CXXII. THE DETERMINATION OF IRON IN ORGANIC SUBSTANCES.

I. MICRO-COLORIMETRY AS FERRIC THIOCYANATE.

II. POTENTIOMETRIC TITRATION WITH TITANOUS SULPHATE.

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IN certain experiments on iron metabolism, involving the iron-containing protein of egg-yolk, lecitho-vitellin, we planned to make a large number of iron estimations. To conserve our small supply of protein, the preparation of which had involved considerable time and labour, we desired to cut down the size of the sample analysed to the smallest amount on which an accurate iron determination could be made. The author and colleagues [1930] had already found that the iron content of egg-yolk protein could be accurately determined by the method of Kennedy [1927]. The samples then analysed contained about 0.05 mg. iron, an amount somewhat greater than that which we now wished to estimate.

The results of a critical study of Kennedy's procedure as the basis of a micro-colorimetric method are recorded in the first part of this paper. The second part is concerned with the determination of small amounts of iron in biological materials by potentiometric titration with titanous sulphate.

I. THE DETERMINATION OF MINUTE AMOUNTS OF IRON BY THE METHOD OF KENNEDY.

Of the recent methods recommended for the oxidation of organic matter preliminary to estimation of iron in organic materials, [Willstätter, 1920; Fowweather, 1926; Kennedy, 1927; Smirk, 1927; Wong, 1928; Elvehjem, 1930] we have found the sulphuric-perchloric acid method of Kennedy to be the most rapid and most convenient. Our adaptation of this method is described later. With some materials "ashing" is the only satisfactory procedure.

The conditions for maximum sensitivity of the ferric thiocyanate reaction when applied to a sulphuric-perchloric acid digest.

Many of the conflicting data on iron estimations [see Nottbohm and Weisswange, 1912; Stugart, 1931; Lásztó, 1931] appear to have resulted from a lack of appreciation of the complexity of the reaction between ferric salts and thiocyanates.

I. *The effect of reduction.* Philip and Bramley [1913] have studied the reduction which occurs in a ferric thiocyanate solution on standing with formaldehyde

This reduction, due to the oxidising power of $\text{Fe}^{\equiv}$ can obviously be retarded by increasing the acidity and the thiocyanate concentration. The reduction in the presence of H_2SO_4 is about twice that in the presence of HCl . When amyl alcohol is used to extract the ferric thiocyanate from aqueous solution (as recommended by Kennedy) it is improbable that reduction can take place to any great extent. However, when determining very small amounts of iron in sulphuric acid solution, and even when using amyl alcohol, the possibility of reduction of ferric thiocyanate (bleaching of the red colour) should not be overlooked.

II. *The effect of acidity.* If the p_{H} is low, the number of undissociated molecules of ferric thiocyanate, and hence the depth of red colour, may be decreased by the union of H^+ with CNS^- , to form comparatively slightly ionised thiocyanic acid.

Solution No.	Amount of 40 % NaOH solution added cc.	Reading of standard (Solution No. 1) when other test solutions were set at 15 mm.
1	3.0	—
2	2.5	15.1
3	2.25	16.4
4	2.0	17.5
5	1.75	15.7
6	1.5	14.8
7	1.0	14.1
8	0.5	13.8
9	Nil	13.8

A solution was prepared which contained in 4 cc. 0.02 mg. ferric iron, 1 cc. concentrated sulphuric acid, 0.5 cc. 60 % perchloric acid and 1 drop conc. nitric acid. Into each of eight 10 cc. glass-stoppered measuring cylinders were measured 4 cc. of this iron-containing solution and varying amounts of a 40 % sodium hydroxide solution¹. All the solutions were brought to the same volume and finally 1 cc. 40 % potassium thiocyanate solution and 3 cc. pure amyl alcohol were added to each. The tubes were shaken, and after standing a few minutes, until the aqueous layer had become colourless, as much as possible of the amyl alcohol layer was transferred to small filters (2.5 cm.). This filtration

¹ Sodium hydroxide prepared from sodium and found to contain no iron.

through paper removes finely divided particles of water dispersed in the amyl alcohol, and permits excellent colorimetry. Without such treatment, the solution of ferric thiocyanate in amyl alcohol always appears very slightly turbid. A colorimeter of the Duboscq type with micro-cups was used.

The maximum intensity of colour was obtained with solution No. 4, which was approximately 1.6 *N* acid. According to Lachs and Friedenthal [1911] the number of undissociated molecules of ferric thiocyanate reaches a maximum in 2 *N* HCl.

