low results.

Since coal can be prepared in any desired amount with trivial trouble and expense, it was decided to use a fresh piece of coal for each determination. The method now employed is to use a piece of coal about 0.5 cm. in diameter and, after the solution has been boiled and cooled, it is removed by means of a small shovel made from a piece of sheet silver attached to a glass rod. This procedure is very easily carried out, the coal does not reduce iodic acid when used but once, and if it is removed from the solution a perfect blank can be obtained in the absence of iodine.

The method now possessed a high degree of accuracy except for the possible presence of substances other than iodic acid which were in the water or resulted from the solution of the fusion. In attempting to remove such oxidizing agents the solution was boiled with a considerable excess of sodium bisulfite and it was found that when iodine is present in amounts of from 5 to 10 mg., which is the upper limit of amounts that are desired to be determined by this method, the boiling in acid solution with sodium bisulfite does not result in the loss of any iodine. Boiling with sulfite reduces all nitrites and other oxidizing agents and prepares the solution for oxidation. Oxidation of such a solution with bromine causes a quantitative production of iodic acid.

Another point which is essential to control is the hydrogen ion concentration of the solution. This we find to be of greatest importance. In the presence of too high a concentration of hydrogen ion, hydrobromic acid will react with iodic acid, causing low results. Even 5 cc. of 50 per cent phosphoric acid in excess may cause an appreciable loss of oxidizing power due to reduction of iodic acid. It was eventually found that titration to the first pink color with methyl orange, after which 5 cc. of 20 per cent phosphoric acid are added, produces a proper acidity for both the reduction with sodium bisulfite and the subsequent boiling with bromine.

In the original method it was recommended to use a 10 per cent solution of potassium iodide. All commercial potassium
iodide will liberate traces of iodine on long standing in aqueous solution. This iodine could be removed with thiosulfate, but the addition of potassium iodide in solid crystalline form to the solution has been found to be the most convenient method for the addition of this salt.

The last point which requires control is the length of time of boiling the solution. It has been found that the solution may be boiled in the presence of sodium bisulfite until a large part of the water has been evaporated without loss of iodine. The solution must be boiled 10 minutes as a minimum length of time, in order to remove all traces of sulfurous acid. The oxidation with bromine is best carried out in a solution of a volume of 200 cc. It is best therefore to have sufficient water present before boiling off the sulfurous acid so that at least 10 minutes are necessary to bring the volume to 200 cc. This volume is marked on each flask. If more time is required to reduce the volume of the solution to the mark it is without effect on the subsequent determination of the iodine. The only essential point is that each flask boils at least 10 minutes.

Although there is a wide limit to the time for boiling with sulfite, it is essential to regulate closely the time of boiling with bromine. If the boiling with bromine is prolonged after the excess has been removed from the solution, low results are produced. A series of experiments has shown that the best time for boiling with bromine is 5 minutes after the solution is colorless. It requires approximately 3 minutes for the bromine to be expelled from the solution after boiling begins, and if the boiling is continued for 5 minutes more a perfect blank will result. As a further precaution, however, salicylic acid is added just before the flask is cooled. Although this may be unessential in most cases, its adoption is a safeguard which assures a uniform removal of the last traces of bromine from all determinations.

With the adoption of these six modifications of the original method, the determination of iodine after fusion in sodium hydroxide can be made with a high degree of accuracy. The method is of the greatest value, however, in the determination of minute amounts of iodine. The water-clear solution resulting by elimination of talcum permits a titration of amounts measured in hundredths of a cubic centimeter, and the removal of all
substances which retain bromine and therefore cause slight liberation of iodine permits the determination of small traces of iodine with a much higher degree of accuracy than before. For example, it has been found that talcum, even after boiling for 10 minutes, will retain bromine equivalent to as much as 0.08 cc. of 0.005 N thiosulfate. This amount of iodine is approximately that found as the normal iodine content of 100 cc. of blood, so that any results which have been obtained in the past with talcum are questionable, at least to within 0.05 to 0.08 cc. of 0.005 N thiosulfate. The method in detail as now employed in our laboratories is as follows:

The first step is the destruction of organic matter and the retention of the iodine as sodium iodide. This is accomplished by fusion with sodium hydroxide in a nickel crucible. In order to maintain a proper degree of temperature and to prevent loss of iodine by volatilization, the crucible is heated indirectly by placing inside a larger crucible, the bottom of which is covered with a layer of sand 0.5 cm. in thickness. The details of the construction of the heating apparatus have been described elsewhere (2). The supporting cylinder is 9.4 cm. in diameter and 30 cm. high; the cross bars which support the large crucible are 7.5 cm. from the top. The larger crucible is of iron and is 7.8 cm. in diameter. The smaller one is of pure nickel and is 5.9 cm. in diameter. The burner is preferably a 15.6 cm. (No. 3) Meker burner.

