# DETERMINATION OF IODINE IN BLOOD

# THE DETERMINATION OF IODINE IN BLOOD.

BY

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THESIS

submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the Degree of Master of Science.

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McGill University,
Montreal, Canada.

September 5, 1936.

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# THE DETERMINATION OF IODINE IN BLOOD

The important part that iodine plays in the body economy has long been appreciated. Definite proof of this was established in 1914 when Kendall separated from the thyroid gland an amino acid, thyroxin, which contains 65% iodine. Since that time interest in the metabolism of iodine has greatly increased, and studies of its relationship to thyroid function have been numerous.

Analyses of the thyroid gland for iodine have been successfully carried out for many years. The distribution of iodine compounds, and the thyroid hormone, in other tissues has been considerably more difficult to determine, but a knowledge of it is essential to an understanding of the health and disease of the thyroid. Blood analyses especially have been unsatisfactory, by reason of the great difficulties of estimating ten millionths of a gram (ten micrograms) of iodine per 100 ml. of that tissue.

The following study was made with a view to examining the methods of analysis for iodine in blood and, if these proved unsatisfactory, developing the one which showed the greatest possibilities.

## I. HISTORICAL INTRODUCTION

The determination of minute amounts of iodine (by other than spectroscopic methods) can be carried out only when the material examined is free of certain interfering substances, both organic and inorganic. Blood is characterized by an extremely high ratio

between organic material and iodine, and this factor is reflected in the laborious methods of analysis. The removal of inorganic substances is less demanding of chemical manipulations, and often may be accomplished with the separation of organic materials.

For purposes of discussion each method of analysis may be divided into two stages. The first of these involves the destruction or removal of interfering substances in preparation for the final stage of actual estimation. The individual methods consist of various combinations of specific techniques for accomplishing these two stages.

First stage of Analysis--Ashing.

## I. ALKALINE ASHING IN AN OPEN VESSEL

The earliest method for the analysis of iodine in organic matter is that of Rabourdin (67) published in 1850. Destruction of organic matter was secured by ashing with sodium hydroxide and potassium nitrate, following which free iodine was liberated by the addition of sulphuric acid and sodium nitrite or potassium dichromate. The liberated iodine was removed by chloroform, carbon disulphide, or carbon tetrachloride, and subsequently estimated colorimetrically. Since this original method of purification was reported there have been few radical departures from it. However, the number of minor changes is considerable.

Rabourdin and other early investigators of iodine were accustomed to work with relatively iodine-rich materials. Early analyses of blood gave no constant result. In 1900 Gley and Bourcet failed to detect iodine in blood samples of 100 and 200 ml.

Using a liter they detected 1.3 to 11 micrograms per hundred millilitres.

The first method adequately adapted to the analysis of blood was developed by Hunter (28) in 1910. It was originally described for the analysis of thyroid gland and detected as little as 10 micrograms of iodine. Hunter overcame many of the difficulties of the earlier methods, particularly that of Baumann (4) who carried out oxidation by fusion with sodium hydroxide, and subsequent oxidation of the char with potassium nitrate. In place of this process which was tedius and disagreeable Hunter used anhydrous sodium potassium carbonate with potassium nitrate. He heated the mixture of oxidant and blood in a crucible with a Bunsen burner to a bright red heat. Ignition was followed by filtration and estimation of iodine.

Since the publication of Hunter's method there have been numerous variations of it described. A year later Kendall (34) published a modification that has been extensively used for biological material, but particularly thyroid tissue. In 1920 Kendall modified it for the determination of iodine in 100 ml. of blood. Three grams of sodium hydroxide were used as a flux. Following ashing, the fuse was extracted with water and barium hydroxide was added. This regenerated the sodium hydroxide which had been converted to carbonate. The barium was then precipitated with sulphuric acid, the solution filtered, the filtrate evaporated in a specially constructed hot air chamber and subsequently fused. The lower limit of detectability for this method

was approximately 5 micrograms.

About the same time Blum and Grutzner combined alkali ashing with barium peroxide oxidation. The barium was subsequently removed by saturation with carbon dioxide and filtration. Potassium pemanganate was added to the alkaline filtrate and boiled. Complete destruction of organic matter and also oxidation of the iodide to iodate for subsequent estimation was thus carried out. At least 150 ml. of blood were needed for these estimations and results were variable.

## II. ALKALINE ASHING IN A CLOSED SYSTEM

The next advance was made a few years later by McClendon (49), who introduced ashing in a closed system in an atmosphere of oxygen, the fumes being passed through a solution of sodium hydroxide which recovered any escaping iodine. Several changes in the ashing were later proposed by McClendon (51) and others. The material (tissue or blood) was dried and mixed with calcium oxide and introduced into a heated silica tube. Collection of the ignition vapours in sodium hydroxide in wash bottles and the use of a Cotrell precipitator were recommended. After pooling the material (now almost completely inorganic), evaporation and extraction of the resulting thick paste with alcohol reduced the volume and impurities considerably. The complexity of this ashing process decreased the value of the method. Consequently in his last paper (71) McClendon discarded the precipitator and the silica tube. An oxy-hydrogen blow torch was used in an atmosphere of oxygen and the vapours passed over a glowing platinum wire, into sodium bisulphite and sodium hydroxide

solutions, and through an alundum filter. In this way nitrite, from the oxidation of atmospheric nitrogen, free iodine and smoke were absorbed.

Ashing in a closed system was also used by others. Schwaibold (78) devised a heated silica tube 95 cms. long. The material to be analyzed was dried, and placed in a boat in the tube. Oxygen was introduced at one end and at the middle of the tube. Fumes were passed into alkali. This method was especially adapted for ashing large amounts of material which are difficult to ignite. Widman (86) modified it by the absorption of organic iodine in carbon disulphide. He claimed that otherwise this iodine was lost. His recoveries of iodine from 100 ml. of blood were thus approximately 32 micrograms instead of the usual 10-12 micrograms.

A closed method for the estimation of iodine in 5 ml. of blood was described by Elmer (12). The sample was first dried and carbonized with 1 ml. of 50% potassium hydroxide on a sand bath at 400-500$^{0}$ C. It was then pulverized and placed in a boat which was introduced into an ignition tube (30x2.5 cms.). Heat was applied and a stream of oxygen passed through. The vapours were not collected, as, according to the author, only a negligable amount of iodine was contained in them. The residue from ignition was then extracted with alcohol. The determination could be completed in 2-2½ hours.

Of the closed methods the most recent is that of Karns', and its modification by Von Kolnitz and Remington (39). The dried

organic material is ignited in a spherical globe into which oxygen is passed. The advantages over McClendon's method are slow ignition, less expensive apparatus, and the use of large amounts of material.

Almost identical, but somewhat simpler, is the method described by Baumaun, Matzger and Baldauf (3) for burning in oxygen. Absorption is by two Drexel wash bottles and a smoke condenser, consisting of two funnels placed mouth to mouth with several layers of filter paper between. Before being burned the blood must be dried in an oven at 80-85° C. for 6 hours. Determinations on many samples of the same blood specimen will probably yield a variation of ±10-15%.

The method which has been used most extensively, and also copied by many workers, is that of Von Fellerberg (16), first described in 1923, and later adapted to various materials. This author described his most accurate method for blood in 1930 (17). Ten ml. of oxalated blood are mixed with one ml. of saturated potassium hydroxide solution and are dried in a nickel dish (8.5x2 cms.). Roasting in a muffle furnace is carried on at a red heat. This is followed by filtration. The carbon containing residue is re-ashed in an ignition tube, re-extracted with water, and the filtrate added to the first. After evaporation over an open flame the residue is heated in a muffle furnace, finally made up into a thick paste with a little water, and repeatedly extracted with 95% alcohol. Evaporation, re-ignition in a furnace, and re-extraction completes the ashing process. The error in determination is approximately ±15%.

