THE DETERMINATION OF IODINE IN FOOD, DRINK,
AND EXCRETA.

BY J. F. McCLENDON.

(From the Laboratory of Physiological Chemistry, The University of Minnesota, Minneapolis.)

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The older determinations on minute quantities of iodine may
be divided into two classes: those that gave too high results,
and those that gave negative results. There is certainly room
for improvement in methods, but the method described in this
paper is believed to be the most accurate now known. The pos-
sible error depends on the percentage of iodine. The adequacy
of the method is based on two criteria: first, the yield is in direct
proportion to the size of the sample, and second, iodine added
in inorganic form is recovered.

This work was done entirely independently of that of von Fel-
lenberg who designed a somewhat similar method. It is signi-
ficant also that von Fellenberg had difficulty with losses of iodine
in ashing:

"Seit Beginn meiner Arbeiten sehe ich die grossen Schwierigkeiten, die
darin liegen, die organischen Substanzen vollständig zu verbrennen ohne
dabei Jod zu verlieren. Die Verbrennung in einem Rohr schwebte mir
auch als Ideal vor; . . . . Nun sehe ich, dass Sie das Problem gelost
haben. Wenn ich dazu komme, werde ich gerne nach Ihrer Methode Ver-
suche unternehmen.

"Ich suchte den Jodverlust bis jetzt zu vermeiden durch verhältnismä-
ssig grosse Zusätze von Pottasche. Dadurch wird aber die Verbrennung
erschwert, so dass man die Kohle meist noch zwei mal mit Wasser aus-
ziehen muss, um sie ganz zu veraschen.

"Bei den meisten Substanzen scheint mir diese Methode ziemlich sicher
zu sein; bei NaCl-haltigen Materialien, wie Harn, sind die Verluste schwerer
tzu vermeiden." (Private communication from von Fellenberg.)

1 von Fellenberg, T., Untersuchung über das Vorkommen von Jod in der

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Some points on the difficulties of ashing may be considered. Sørensen observed that the total solids of sea water could not be estimated by the method of "ashing." In 1908 I observed that on the evaporation of sea water, certain secondary reactions took place. These observations were extended\(^2\) in 1916, but perhaps all that it is necessary to mention here is that when sea water is evaporated nearly to dryness, hydrochloric acid is given off, although at the beginning of evaporation in these experiments the pH was 8.2 and this increased in value for a considerable period. Since hydrochloric acid is given off, it is probable that at a certain stage the pH is just right for the reaction between iodate and iodide to liberate iodine, according to the reaction \(\text{IO}_3^- + 6\text{H}^+ + 5\text{I}^- = 3\text{I}_2 + 3\text{H}_2\text{O}\). Furthermore, if any excess iodide (which is improbable) remained, some of it might be evolved as hydriodic acid; any remaining as iodate would be retained completely in the salt if the temperature did not exceed 110°. In evaporating sea water to retain all of its constituents, it was not evaporated to dryness, but was bottled in the wet stage. In 1920 it was pointed out that iodine in sea water might be utilized for the nutrition of the thyroid gland.\(^3\),\(^4\) It must be admitted, however, that the method described retained only part of the iodine in sea water as iodate. A better method was described in 1922, by the addition of enough soda to precipitate all the calcium and magnesium as carbonates before evaporation.\(^5\) This method was checked by determinations of iodine in sea water, and the iodine in the salt.

In the presence of much organic matter in a sample for iodine determination, however, large quantities of CO\(_2\) and other organic acids are produced in the ashing, and it is very difficult to prevent entirely the loss of iodine merely by the time-honored method of adding alkali before ashing. Furthermore, this alkali interferes with the ashing process, and since in many foodstuffs it is required to use a sample weighing several kilos in order to ob-