For the determination of iodine in thyroid preparations it is best to use not more than 0.5 gm. of material. Whether this is in the form of a dry powder, a solution, or a moist precipitate on a filter paper, the same procedure is carried out. The material is placed in a 5.9 cm. nickel crucible and moistened with a few drops of 30 per cent sodium hydroxide; 5 to 10 gm. of stick sodium hydroxide which has been broken into small pieces are added and the crucible is placed on a hot plate until the excess of water is evaporated and the contents have a thick, syrupy consistency. If but little organic matter is present, there is a tendency for spattering of fine drops during the evaporation of the excess water. If some organic substance is dissolved in the solution this spattering is prevented. A small amount of lactose is suitable and sufficient for this purpose.
For the fusion of the organic matter with the sodium hydroxide it is necessary to heat the bottom of the large crucible to a red heat. If the crucible is heated too much the fusion in the small crucible will creep up the sides and sodium hydroxide will volatilize with loss of iodine. If the large crucible is heated insufficiently the destruction of organic matter will not be complete. However, there is a wide range of temperature between the two limits and after a little experience no difficulty is encountered.

When the sodium hydroxide is first heated in the presence of water considerable foaming is produced. This, however, does not extend more than half way up the sides of the crucible. As the heating continues the foaming becomes less and after from 5 to 10 minutes the melt settles to the bottom. Bubbles continue to be given off for some time (5 minutes or so), depending on the nature of the organic matter.

When the melt has settled to the bottom and only a few bubbles of gas are being liberated, the small crucible is removed with crucible tongs and partially cooled by agitating the contents with a rotary motion. This will also remelt and carry to the bottom any particles of the fusion which have solidified on the cooler sides of the crucible.

5 to 10 mg. of potassium nitrate are now added. This oxidizes the remaining organic matter and causes a liberation of bubbles. If only a few bubbles appear, a second addition of from 5 to 10 mg. of potassium nitrate will not cause a further liberation of bubbles and the oxidation of the organic matter is complete. If the second addition of nitrate causes a further oxidation, repeated additions of from 5 to 10 mg. of the nitrate are made until no more bubbles of gas are produced by the addition of the nitrate. The melt is now poured into the shallow cover of the 5.9 crucible and allowed to cool.

The entire time required for the fusion is from 10 to 15 minutes. It is most convenient to use two crucible-supporting cylinders and to carry on two fusions at the same time.

When the melt and crucible are cool, they are placed in a beaker of from 600 to 800 cc. capacity and 125 to 150 cc. of water are added. The beaker is then placed on a hot plate. After the melt is dissolved it is transferred to a 500 cc. Erlenmeyer flask.
It should be a colorless, clear solution with a volume of about 200 cc. To the solution 5 cc. of 20 per cent sodium bisulfite and just two drops of a saturated water solution of methyl orange are added. The solution is cooled by immersing the flask in cold running water. When it is cool, 85 per cent phosphoric acid is added by allowing the acid to run directly into the flask from a pipette or syphon having a small delivery tube. The flask is vigorously and constantly shaken with a rotary motion to expel the carbon dioxide. As the indicator begins to turn pink the neutralization is finished slowly, and the addition of acid is stopped at the first definite change of the indicator to pink.

5 cc. of 20 per cent phosphoric acid and a small piece of hard coal, about 0.5 cm. in diameter, are added. The volume is adjusted to about 250 cc. and the water is boiled on a hot plate for at least 10 minutes, and longer if necessary, until the volume of the solution is about 200 cc. After the flask has been cooled in water bromine is added and the solution shaken until the bromine imparts a distinctly yellow color. This is essential, as the addition of too little bromine will prevent the subsequent determination of iodine. The flask is again placed on a hot plate and the solution is boiled, the time when all visible bromine is expelled being noted. This will require about 3 minutes of actual boiling. The solution is boiled for just 5 minutes after it is colorless. It is then removed from the hot plate, a few crystals of salicylic acid are added, and the flask is immersed in cold water. The volume of the solution after boiling should not be less than 175 cc. as the high concentration of the salts makes the end-point less sharply defined. 5 cc. of reduced 20 per cent phosphoric acid and about 1 gm. of pure potassium iodide crystals are added.