An ingeneous ashing process has been described by Middleton (53). To the material for analysis in a nickel crucible is added an excess of sodium carbonate. This crucible is then inverted in a larger one, and sodium carbonate filled into the space between. Heat is then applied with a Bunsen flame.

In each of these methods before the iodine may be set free acidification is necessary, and if most of the alkali has not been removed (as by alcohol extraction) a considerable bulk of material must be manipulated. Sheffer (77) reduces this amount by neutralizing the potassium hydroxide with ammonium carbonate. Ammonium hydroxide is then removed on a water bath.

Turner (85), in a method comprising alkaline ashing in oxygen and alcohol extraction, introduces, also, a means for removing organic chromatic substances. To an iodine containing filtrate (less than 40 ml.) are added 2 ml. of saturated potassium sulphate and 5 ml. of 10% barium chloride. Centrifuging removes the barium sulphate leaving a clear fluid. Turner's method is adapted to the analysis of 10 ml. blood with an error of 10-15%.

## III. ACID ASHING

During the years when von Fellenberg's method was being perfected several attempts at acid destruction of organic matter were described. In Pfeiffer's method (61), first published in 1928, the oxidation is carried out in a special flask with sulphuric acid and 30% hydrogen peroxide.

The fumes evolved in the process are passed into potassium hydroxide solution. With some materials (e.g. blood) this treatment fails to destroy fatty acids. This difficulty is overcome (63) by the use of a quartz tube containing platinum gauze. The tube is heated to 900° C. and complete destruction of organic material is reported to take place. Other methods of eliminating this difficulty have also been devised.

Equally complicated and very similar to Pfeiffer's apparatus is that used by Eyckerman (14) for oxidation with sulphuric acid and 30% hydrogen peroxide. Ten to fifteen ml. of blood are required and uniform results given (on the basis of five determinations).

Also similar in principle, but slightly different in construction, is the apparatus used by Glimm and Isenbruch (24). Analyses require 10 ml. blood.

It may be noted here that wet ashing with sulphuric acid and hydrogen peroxide in the hands of these and other workers, with the exception of one, has produced results uniformly higher by several micrograms per 100 ml. than those given by other methods.

Acid oxidation of organic matter by means of fuming sulphuric acid, potassium persulphate and copper sulphate (catalyst) is recommended by Willard and Thomson (87). The halogen is distilled into alkaline arsenite solution.

Sulphuric acid and chromic acid are used by Leipert (44) as the oxidixing agent, and he recommends cerium sulphate as

catalyst. These reagents are heated with the blood, and all iodine is changed to iodate which does not escape. Following oxidation arsenious oxide is added to complete reduction of chromate and iodate. The iodine is then distilled into sodium hydroxide solution with steam. Samples of 20 ml. of blood are used for analysis. The complete process is carried out in an all glass apparatus.

A modification of the above method has been described by Trevorrow and Fashena (84) who use almost identical apparatus. After attempting to repeat Leipert's work they "became convinced that there were many sources of error in the method which made it undependable and therefore useless," a not uncommon statement in the literature about any iodine method. The chief difficulty lay in the transfer of arsenic, chromyl chloride and acetic acid into the distillate, with subsequent interference with estimation. These difficulties were overcome by removing chromyl chloride and acetic acid by boiling before distillation, and by substituting phosphorous acid for arsenious oxide. Minor changes in the apparatus were initiated. Estimates are made on samples of 15 ml. with an approximate variation of 15%. (Adequate figures are not given.)

A year before the publication of Trevorrow and Fashena, McCullagh (52) developed a method which included both alkaline ashing and acid distillation. Twelve ml. of saturated potassium hydroxide are fused with 10 ml. of blood, first over a Bunsen flame and then in a muffle furnace, the tempera-

ture finally being raised to 360°C. The fuse is then made into a paste with a little water and extracted with 65 ml. alcohol in five portions. After evaporation of the alcohol (made alkaline with 1 ml. saturated potassium hydroxide) the residue is fused in the furnace at 385°C.for 10 minutes. Originally a stream of oxygen was passed into the furnace, but this has since been found unnecessary (personal correspondence). This completes the ashing process. The material is then filtered into a 500 ml. Claisen flask and sulphuric acid, hydrogen peroxide and ferric sulphate added. The distillate is collected in acid sulphite in a Fresenius flask. About 175 ml. of water are distilled over. Subsequently this is neutralized, evaporated and iodine estimated by the Dupre-Winkler method. McCullagh's recoveries of added iodine would seem to vary approximately ±10% but a greater number of estimations on one sample would be desired.

## SECOND STAGE OF ANALYSIS--ESTIMATION

The second stage of analysis, namely estimation of iodine may be begun once the material has been purified and concentrated. This may be done either colorimetrically or titrimetrically, both of which methods have been well established on a macroscopic scale for many years.

### I. COLORIMETRIC METHODS

Rabourdin (67) in his classical method, estimated the iodine colorimetrically. Following the ashing process sulphuric acid and either sodium nitrite or potassium dichromate

were added. The free iodine thus liberated from iodide (in which form all of the iodine is assumed to be before oxidation) is taken up in chloroform, carbon disulphide or carbon tetrachloride, and the amount of iodine may be established by comparison of the color with a series of solutions containing varying amounts of iodine. The different phases of this process and the factors affecting them have been studied by many workers.

(a) Preliminary Reduction of Iodates.

This method of estimation is based on the assumption that all the iodine is in the form of iodide when the oxidizing agent is introduced, an assumption that has been questioned by several workers. In order to make certain of this McClendon (50) has recommended that arsenious acid be added before the introduction of nitrate or other oxidant. In this way iodate, which would otherwise remain as such, is reduced and then subsequently oxidized to iodine. The same thing has been accomplished by Lange and Ward (43) by the use of hydrogen sulphide in the analysis of water. For the same purpose Reith (69) uses azite, and Baumann, Nietzger and Baldauf (3) sodium bisulphite.

(b) Production of Free Iodine.

At the completion of ashing in nearly all the methods, the iodine is in the form of an alkyl iodide. Before colorimetric estimation this is oxidized to free iodine. Oxidation takes place in acid solution and many reagents have been used

for this purpose. Rabourdin (67) originally made use of sodium nitrite or chromic acid, and many have used these oxidants, more especially the first. McClendon (49) at first used sodium nitrite and took up the free iodine in carbon tetrachloride.

A variety of other oxidizing agents has been used. McClendon later recommended the use of nitrosyl sulphuric acid. Remington, McClendon and von Kolnitz (70) used phosphoric sulphurous acids for that purpose. In acid solution potassium permanganate releases iodine and Rupp and Horn (74) have made use of this. Winterstein and Herzfeld (88) together with other workers (10, 43, 47) have used acid hydrogen peroxide.

(c) Organic Liquids for the Detection of Iodine.

The use of organic liquids for this purpose depends on two factors, the greater solubility of free iodine in them than in aqueous solutions (favourable partition coefficient), and the property of giving a marked color with free iodine. The partition coefficients have been determined for all the liquids commonly used, those for chloroform, carbon tetrachloride and carbon disulphide being 131, 385, and 590, respectively. In these solutions the limit of visibility of the red-violet color is greater than that of even the starch iodine complex. Kolthoff (40) reports that with an end volume of 50 ml. he was able to detect $4 \times 10^{-5}$ N iodine in 10 ml. of chloroform.

(d) Indicators for Colorimetric Determination.

Organic solvents have not alone been used for the colorimetric determination of iodine. Early attempts were made to use the starch iodide reaction for this purpose. Results have been very disappointing, however, and starch has been discarded for this purpose. (71) Lately, however, Woodard (89) reported that the greatest source of error with starch is due to the dissociation of the starch-iodine complex. He gives a series of correction factors. The effect of the concentration of starch, potassium iodide, and of the presence of contaminating acids and salts were also investigated.