\(^3\) McClendon, J. F., J. Biol. Chem., 1921, xlvi, p. xxvii.
tain enough iodine for a quantitative analysis, the process of
ashing with large additions of alkali is very tedious and expensive.
Kendall\(^6\) greatly improved the technique of determining iodine
in organic matter, and his method was tried on cereals,\(^7\) but it
was found that the iodine was too low for Kendall's method, since
if the size of the sample was increased, the size of the yield was
not increased in proportion. A method was tried to reduce the
content of organic matter by the addition of yeast, then driving
off the water after making the sample alkaline, and in the com-
busion passing the fumes through an alkaline solution to catch
the iodine.\(^7\) Later developments have omitted the fermentation
but depended on the alkaline solution to prevent loss in ashing.
Many methods of ashing were tried. It was found that samples
containing relatively large amounts of iodine could be ashed in
the bomb of a calorimeter. In increasing the size of the sample,
however, up to about 25 gm., the ignition plug of the bomb blew
out. If the organic matter was small in amount as in water
residues, it would not burn in the bomb. A very heavy steel
bomb was made and heated from the outside to burn water resi-
dues.\(^8\) A number of analyses were made in this way, using a
copper-asbestos gasket to prevent the escape of iodine during
the heating. This method, however, was given up. Cereals
were destructively distilled in a steel retort, passing the gas
through an alkaline solution, but the difficulty in ashing the
remainder was almost as great as ashing the whole sample. There-
fore, the following procedure was adopted as the final method.\(^9\)–\(^14\)

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\(^8\) McClendon, J. F., \textit{J. Biol. Chem.}, 1923, lv, p. xvi.
1922–23, xx, 286.
\(^12\) McClendon, J. F., \textit{Science}, 1924, lix, 21.
1923–24, xxi, 129.
1923–24, xxi, 347.
Method of Iodine Determination in Water.

The unique characteristic of this method is the use of the combustion tube and micro colorimeter. In case of water samples it is necessary to use at least 100 liters unless the iodine content is high. This is made alkaline with the addition of sodium carbonate, sodium bicarbonate, or sodium hydroxide, and it must remain alkaline to phenol red paper during the entire period of evaporation. The easiest method of evaporation is to insert a 1 inch pipe into a barrel, place the water in the barrel and lead the pipe down into a large dish-pan into which 2 gm. of sodium bicarbonate have been placed. By means of a very large gas flame, or other heater, evaporation is quickly effected. It is not probable that any iodine is acquired from the gas since practically all the iodine in coal remains in the coke and in the ammoniacal liquids of the gas works. If any escape these, they would be caught in the lime over which the gas is passed in purification. Since ordinary coal gas contains some water gas, the carbon monoxide should reduce any iodine to iodide and prevent it from evolving in a vapor state. Another convenient method of evaporation is as follows: A zinc tank in which 100 liters could be accurately measured was rigged up with a float valve to deliver the water to the dish-pan, and attached to this float valve was a trigger to turn off the gas when the evaporation was completed. By this method the water is evaporated to about 1 liter. A considerable amount of carbonates of alkaline earths are incrusted in the pan or precipitated to the bottom. These are filtered off as it was found they never contained more than 2 per cent of the iodine. The sample is then evaporated to dryness, powdered, and placed in the boat, D (Fig. 1). This boat is best made of silica, but a sheet of nickel or even iron can be used. The boat is inserted into the silica tube, C. (A Pyrex tube may be substituted for this silica tube, but is not so convenient.) 10 cc. of a 10 per cent sodium hydroxide solution are placed in the Pyrex test-tube, B. The end, A, of the silica tube is inserted into this sodium hydroxide solution. The water-cooled stopper, F, closes the other end of the silica tube. Through a glass tube passing through the stopper, F, a stream of oxygen is passed over the sample and out through the sodium hydroxide solu-
tion. The silica tube is heated to dull redness for the shortest length of time required to burn all the organic matter in the sample. This varied with the depth of the layer of powder and the proportion of organic matter in water. If on removing it from the tube it is not completely burned, it can be reinserted and the operation repeated. In case the water cooling was not ef-