1 The sulfite and bromine destroy the color of methyl orange if only two drops are added. If more than two drops are added the solution may retain a pink color.

2 Experience has shown that practically all samples of distilled water take up small amounts of iodine. As this reducing action is destroyed by boiling with bromine this source of error is entirely controlled under the conditions of the method described above. However, a considerable error may result in the determination of iodine if any distilled water is added after the bromine has been boiled out of the solution.
The liberated iodine is now titrated with 0.005 N sodium thiosulfate. The titration is finished with the aid of the blue

3 The best strength of sodium thiosulfate for amounts of iodine ranging from 0.1 to 5 mg. is 0.005 N. The stability of the thiosulfate solution depends in large part on its manner of preparation. The most satisfactory method is to boil distilled water for at least 30 minutes, leaving the final volume about 4 liters. 5.25 gm. of sodium thiosulfate crystals are added to about 1 liter of this boiled water and this solution is boiled for about 30 minutes. The solution is cooled and made to 4 liters with the boiled water and is protected from carbon dioxide by a sodium hydroxide tube. This solution must be restandardized frequently. A convenient method proposed by Hunter is to prepare a solution of potassium acid iodate which is equivalent to a known weight of iodine. The strength of any sample of thiosulfate is readily found by titrating the iodine liberated by the acid iodate solution, which retains its strength indefinitely. The iodine equivalent of the potassium iodate is found as follows:

Prepare a 0.1 N solution of potassium acid iodate, KIO₃HIO₆, by dissolving 3.249 gm. of the salt in 1 liter of water. This solution diluted twenty times will be approximately 0.005 N. Dissolve a known weight of pure iodine (approximately 1 gm.) in 1 liter of water containing from 5 to 6 gm. of sodium hydroxide. Dilute this ten times. 1 cc. of this solution will contain 0.1 mg. of iodine. Measure 25 cc. of this solution into a 500 cc. flask and dilute to 200 cc.; add 10 cc. of 30 per cent sodium hydroxide; and neutralize as in the determination with 85 per cent phosphoric acid to a definite pink color with methyl orange. Add 5 cc. of 20 per cent phosphoric acid, a small piece of hard coal, 5 cc. of 20 per cent sodium bisulfite, place the flask on a hot plate, and boil for at least 10 minutes. Cool, add bromine, and boil as described in the determination. Add salicylic acid, cool, then add 5 cc. of 20 per cent reduced phosphoric acid, 1 gm. of potassium iodide crystals, and titrate the liberated iodine with approximately 0.005 N thiosulfate. This will establish the relation between "original iodine" and the 0.005 N thiosulfate, and from this the iodine equivalent of the potassium acid iodate can be found as follows:

Add a known volume, 10 or 15 cc., of the acid iodate solution to a 150 cc. flask. Add 0.25 gm. of potassium iodide crystals, five drops of a 20 per cent reduced phosphoric acid solution, and titrate with the thiosulfate, using starch at the end. When a small amount of iodine acid is in a solution which contains but a small amount of salts, the reaction with potassium iodide is retarded and the end-point of the titration with thiosulfate is uncertain. In the original method the volume of water present with the potassium acid iodate was about 150 cc. and to this sodium chloride was added in order to increase the distinctness of the end-point. It has been found that the salt is a source of error and that more accurate results are obtained by not diluting the iodate solution. The potassium iodide added should be reduced to a minimum, as large amounts will cause a pink-colored end-point instead of blue with starch.
starch-iodine color. For this a 0.5 per cent solution of soluble starch is recommended (3).

The most convenient procedure if a number of determinations are made each day is to use six or seven crucibles, each marked with a letter for identification. Each crucible has a corresponding 500 cc. Erlenmeyer flask marked with the same letter. The crucibles are used in rotation. Frequent blanks should be made on all reagents used, and the reduced phosphoric acid should be tested at intervals with potassium iodide.

Accuracy of the Results.

The following determinations were made with iodine in the form of potassium iodide added to water. Titrations were made with 0.005 N thiosulfate.

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<th>Amount found.</th>
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<td>1.0 mg. iodine added.</td>
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