Histological methods have used both platinum and palladium for iodine detection. Platinum has had a very restricted use in colorimetric determinations. Krauss (41) utilized the color developed by the addition of palladium chloride in an acid solution of acetone, alcohol, and water. He records that in volumes of 2-5 ml. as little as 0.1 microgram may be detected and estimated in a colorimeter. Lucas (46) has also used palladium.

(e) Interfering Substances.

According to Maljaroff and Matskiewitsch (48) sodium sulphate and magnesium sulphate in concentrations up to 200 g. per litre do not interfere in the colorimetric determination with chloroform. However, errors were introduced with concentrations of sodium chloride, calcium chloride and magnesium chloride above 15, 12.5, and 10 g. per litre respectively.

(f) Precautions.

Maljaroff and Matskiewitsch (48) have examined the various factors in the colorimetric determination using chloroform. They point out that volumes of the solvent and of the solution under examination must be constant for all tests and that the volume of chloroform must not be too small. If the volume of chloroform is one-fourth that of the test solution a theoretical error of 3% is introduced as a result of the partition coefficient of 131 . The method can be used for concentrations as low as 0.125 microgram per millilitre.

## II. TITRIMETRIC METHODS

(a) Production of Free Iodine.

At the beginning of the estimation all the iodine is in the form of iodide. Estimation consists of quantitative change to free iodine and subsequent titration with a suitable indicator. The change to free iodine may be accomplished either by simple oxidation with a reagent such as

nitrite, or according to the Dupre-Winkler method by oxidation first to iodate, and then by interaction with added iodine to release six times the amount of iodine originally present, according to the following equation. -

$$6H^{+} + 5I^{-} + IO_3^{-} \longrightarrow 3H_2O + 3\ I_2\ .$$

(I) Simple Oxidation.

This type of oxidation has played a relatively minor role in titrimetric estimations of small amounts of iodine. The various means employed to secure oxidation have already been discussed. (Page11). It may be pointed out, however, that when a titrating fluid is to be added it is necessary to prevent interaction with the oxidizing agent. Thus, in the method of Norris and Rao (57) the iodine is released with nitrosulphuric acid, taken up in carbon tetrachloride, which is washed in water, freed from sulphuric acid with sodium acetate, and titrated. Losses may occur at several points in this process. A similar method is used as an alternative to the colorimetric one by Pfeiffer (61), and by Lenz (45. Rupp and Horn (74) have oxidized an acid solution to free iodine with potassium permanganate which before titration is decomposed with oxalic acid.

(II) Dupre-Winkler Method.

Of prime importance in the estimation of minute quantities of iodine has been this method whereby one titrates six times as much iodine as was originally present.

Oxidation of iodide to iodate may be carried out by means of a large number of reagents. Hunter (29) used

chlorine in 1910. Hypochlorites were recommended by Kendall (33) and others (22). In order to prevent the oxidation of added potassium iodide it is necessary to remove all oxidizing chlorine compounds. This is usually accomplished by boiling. The danger of incomplete removal has been stressed by many workers (76) and has led to the use of other reagents, and particularly bromine. As in McCullagh's method a small drop of bromine is added to the fluid to be examined (10 ml.). The flask is shaken to color the solution yellow and boiled to a small volume for titration. During this boiling all the bromine is removed, and the iodate is concentrated.

An alternative oxidation to iodate is that of Bellucci and Vigni (5) and others (38) whereby potassium permanganate in alkaline solution is used.

The reduction of the iodate is elicited in acid solution by the addition of a minute crystal of iodide. Sandusk and Ball (76) report that the hydrogen ion concentration of the solution must be over pH 2 for the reaction to go to completion.

(b) Titrating Reagents.

The titration of free iodine could theoretically be carried out by all reagents which reduce free iodine to iodide. In practise only a few of these have been used. By far the most important is thiosulphate, which is used as the sodium salt. Reaction proceeds with the formation of iodide and tetrathionate. Kolthoff (40) points out that with re-

duced acidity an iodine solution may oxidize the thiosulphate to sulphate. The error thus induced becomes particularly large in the titration of very dilute iodine solutions. This is avoided by increasing the acidity. In the micro methods described here, and particularly in the Dupre-Winkler method the acidity is well above the danger level, since a pH of 2 is required for the oxidation of iodide to go to completion (76).

(c) Determination of end point

(I.) Organic Solvents

The organic solvents which have been used extensively in colormetric work may also be used in titrimetric estimations. In general these solvents have been replaced but as late as 1934 Bellucci and Vigni (5) have recommended the use of carbon disulphide. Pfeiffer recommends (61) the use of chloroform, and Norris and Rao (57) carbon tetrachloride.

(II) Indicators.

At an early stage in the history of iodine analyses starch was used as an indicator of free iodine, and it is still the most common one. According to Kolthoff (40) its sensitivity depends on several factors, of which salt concentration, acidity, the presence of organic substances, and temperature are the most important. Iodides are necessary for the blue color and this factor is optional at $4x10^{-5}$ N. in iodide. Concentration below this decrease the sensitivity, a fact which Allot, Dauphinee and Hurtley (1) have emphasized. With inadequate concentrations a dirty

purple is given instead of a clean blue. They also stress that an acidity of 0.002N. to 0.05N. is most satisfactory since excessive acid leads to oxidation of iodide. With regard to temperature Sandusk and Ball (76) report that the sensitivity at 17°C. is twice that at 31°C. Titrations are thus best carried out in cold solutions. The presence of salts, and organic matter are detrimental to good color production. Under the best conditions starch is sensitive to about 1 to 2 micrograms per millilitre.

In addition to starch many other indicators have had a restricted use. Erdmann has used the yellow coloration of mercuric chloride but this is probably unsuitable for minute amounts of iodine. Lucas (46) reports that at a dilution of $1:4x10^{-16}$ in which starch and chloroform are negative iodine gives a dark yellow color with palladium chloride. This reaction is not specific. Bromine also gives it. Stanek and Nemes (82) have recommended sodium nitroprusside. Praesodymium acetate has also been suggested (37).

Organic indicators have been used to some extent. Lange and Ward (43) in analyzing water use o-tolidine which they claim gives a blue green color with less than one microgram per millilitre. Reith (68) has reported that alpha-naphthoflavone gives a color reaction five times as sensitive as starch. It indicates two micrograms per millilitre. Corrections must be made for potassium iodide concentrations. Basic lanthanum is also a sensitive indicator for iodine (37). Dubsky and Trlilek (11) recommend diphenylcarbazone as an

indicator in the mercurimetric titration on account of the intense violet color formed with mercuric ions.

III. Potentiometric Determination.

The potentiometric delection of iodine was early applied on a macro scale. In 1925 Popoff and Whitman (66) studied the potentiometric standardization of solutions used in iodometry.

Faulk and Bowden in 1926 (20) described a "dead stop end point" which is more sensitive than starch. It depends on the use of so low a potential (10-15 mv.) between two platinum electrodes that the back potential of polarization balances it. This is the condition at the end point, i.e. no deflection on the galvanometer. Free iodine, however, behaves as a depolarizer with the result that there is a flow of current. Thus, in practise, titration is continued till the galvanometer ceases to record.

Hahn and Weiler (27) describe an electrometric method and recommend the addition of carbon tetrachloride to prevent side reactions and to secure a sharp end point.