Fig. 1. Apparatus for determining iodine in water residues.
A-C = Silica combustion tube.
B = Pyrex tube containing alkaline absorption solution.
D = Combustion boat.
E = 12 cc. separatory funnel.
F = Water-cooled rubber stopper admitting oxygen into silica tube.
G = Colorimeter cups holding 1 cc. at 2 cm. depth.
H = Bausch and Lomb micro colorimeter.
fected on the rubber stopper, it sometimes caught fire. The water cooling device is simply a piece of tin or lead pipe 5 mm. in diameter inserted through one hole, looped around and passing out another hole in the rubber stopper. The passing of cold water through this tube shielded the stopper from the great heat. This shielding is not necessary in the ordinary combustion tube, but by means of this water cooling device it is possible to use tubes of much larger diameter and shorter in length, therefore more easily handled. The ash is powdered, and the sodium hydroxide solution together with the rinsings of the tube are evaporated to dryness and powdered and then both mixed in the mortar. A measured portion of water (for instance, 15 cc.) is added and the powder ground in the water so as to extract the iodides and iodates. This is filtered and an accurately measured aliquot taken (for instance, 7.5 cc.). This aliquot is neutralized with concentrated hydrochloric acid and the volume made up to 10 cc. It is then placed in the 12 cc. separatory funnel, E (Fig. 1), and 1 drop of concentrated hydrochloric acid and 1 cc. of purified carbon tetrachloride are added and the funnel is shaken. If any pink color appears it denotes that iodate as well as iodide is present in the ash. 1 drop of 0.1N arsenous acid is added and allowed to remain for 20 minutes. This is to reduce any excess iodate that might be present. 1 drop of nitrosyl sulfuric acid is added to oxidize iodide to iodine and the separatory funnel shaken hard for 2 minutes to extract the iodine. The carbon tetrachloride is allowed to run into a glass-stoppered bottle and centrifuged for 1 minute to remove any water droplets. It is then transferred to one cup of the Bausch and Lomb micro colorimeter, H, and into the other cup is placed carbon tetrachloride, 1 cc. of which contains 0.1 mg. of pure iodine. This micro colorimeter has the advantage of measuring very small amounts of iodine, since 1 cc. of carbon tetrachloride fills the cup to the depth of 2 cm. The iodine extracted is determined colorimetrically and another cubic centimeter of carbon tetrachloride added to the separatory funnel and another extraction made and determined in the colorimeter. By repeated extractions practically all of the iodine may be recovered and measured and the results of the different extractions added together to determine the amount in the aliquot, and knowing the relative volume of the
aliquot, the iodine in the total sample may be calculated. It was possible to obtain a fair degree of accuracy with a single extraction on the assumption that about 80 per cent of the iodine in the separatory funnel was extracted from 10 cc. of an aqueous solution by 1 cc. of carbon tetrachloride. The partition coefficient of iodine between these two solvents varies with the electrolyte content of the water. In some cases as high as 86 to 87 per cent of the iodine was obtained in the first extraction. Under uniform conditions a factor can be determined for this partition coefficient and one extraction is all that is necessary.

It is always possible to have the water phase saturated with NaCl, and if other electrolytes are small in amount, the conditions approach uniformity.

Carbon tetrachloride contains reducing substances which must be removed. The simplest method is to take an entire drum of the commercial product, place it in a large glass vessel in the sunlight and add bromine to it as this bromine is decolorized, for several days or until it will remain for several hours without diminution in the bromine color. The excess bromine must be removed in some way as by shaking the carbon tetrachloride with an alkaline solution. After this it is washed with pure water several times or continuously by inserting a tube to the bottom and allowing pure water to bubble up through it. The water is then separated off, and plaster of Paris added to absorb the remaining water. It is then filtered through paper and distilled. It is usually found that some water and other impurities come off in the first portion of the distillate, and this is rejected. It is also necessary to stop the distillation before the end to guard against the effect of excessive heating on non-volatile impurities. Carbon tetrachloride is stored in the dark.

Iodine is purified by mixing it with KI, subliming it, then re-subliming it, and drying in a desiccator. With this iodine dissolved in carbon tetrachloride that has been treated in the above manner, the color gradually changes from a pink to a yellowish. This yellowish substance is apparently a compound of iodine and chlorine, but whatever be its nature, its development must be prevented. This is most easily done by overlaying the iodine solution in carbon tetrachloride with a layer of water containing 1 drop of nitrosyl sulfuric acid in 50 cc. and containing iodine at
the same solution tension as that in the carbon tetrachloride. If iodine in carbon tetrachloride is sealed in glass tubes, the color disappears entirely in a few minutes and cannot be brought back by shaking with oxidizing or reducing solutions, but if after placing the carbon tetrachloride solution in the tube, it is overlaid, as is directed above, with an aqueous solution, the sealing can be effected without bleaching of the iodine. The reason for this is probably that the vapor of carbon tetrachloride is overheated during the sealing and the reaction products react with

Fig. 2. Apparatus for combustion of large quantities of organic matter preliminary to iodine determination.

A = Water-cooled rubber stopper.
B = Silica combustion tube, 10 cm. bore.
C = Absorption apparatus containing alkaline solution.
D = Cooling coil.

the iodine, whereas the overlying aqueous solution prevents the evaporation of carbon tetrachloride and prevents any of its vapor from being overheated. Directions for preparing the reagents may be found in Treadwell and Hall.15

Determination of Iodine in the Presence of Large Quantities of Organic Matter.