Muller and Gorne (56) recommended a potentiometer for the accurate determination of the end point in the titration of iodine by stannous chloride. Shchukarev and Suisoev (81) described a more sensitive detection than by starch. It depends on the effect that free iodine has on two platinum electrodes dipping into a solution of 0.1 N. potassium iodide which is placed between the poles of an electro-

magnet. The iodine is considered to act as depolarizer. By potentiometric titration with silver nitrate, Tomicek and Jansky (83) are able to detect as small concentrations of iodide as one microgram per millilitre, in the presence of chloride and bromide. Flatt and Boname (18) also have a method for titrating iodine with potassium permanganate using a potentiometer. For titration in a very small volume of solution (as little as one drop) Schwarz (79) has a potentiometric method which can be applied to the titration of iodine with thiosulphate.

Paol and Motz (59) have developed a rather complicated electrometric method for use with 20 ml. of blood. Added iodine is estimated with less than 10% error.

IV. Photoelectric Determination.

King and Jacobs (37) have reported a very sensitive apparatus for detecting free iodine. It consists of a photoelectric cell and a balanced galvanometer. They use it with water and report that it is more sensitive than starch, basic lanthanum or praesodymium acetate. It will probably detect 0.02 micrograms per millilitre.

(d) Removal of Interfering Substances.

Interference takes the form of reaction with iodides, iodates or thiosulphates. Most important of the interfering substances are those that oxidize iodides to iodine.

Certain materials may contain small amounts of iron. Ferric ions will oxidize iodide to iodine, and also exert a catalytic influence on the oxidation of iodide by oxygen from the atmosphere. Methods applied to blood which include alcoholic extraction have as a result little iron present, as also have those employing distillation (14, 24, 52). Sandusk and Ball (76) recommend acidification with phosphoric acid when iron is present, since ferric ions are thus reduced to a minimum. However, the paler color that starch gives in the presence of phosphates is a disadvantage. With large amounts of iron special precautions have to be taken (90).

If the ashing has been facilitated by the addition of nitrate, nitrites are very likely to interfere by oxidation of iodides. The use of azite for their removal, as recommended by Reith (69), is probably the best. Its use has been recognized by other workers (70). Sodium bisulphite (35), and zinc in alkaline solution (80) have also been used. Boiling with urea in acid has frequently been recommended (5, 26) and boiling with acetic acid (21, 15) has been reported. In a discussion of this subject von Os (58) points out that when nitrates are present the use of chloroform for extraction may lead to difficulties. This he attributes to the ethyl alcohol which may be present as an impurity, becomes nitrated, and subsequently liberates iodine from the sodium iodide formed during titration.

Potassium permanganate is another substance which causes difficulty when present. It may be destroyed in either alkaline or acid solution. In the alkaline state boiling with ethyl alcohol has been widely used (6, 7, 38), and one worker has employed charcoal (64). In acid, nitrate (26), hydrogen peroxide (2) or oxalic acid (14) have been suggested for removing it.

For the removal of bromine and chlorine, added in the Dupre-Winkler method, boiling was recommended at first but its use was criticized by many workers. In this respect, chlorine presents great difficulties. Besides, such reagents often contain chlorates (54). Kendall (33) recommends the addition of phenol which reacts with free chlorine but not iodine. Similarly phenol has been used widely for the removal of bromine (25, 54). Care must be exercised to add phenol in one portion, otherwise tribrom phenol or tribromphenol bromide, both of which oxidize iodide, may be formed. Salicylic acid, although of fairly general use (35) for the same purpose, has been condemned (25, 54) because it is less efficient than phenol, and also acts as a reducing agent for iodine (76). Formaldehyde (30) and xylene (55) have each been used, and sodium cinnamate, maleic acid and formic acid reported unsatisfactory (54).

Atmospheric oxygen will oxidize iodides if the acidity of the solution is high, even in the absence of iron. Sandusk and Ball (76) contend that above pH 1 this oxidation is sufficiently rapid to cause inaccuracy.

## III. CATALYTIC METHODS

The catalytic method of determining small amounts of iodine has been suggested by several workers. Lang, (42) in a study of the reaction between arsenious acid and potassium permanganate, reported that iodine compounds were excellent catalysts and that simple proportionality might exist between rate of reaction and catalyst concentration.

Sandell and Kolthoff (75) have studied the reaction between arsenious acid and ceric salts. The end point is indicated by a disappearance of the yellow color due to ceric ions (and may be made more pronounced by the use of o- phenanthroline ferrous sulphate). They report a procedure which will determine with 20% accuracy quantities of iodine ranging from 0.001 to 1 microgram in dilutions of $1:10^7$ in the presence of $10^5$ times the amount of chloride and bromide.

## IV. X-RAY METHOD

Codino (9) has reported a method applicable to urine which depends on the absorption of x-rays by iodine containing solutions.

## V. SPECTROMETRIC METHOD

Dr. J.S. Foster personally has suggested to me that analysis of iodine in blood in amounts of the order of 10 micrograms would probably be possible with an accuracy of 15-20% using a technique similar to that for lead (19).

## VI. CHRONOMETRIC METHOD

Riegler (73) has developed a chronometric method for urine which depends on the reaction between ethyl aceto-acetate and free iodine. A formula is given.

## VII. NITROMETRIC METHOD

Riegler (72) has estimated as little as 10 micrograms of potassium iodide by oxidation to iodate, reduction with hydrazine, and measurement of the nitrogen evolved. The very small amounts of iodine in blood make it inapplicable.

## II. EXPERIMENTAL INVESTIGATION

The following experiments were commenced in order to perfect a method of iodine analysis for use in research and clinical laboratories. Of the methods described that of McCullagh (52) seemed the most promising. An attempt was therefore made to repeat McCullagh's work. When the desired accuracy was not attained by the use of his method attempts were made to improve it.

(1) Repetition of McCullagh's Work.

Attempts to duplicate McCullagh's results by his method were unsuccessful. From early experiments it seemed that the difficulties were chiefly those of ashing and titration. The description of ashing that McCullagh gives is incomplete, important details being omitted. In connection with titration, it was found that a personal idiosyncrasy made McCullagh's method unsatisfactory. Experiments conducted to overcome this difficulty are described in the section on

"Modification of Titration".

During the period of research, an opportunity was presented for visiting McCullagh's laboratory in Cleveland, and carrying out analyses under his direction. Several variations from the original description of the ashing process were observed. Consequently the following outline of ashing was prepared at McCullagh's request.

"The most difficult part of McGullagh's method of iodine analysis is probably the destruction of organic matter with potassium hydroxide. As recorded, 10 ml. of oxalated blood, and 12 ml. of saturated potassium hydroxide are heated in a 250 ml. nickel crucible over a Bunsen flame. The temptation at this point is to excessive heating. The blood alkali solution should be gently boiled. Two crucibles may be manipulated in one pair of tongs and the fuse cautiously swirled up on the sides of the crucibles. As the volume of the material is reduced foaming will decrease and the color will change from reddish-brown to a dark grey. Most of the water has been removed when a slight tendency to give off blue fumes is exhibited. It is best now to wash the organic matter from the sides of the crucibles with a few millilitres of water cautiously directed from a wash bottle. The same process is then repeated at least twice. In the final stage the material changes from a dark foaming fluid, which contains water, to a less viscid fuse with no reddish-brown tinge, and on further heating the fuse again begins to foam, though not excessively. It is quite black. The ashing to this point

may be completed in 15 minutes, but usually considerably longer (about 25 minutes) is required when one is learning.

"The crucibles are now placed in a muffle furnace at 250° C. If examined from time to time the fuse will be found to become much lighter in color and quite crusty. It is well to swirl the liquid up onto the walls of the crucibles before replacing them in the furnace. Progressive bleaching and solidification takes place. Finally the material is quite dry, yellowish-white, and is now ready for higher temperatures. The ashing in the furnace at 250° C. may be completed with experience in 15 to 20 minutes, but otherwise 40-50 minutes may be required. It is better not to rush the decomposition. The temperature of the furnace is now raised to 360° C. and is held there for 10 minutes. A little charring may take place. The use of oxygen is unnecessary.

"In the following process, that of alcoholic extraction, it is not easy to go astray. Usually only sufficient water is added to form a fluid paste. The time spent in reevaporation is thereby saved. The alcohol, after being decanted, should show no color. If colored, the destruction of organic matter has not been complete. After extraction, the alcohol is made alkaline by the addition of 1 ml. of saturated potassium hydroxide. The crucible is then placed on a steam bath or hot plate and evaporated nearly to dryness. Ten or fifteen minutes in the muffle furnace at 400° C. com-

pletes the ashing process.

McCullagh reports a recovery of added iodine with an error of 5%. The following tables represent the recoveries made using McCullagh's method, with the titration process alone modified.

Analyses of Horse Serum and Added Iodine

| Sample | Iodine Added (Micrograms) | Iodine Found (Micrograms) | Percentage Recovery of added Iodine |
|---|---|---|---|
| 10 ml. horse serum | | 0.4 | |
| 10 ml. " " | | 0.3 | |
| 10 ml. " " | | 0.4 | |
| 10 ml. " " | | 0.2 | |
| 10 ml. " " | | 0.4 | |
| 10 ml. " " | | 0.4 | |
| 10 ml. " " | 0.845 | 1.2 | 98% |
| 10 ml. " " | 0.845 | 1.1 | 91.5% |
| 10 ml. " " | 0.422 | 0.8 | 99% |
| 10 ml. " " | 2.535 | 2.7 | 93.5% |

Average of six determinations on 10 ml. of serum alone: 0.37 micrograms.

Analyses of Dog Blood and Added Iodine

| Sample | Iodine Added (micrograms) | Iodine Found (micrograms) | Percentage Recovery of added Iodine. |
|---|---|---|---|
| 10 ml. dog blood | | 1.0 | |
| 10 ml. " " | | 1.7 | |
| 10 ml. " " | | 1.1 | |
| 10 ml. " " | | 1.1 | |
| 10 ml. " " | | 1.1 | |

| | | | | |
|---|---|---|---|---|
| 10 ml. dog blood | | | 0.9 | |
| 10 ml. " " | | | 1.1 | |
| 10 ml. " " | | 1.69 | 2.2 | 74% |
| 10 ml. " " | | 1.06 | 2.1 | 104% |
| 10 ml. " " | | 1.06 | 1.8 | 77% |
| 10 ml. " " | | 1.06 | 1.7 | 68% |

Average of seven determinations on 10 ml. blood alone:

0.99 micrograms.

The results of these determinations reveal that there is considerable loss, and suggest that the ashing process is the chief fault. The satisfactory recoveries from horse serum, which contains less organic matter than whole blood, indicate that the determination from the distillation stage to the estimation is reasonably satisfactory.

(2) Modifications of Ashing.

McCullagh's method of ashing and those of other workers are characterized by being long and tedious. Many of them fail to retain all the iodine. Attempts were thus made to obtain a rapid, complete, and accurate ashing method by the use of closed ashing under pressure. Two types of bombs were used.

The first bomb was of special composition steel and adapted to ashing with oxidizing reagents. Its capacity was 100 ml., and the walls were approximately .75 in.thick. The cover was secured tightly against the body of the bomb by a heavy screw cap. A copper gasket was used to prevent

escape of gases. The bomb was estimated to withstand a pressure of 100 atmospheres with safety. In practise, the material to be ashed and the reagents were enclosed in the bomb, which was then placed in an electric furnace. The furnace was not, however, large enough to receive the whole bomb. The screw cap projected somewhat, and was covered with loose asbestos to secure heat insulation. No means have been available for determining the temperature of the bomb during these experiments. Experience has shown that a current of 5 amperes developed a temperature of approximately 275° C. Seven amperes heated to 350-400° C.

The material for ashing had to be dried before introduction, because the excessive pressures of steam would be dangerous. The drying process is indicated in each of the following experiments. The amounts of oxidant added are in excess of those needed to completely oxidize the organic material on a basis of 2 grams per 10 ml. of blood.

Bomb Experiment I.

Ten ml. of dog blood and 10 ml. of saturated potassium hydroxide were heated in a nickel crucible for thirty minutes in an oil bath, the temperature finally reaching 260° C. The fuse was broken into small pieces and placed in the bomb. The furnace was run at 10 amperes for 20 minutes, and at 7 amperes for 2 hours. After cooling the fuse was examined. It was brown, suggesting undecomposed organic matter. The material was dissolved in water and boiled down. Foaming indicated fatty acids. The material was thus

discarded.

Bomb Experiment II.

The blood was dried as above, introduced into the bomb, and well mixed with 2 grams of potassium permanganate. The furnace was turned on at 10 amperes for 15 minutes and 7 amperes for 2½ hours. After solution of the fuse in water, and evaporation, foaming and a characteristic odor indicated that fatty acids had not been completely decomposed. The material was discarded.

Bomb Experiment III.

The blood was prepared as above. Five grams of potassium permanganate were used. Heating at 10 amperes for 15 minutes and at 7 amperes for 3 hours was carried out. Fatty acids were still in evidence, though fewer than in experiment 2.

Bomb Experiment IV.

Animal charcoal was used in place of blood residue. Two grams of animal charcoal, two grams of anhydrous potassium carbonate and 20 grams of sodium peroxide were well mixed and introduced into the bomb. The furnace was heated with 10 amperes for 15 minutes, and 7 amperes for 2 hours. The resulting fuse was nearly white but contained pieces of unburned carbon.

Bomb Experiment V.

Two grams of animal charcoal, two grams of potassium carbonate and 26 grams of sodium peroxide were well mixed. The furnace was heated at 10 amperes for 30 minutes, and

eight amperes for five hours. The fuse was dissolved in 100 ml. of water, saturated with carbon dioxide and evaporated. A considerable amount of brown precipitate was present. This was probably ferric hydroxide. The inside of the bomb showed signs of marked corrosion by the fuse. For this reason the use of sodium peroxide had to be discontinued.

Bomb Experiment VI.

Ten ml. of blood and 2 grams of potassium carbonate were heated to 250° C. on an oil bath and kept at that temperature for 10 minutes. The residue was then ground, mixed with 13.7 grams of potassium nitrate, and placed in the bomb. The current was turned on at 10 amperes for 30 minutes, and 8 amperes for 3½ hours. The fuse was dissolved in 50 ml. of water, evaporated to a thick paste in a beaker, and extracted with four 10 ml. and one 5 ml. portion of alcohol. The alcohol was evaporated on a water bath. Two millilitres of water were added. The solution was then acidified to methyl orange with sulphuric acid. There resulted the evolution of nitrogen peroxide. Waxy flakes (fatty acid) appeared indicating the incomplete destruction of organic material.

The second bomb was originally part of an Emerson bomb colorimeter for the determination of fuel values. It consisted of two stainless steel hemispheres, which were held together by a large nut. Inside the bomb, which had a volume of 400 ml. was a small pan approximately one inch

across. Two binding posts were provided for a fine iron wire which was heated electrically, and ignited the charge placed in the pan. The bomb was filled with oxygen at a pressure of 20 atmosphere from an oxygen cylinder by means of metal connections. The bomb was fired under water (in this case to avoid overheating).

Bomb Experiment VII

The charge for the colorimeter bomb was prepared by heating 10 ml. of blood and 1 gram of potassium carbonate in a nickel crucible placed in an oil bath. The temperature was raised to 270° C. in 30 minutes. The residue was then ground to a powder, and burned in the bomb in three portions. The bomb was opened and the lining washed into a beaker with 50 ml. of water, filtered from particles, and boiled down to one or two millilitres. The resulting syrup was brown, and foaming, which indicated incomplete oxidation of organic matter. This method was thus discarded.