This method is the same as that described above with the exception of the first ashing process. It is sometimes necessary to use several kilos of foodstuff in order to obtain 0.01 mg. of

Food is placed on a piece of sheet iron in the large silica tube, B (Fig. 2). This tube is 10 cm. bore and more than a meter long. The length, however, could be greatly reduced. In the smaller end of this silica tube is inserted a double coil of tin or lead pipe, D, through which cold water is circulated. 1 liter of 10 per cent NaOH solution is placed in the Pyrex absorption vessel, C, and this is adapted over the downwardly directed end of the silica tube containing the cooling coil. It is practically necessary to have a notch or separate opening at the top of the absorption vessel, C, through which the cooling tubes can pass. A separate opening can be made air-tight by means of a double bored rubber stopper which is either passed downward over the free ends of the cooling tubes or is fitted around them by splitting it. The opening between the silica tube and the vessel, C, is closed by means of a wet mass of asbestos fibers. By means of a side neck on the vessel, C, connected with a rotary exhaust pump, a very rapid air current is sucked out of the silica tube and through the alkali solution. At the same time, cold water is passed through the cooling coil. Oxygen is allowed to spurt into the open end of the tube, B. By means of a gas flame the material in the tube, B, is ignited. The combustion is always very rapid and it is necessary to use a great deal of oxygen in order to accomplish complete combustion. This necessitates a very rapid action of the air pump in order to draw out all the products of combustion through the alkaline solution. It is then difficult to get complete absorption and it is practically necessary to insert an additional absorption vessel between the vessel, C, and the air pump. The most rapid portion of the combustion process is usually completed within half an hour. If it is desired to slow up this process the sample may be divided and only part of it inserted at a time. In case of a liquid like cod liver oil it is impossible to get complete combustion if the whole sample is placed in the tube at once. The only practical method is to drop or spray it into the tube during the combustion.

During the earlier experiments the air pump was not used, but the large end of the silica tube was closed by means of the water-cooled rubber stopper, A, and the pressure of oxygen from the tank was depended on to force the products of combustion through the alkaline solution. It was necessary to ignite the sample after inserting the rubber stopper either by means
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of electric wires or by means of a gas flame inserted through a little hole closed by a silica stopper. If the oxygen was not forced in with sufficient rapidity, an explosion was liable to result which would blow out the rubber stopper; therefore, the suction method was substituted. The mixing of a little air with the oxygen is not at all objectionable.

After the volatile products are consumed the carbon is burned more slowly and it is then necessary to apply external heat to the tube. If the ash is reduced to relatively small volume it is sometimes preferable to stop the combustion in this large tube and grind the ash to a powder and complete the combustion of the remaining particles of carbon in the smaller tube used for water residues. The sodium hydroxide solution and rinsings of the tube are evaporated to dryness and usually contain some unburned organic matter. This may be burned in the smaller tube used for water analysis. The remainder of the analysis is the same as that for water analysis given above except that the large volume of ash may require a larger portion of water for extraction and the aliquot transferred to the separatory funnel may necessarily be a smaller fraction of the total. It is very desirable to have a small ball mill for grinding the ash with water.

If only a few analyses are to be made it is possible to use a Pyrex combustion tube. The cracking of the Pyrex tube where the hot portion reached the alkaline solution was avoided in a number of experiments by wrapping a section of the Pyrex tube with a thin sheet of asbestos paper, then winding around it about fifteen turns of small lead pipe through which cold water was flowing during the whole process of combustion.

It is easily possible to detect 0.001 mg. of iodine in 1 cc. of carbon tetrachloride and make a roughly quantitative determination of it in the micro colorimeter. It is desirable, however, to have 0.01 mg. of iodine or a greater quantity in the sample used for analysis. Sometimes there is a slight yellowish tinge to the carbon tetrachloride used in extracting the iodine. It is sometimes possible to make a determination even in this case by transferring this carbon tetrachloride into another small separatory funnel together with 1 cc. of water and 1 drop of 0.1 N sulfurous acid. On shaking, all the iodine will pass into the sulfurous acid as iodide. The carbon tetrachloride is now withdrawn, and about one-tenth of a drop of nitrosyl sulfure acid
added together with 1 cc. of pure carbon tetrachloride. On shaking, the iodine will now pass into the carbon tetrachloride and it may happen that the yellow color does not develop, but only the pink color of the iodine remains. Much trouble that might arise in the determination may be attributed to failure to obtain complete combustion of organic matter, and if the determination is repeated and care taken that the final alkaline solution used in absorption is free from organic matter, the analysis will usually be normal.
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J. F. McClendon

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