These experiments indicate the great difficulty of obtaining complete destruction of the organic matter in blood, even with very powerful oxidizing agents. Sodium peroxide is seen to be the best, but tends to dissolve the steel of the bomb. The use of a nickel or "alloy" bomb would probably overcome this difficulty, but they were not available. The added difficulty of obtaining sodium peroxide free from iodine would probably be great.

(3) Modifications of Distillation

The advantage of a distillation stage in the purification process lies in the fact that it leads to a much less concentrated, and complete solution, for final titration. Inorganic constituents such as iron, which may interfere, are completely removed.

McCullagh and other workers have carried out distillation on a macroscopic scale. Before distillation, the iodine has been concentrated into a volume of one or two millilitres and it must be reconcentrated after distillation, since the distillate amounts to more than 200 ml. In this process some loss must inevitably occur. Considerable time is also wasted.

Several attempts were made to develop a technique for distillation on a micro scale, using several reagents and apparatus. Considerable information regarding the chemistry of iodine determinations is given by these experiments.

The first apparatus used was designed to make best use of the factors of heat, acidity, temperature, and a closed system. The apparatus, as shown in diagram A. consists of a 50 ml. distillation flask D, into which water vapour is led from steam generator, A, the flow being controlled by stop cork B. F is an opening which provides an outlet for steam from A, and ensures that the pressure in A does not reach a high value. C is a tube by which reagents may be introduced into D. The delivery tube of D

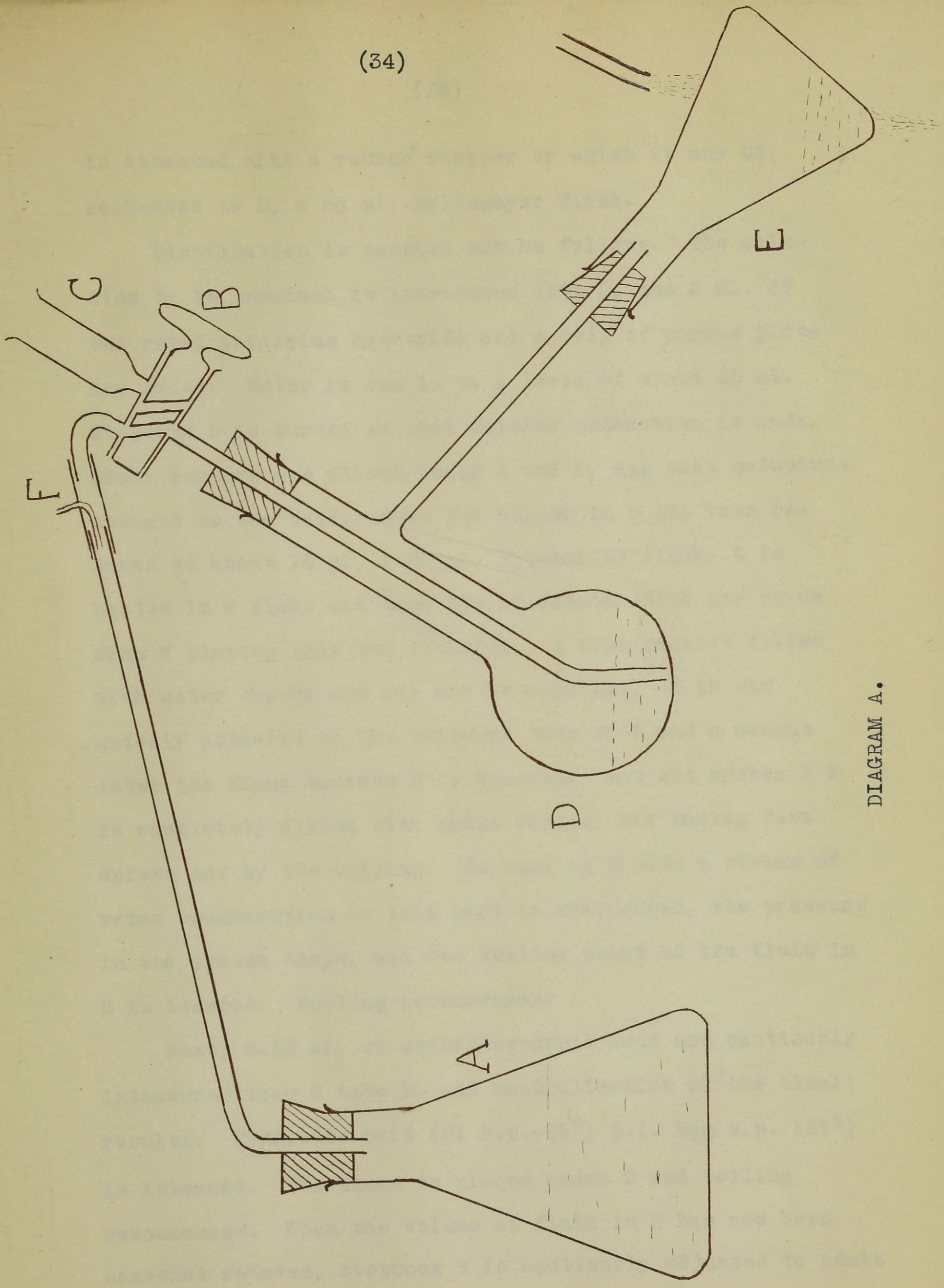


DIAGRAM A.

is attached with a rubber stopper by which it may be connected to E, a 50 ml. Erlenmeyer flask.

Distillation is carried out as follows. The solution to be examined is introduced into D, and 2 ml. of saturated potassium hydroxide and a chip of porous plate are added. Water is run in to a level of about 20 ml. Stopcock B is turned so that neither connection is made. Micro burners are placed under A and D, and both solutions brought to the boil. When the volume in D has been reduced to about 10 ml, a 50 ml. Erlenmeyer flask, E is heated in a flame and held for 10 seconds with the steam from F playing into the interior. E thus becomes filled with water vapour and all air is expelled. E is now quickly attached to the delivery tube of D and a moment later the flame beneath D is removed. Now the system D E is completely filled with water vapour, air having been driven out by the boiling. On cooling E with a stream of water condensation in this part is encouraged, the pressure in the system drops, and the boiling point of the fluid in D is lowered. Boiling recommences.

Next, 5-10 ml. of orthophosphoric acid are cautiously introduced from C into D, and neutralization of the alkali results. Hydriodic acid (HI b.p.-36°; H.I. $H_2O$ b.p. 127°) is released. The flame is placed under D and boiling recommences. When the volume of fluid in D has now been somewhat reduced, stopcock B is cautiously adjusted to admit

live steam into D. The pressure in D thus rises to atmospheric pressure and the rate at which steam passes into D is controlled by the condensation at E. The system is thus self-regulating and requires almost no attention. Several sets of apparatus may be run at once. When sufficient condensate (10-12 ml.) has collected in E, air is introduced through C, and E is disconnected. The determination is then completed by oxidation with bromine and titration (see following section).

The apparatus was perfected using iodate solutions and potassium hydroxide. Yields were accurate to 5%. The advantages of such a process are evident. The temperature of the distilling fluid is probably above 200° C. (phosphoric acid loses water at 212° C.), strongly acid, and loss from the system would seem to be impossible.

Attempts were made to replace McCullagh's distillation process with this one, which is more rapid and has the advantages mentioned. The fuse from ashing was washed into the distillation flask with about 20 ml. of water. It was found, however, when the acidification was attempted, that there was a considerable amount of potassium carbonate in the solution, and the carbon dioxide evolved developed pressure, or at least prevented the rapid transfer of water vapour to the condensation flask E.

In an effort to overcome this difficulty the solution was acidified and then made alkaline again before boiling.

However, when the carbon dioxide was adequately evolved, iodine also escaped. Attempts to utilize oxidation of the iodide to the iodate state with bromine were unsuccessful. Iodate can also be produced in the alkaline state by potassium permanganate. Subsequent acid boiling during distillation would probably decompose the iodate at the high temperatures, but under these conditions it was found that an oxidizing agent (perhaps manganese heptoxide or phosphoric acids) was distilled over and later oxidized both methyl orange, and potassium iodide. An alternative method was to reduce the potassium permanganate during acidification. This was attempted with phosphorous acid but on distillation phosphine was set free and interfered with titration.

The difficulty of carbon dioxide evolution might also be overcome by using an open distillation system. This was attempted. A 50 ml. Claisen flask and a six inch condensor were joined by a small rubber tube. The end of the condensor dipped into alkaline solution in a 50 ml. Erlenmeyer flask. It was found advisable to add potassium permanganate to the distilling fluid in order to destroy completely all organic matter. After heating, but before distillation, this was decomposed by hydrogen peroxide. It was found, however, that an oxidizing agent was carried over into the distillate when phosphoric and hydrogen peroxide were boiled together. Ortho-phosphoric acid does not itself oxidize iodides. Under these conditions permonophosphoric and pexdiphosphoric acids are probably formed. These are both powerful

oxidizing agents (65).

Sulphuric acid, a more volatile acid, was then substituted for phosphoric acid, although this reduced the temperature of the distilling fluid and also increased the acid which would distill over. Ferric sulphate was added to oxidize iodides. It was found that in some cases an oxidizing agent distilled over. This may have been one of the persulphuric acids which are powerful oxidizing agents. In order to reduce such substances, acid bisulphite was added to the receiver instead of alkali as was used previously. There then resulted no excess of iodine on addition of iodide, indicating satisfactory reduction. It was found, however, that the yields on blanks were variable and occasionally as low as 65%.

It is not clear from this experiment why the yields were low. McCullagh's method, which is practically the same on a macro scale, is reported to give yields with an error of 5%. A series of determinations on horse serum confirms this. The difficulty in the method here described may be one of inefficient collection of the distillation gases. A more elaborate receiver might have been substituted. It was felt, however, that a complicated apparatus was a disadvantage.

(4) Modifications of Titration.

McCullagh follows the Dupre-Winkler method for estimation of the iodine. After distillation the sodium bisulphite is destroyed by boiling the distillate for three minutes. The solution is then neutralized to litmus and

evaporated to 10 ml. From the 250 ml. beaker, in which this has been manipulated, the fluid is washed into a 50 ml. Erlenmeyer flask, acidified to methyl orange, a drop of bromine added, and the solution boiled, with constant agitation, to a volume of 2 ml. After cooling in ice water, the addition of a minute crystal of potassium iodide releases iodine in six times the amount originally present. This is titrated to a starch end-point with a 0.2 ml. pipette graduated in thousandths. Freshly prepared thousandth normal thiosulphate is used.

This is, in the main, very similar to the technique used by other workers. It was found, however, in several months' work, that it was impossible to carry out the titration accurately. It is unnecessary to record the experiments that were conducted in an attempt to secure satisfactory results. Many of these experiments suggested that there was difficulty with the end-point. When opportunity was presented to visit McCullagh's laboratory, it soon became evident that the fault was one of color perception on the part of the writer. At McCullagh's suggestion a clinical test was made for color blindness, and this was found to exist in some degree, particularly with regard to blue, grey and green shades. For this reason, the use of the starch end-point was deemed inadvisable.

A review of the literature revealed that several other indicators had been used, but that the most promising methods seemed to be electrometric. In particular, that of Faulk and Bowden (20) seemed to be adaptable to the special demands of micro work, although it was originally used with solutions containing large amounts of iodine. The apparatus (diagram B) consists of two platinum electrodes dipping into the solution to be tested. A galvanometer is connected in series to a potentiometer, which is adjusted to deliver 10-15 millivolts. In practise, according to the authors, the key is held down during the whole titration. The end-point is indicated when no deflection of the galvanometer is recorded.

For the titration of the small quantities of iodine that are in blood, a cell of 3 ml. capacity was formed from the lower 2 inches of a 25 ml. Pyrex test tube. The cell was mounted in a large rubber stopper which provided an adequate base. By means of a clamp two platinum wires were held in the cell. The potentiometer was of Leeds and Northrup construction, and adapted for H-ion measurement. A standard calomel cell was used. The solution to be tested was washed into the cell with several portions of water to give a final volume of 3 ml. This was the apparatus originally set up. It was found that a more sensitive galvonometer than that of the potentiometer was required. This was procured. Adequate stirring was necessary and this could be conveniently done only by a stream of air bubbles. A

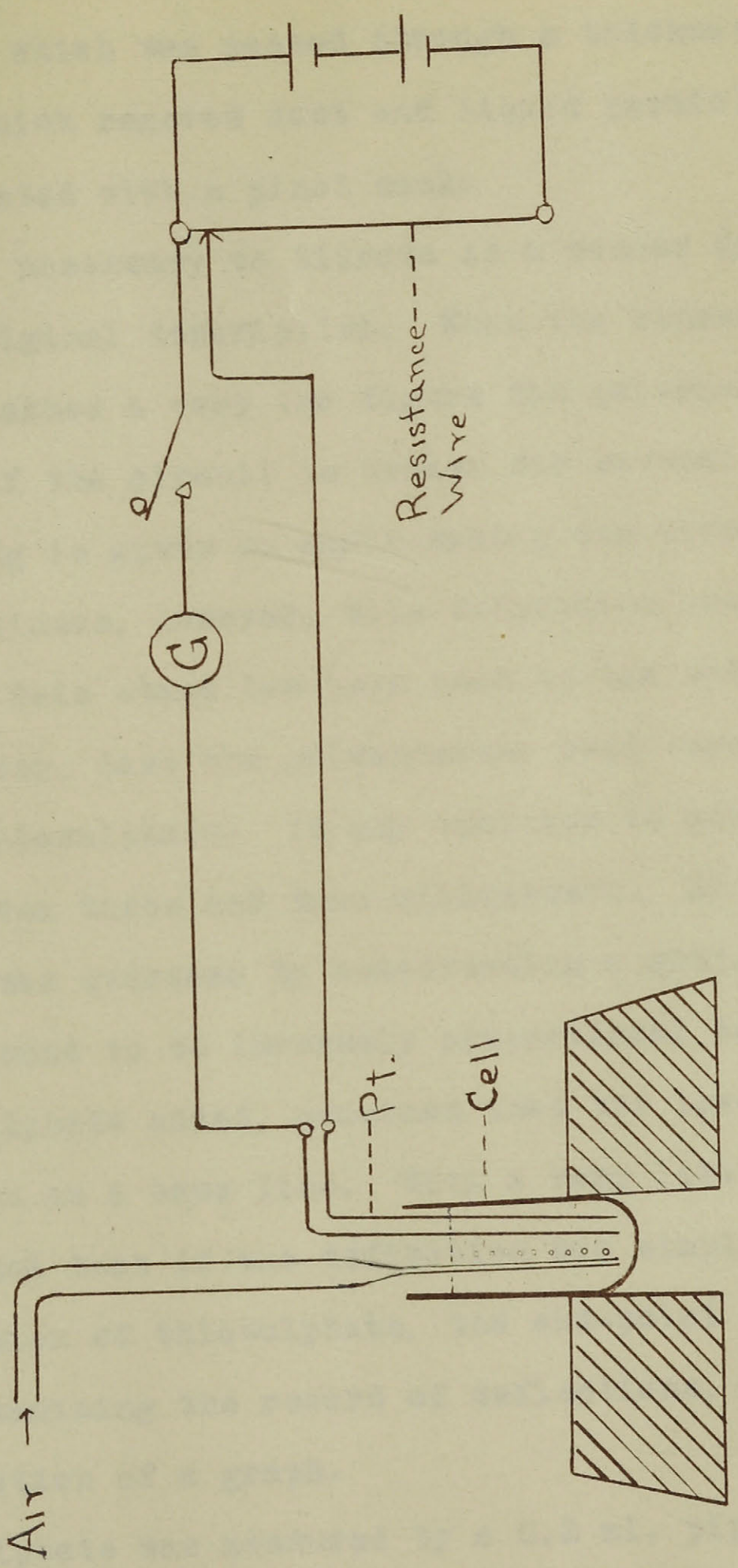


DIAGRAM B.

glass tube was drawn out to a capillary point and clamped with the point near the bottom of the cell. Use was made of compressed air which was passed through a thickness of cotton batting, which removed dust and liquid particles. The flow was adjusted with a pinch cock.

It was found necessary to titrate in a manner different from the original description. When the concentration of iodine reaches a very low figure the galvanometer reads zero, but if the circuit is broken for several moments, a reading is given on again making the circuit. As titration continues, however, this deflection ceases to be recorded. This stage has been used as the end-point. Not always, however, does the galvanometer read zero, even with excess of thiosulphate. It may continue to give minor deflections of even three and four millimeters. At first this difficulty was overcome by constructing a graph. The deflection was found to be inversely proportional to the amount of thiosulphate added, provided that the lowest reading was taken as a base line. With a very little experience it was found that if the deflection was simply recorded after each addition of thiosulphate, the end-point could be determined by examining the record of deflections, but without the construction of a graph.

The thiosulphate was measured by a 0.2 ml. pipette graduated in thousandths of a millilitre. Delivery could be controlled to one thousandth of a millilitre after a little experience. At each delivery the tip of the pipette was

dipped into the fluid.

In practise it was found that the accuracy of the end-point determination was greater than that of pipette delivery. The following titration figures (in thousandths of a millilitre of thousandth normal thiosulphate) were obtained in a series of tests on 1.06 micrograms of iodine (as iodate) made daily over a period of two weeks:- 48, 48, 49, 47, 48, 49, 47, 48, 48, 48, 47, 48, 47, 49. The average is 47.9.

The effect of the presence of salts has been examined. While it does not seem to alter the titration figure, yet the end-point is less sharp than with simple iodate solutions. If the solution to be titrated is warmed, a very definite end-point is given, but there is marked loss of iodine under these conditions.

To test the final titration method under working conditions, blanks were run on known amounts of iodate solution, in the presence of the standard amount of salt and after the usual oxidation. Quantities of iodate solution were run into 5 ml. Erlenmeyer flasks, 10 drops of 10% potassium hydroxide were added to each, and the solutions were neutralized to methyl orange with 3% sulphuric acid. A drop of bromine was immediately added, and the solution boiled to 2 ml. with constant agitation to avoid overheating. It was then washed into the titration cell, where estimation was made as described. At first four determination of this type would be run together, the titrations being done in turn.

This meant that some solutions stood aside for a few minutes before estimation was made. The following two series show the type of results that were obtained.

| | Amount of Iodine Present (micrograms) | Amount Recovered (micrograms) | Percentage Recovery |
|---|---|---|---|
| Series I | 1.91 | 1.92 | 100% |
| | 1.27 | 0.52 | 41% |
| | 1.70 | 0.63 | 37% |
| | 0.85 | 0.19 | 22% |
| Series II (the first test not titrated immediately) | | | |
| | 1.46 | 1.34 | 90% |
| | 2.54 | 1.39 | 55% |
| | 1.06 | 0.52 | 49% |
| | 2.12 | 1.41 | 67% |

These results suggest strongly, that the longer a solution stands after bromine oxidation, the less will the recovery values approach to the amounts of iodine present. The following series represents determinations carried out individually and titrated immediately.

| Amount Iodine Present (micrograms) | Amount Recovered (micrograms) | Percentage Recovery |
|---|---|---|
| 1.48 | 1.39 | 93% |
| 1.27 | 1.28 | 100% |
| 2.12 | 2.26 | 100% |
| 2.12 | 2.10 | 99% |
| 0.85 | 0.86 | 100% |
| 1.27 | 1.24 | 98% |

The error is roughly 7%, and as part of this may be attributed to the oxidation process, the titration is presumed to be of considerably better accuracy.

Several months experience has shown that some care of the electrodes is required to ensure accurate titration. Before and after use the electrodes should be washed with water. From time to time it is necessary to dip the electrodes in chromic sulphuric acid solution to restore the sharpness of the end-points. After some time this ceases to be sufficient. If then the electrodes are removed, and lightly scraped with a knife it will be found that they work as well as new. These precautions alone ensure continual satisfaction.

## III DISCUSSION

A survey of the literature on iodine determinations reveals that many methods (accurate within 15%) have been devised, but that the very large majority of these, in the hands of other workers, have failed to justify the claims made. For purposes of clinical and physiological research a method is desired which combines accuracy, with simplicity of technique, and rapidity of determination. No method yet described has filled these requirements, and been found satisfactory in more than one or two laboratories.

The experiments that have been carried out in an attempt to repeat McCullagh's work, suggest that much improvement of his method is necessary. While it has been possible to make recoveries, with reasonable accuracy, of iodine added to certain materials (e.g., horse serum) the recovery of iodine added to blood, has been poor. In fact, a series of determinations on one sample of blood yielded results of considerable range. Since the technique from distillation to titration is the same for all materials the results suggest that the ashing of the blood must be at fault. Considerable experience has been had with this ashing technique. It can thus not be considered a suitable method for general laboratory use.

The experiments on ashing in bombs with oxidizing reagents have not been very successful. Not all the possibilities have been fully exploited, however, and it may be that further investigation would yield a satisfactory method of ashing. Of the oxidants used, sodium peroxide was superior

for destruction of organic matter. With a bomb of nickel, or some non-corrodable alloy, an ideal ashing method might be elaborated.

The process of distillation, as described by McCullagh, is time consuming, and involves some loss of iodine. The advantages of conducting this on a micro scale in a closed system have been appreciated. Attempts to do so have been unsuccessful. However, considerable information pertaining to the chemistry of this stage of analysis has been gained. The chief difficulties lie in the inclusion of carbonates in the fuse, and the production of volatile oxidizing agents in the reaction of hydrogen peroxide (or other oxidants) with sulphuric and phosphoric acids. This latter difficulty may be eliminated by using sodium bisulphite as a reducing substance in the receiving flask. The possibility that inefficient collection of distillation gases may also cause loss has not been eliminated.

The modification of the final stage of titration by the potentiometric determination of the end-point has been very successful. This method is considerably more satisfactory than titration to the indefinite starch end-point.

## IV SUMMARY.

1. A review has been made of the literature on the determination of iodine in blood. Alkaline and acid ashing in open and closed systems have been described. The various stages of estimating by both colormetric and titrimetric methods, have been considered.

2. The method described by McCullagh has been examined in detail. Attempts to obtain the same accuracy that he reports on determinations of iodine in blood have not been successful. Evidence has been presented that the ashing and titrimetric processes require modification.

3. Ashing of blood has been carried out in two types of bomb with several oxidizing agents. The use of sodium peroxide offers the greatest possibilities.

4. The use of a micro method of distillation has been suggested. Some preliminary experiments have been conducted to determine the form this should take.

5. McCullagh's method of titration has been improved by the development of a potentiometric determination of iodine. The apparatus described is sensitive to less than $10^{-7}$ grams of iodine. Personal idiosyncracy is thus excluded as a source of error.

ACKNOWLEDGMENT

I wish to express my appreciation to those in the Department of Biochemistry whose criticism and encouragement has been of aid to this investigation. In particular I am indebted to Dr. J. B. Collip and Dr. D. L. Thomson for their constant assistance and advice.

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