III. *The effect of thiocyanate concentration.* Solutions containing varying amounts of potassium thiocyanate were prepared. A series of iron solutions was prepared, each having the same composition as Solution No. 4 in the pre-

Fig. 1.

ceding experiment. Potassium thiocyanate in varying dilutions was added to the iron solution, 1 cc. to each cylinder. The colorimetric determination was carried out as already described.

It may be concluded from the results shown in Fig. 1 that the maximum intensity of colour with equal amounts of iron (0.02 mg. in 1.6 *N* acid) is obtained with 1 cc. 3.5 to 4.0 *M* potassium thiocyanate solution. This concentration is obtained with approximately 1 cc. 40 % potassium thiocyanate solution. The shade of colour changes slightly towards a brownish-red with increasing amounts of potassium thiocyanate.

IV. *The effect of phosphate.* The lecitho-vitellin which we prepared contained 0.59 % phosphorus. When determining iron as ferric thiocyanate in the ash of biological materials, Elvehjem and Hart [1926] have given particular attention

to the interfering action of large amounts of phosphate. According to Leeper [1930], the interfering action of orthophosphate has been overemphasised, the effect being primarily due to pyrophosphates, which are formed only on ashing at high temperatures. According to Kennedy and to Elvehjem and Hart, pyrophosphates are not formed during sulphuric-perchloric acid digestion. Leeper has recorded that high concentrations of phosphate do, however, "turn the red colour of ferric thiocyanate progressively towards a straw yellow" which cannot be matched in tint with phosphate-free solutions. This effect should be minimised by using the high concentration of thiocyanate which we have found necessary for the full development of the red colour. Quantitative determinations of iron in varying quantities of lecitho-vitellin show that the presence of phosphate has no effect on the results obtained (see Table I).

V. *The effect of varying amounts of iron (0.0025–0.025 mg.) on the intensity of the red colour.* A series of iron solutions was prepared, each solution containing the same amount of sulphuric acid and perchloric acid. The reaction in each

Fig. 2. Line A-B=Theoretical proportionality.

case was adjusted to approximately 1.6 *N* acid with 40 % sodium hydroxide. To 5 cc. of each dilution in a 10 cc. glass-stoppered measuring cylinder were added 1 cc. 40 % potassium thiocyanate solution and 3 cc. amyl alcohol.

The colorimetry was carried out as follows. The solution chosen as standard, which contained 0.0105 mg. iron, was always adjusted to match the other test solutions, which were set at 15 mm. The advantage of this procedure, as pointed out by Hunter and Eagles [1925] is that any divergence from proportionality is more easily seen when the graph is a straight line instead of a logarithmic curve. In this way, the colorimeter readings are also shown as increasing with increasing concentration of the coloured substance.

Within the range of iron concentrations employed, the intensity of the red colour is not directly proportional to the amount of iron present (see Fig. 2). When applying the thiocyanate reaction to the estimation of minute amounts of iron (0.0025 to 0.025 mg.) under the conditions just described, a correction must be made for this deviation from Beer's Law. In estimating the iron content of lecitho-vitellin, this correction was made directly from Fig. 2.

The estimation of iron in lecitho-vitellin.

Samples of lecitho-vitellin (18.65 to 127 mg.) in small glass tubes, were weighed on a micro-balance, and transferred to micro-Kjeldahl flasks, and the weight of the sample was obtained by reweighing the tube. 1 cc. of concentrated sulphuric acid, 0.5 cc. 60 % perchloric acid, and a glass bead were added, and the digestion was carried out over a micro-burner. Towards the completion of oxidation (10 to 15 minutes) the digest becomes almost colourless and boils very slowly. This is immediately followed by sudden ebullition and the appearance of a fairly intense yellow colour in the solution. The evolution of dense white fumes at this stage marks the end of the digestion. After cooling, 1 drop of concentrated nitric was added and the digest again heated to boiling to drive off the excess nitric acid.

The digest was transferred to a 10 cc. volumetric flask and the reaction adjusted so that when diluted to the 10 cc. mark the solution was between 1.5 and 2.0 *N* acid¹. A 4 cc. aliquot was measured into a 10 cc. glass-stoppered measuring cylinder, and the colorimetry carried out as already described, using 1 cc. 40 % potassium thiocyanate solution and extracting the ferric thiocyanate with 3 cc. amyl alcohol.

The standard iron solution was prepared so as to contain the same reagents in the same amount as the unknown test solution. The iron content of the same sample of lecitho-vitellin was also determined by the method of Elvehjem [1930] and by the unmodified procedure of Kennedy [1927].

Table I.

Method of analysis	Weight of lecitho-vitellin mg.	Iron content of a 4 cc. aliquot of digest after subtracting the graph correction mg.	% iron in lecitho-vitellin
Modified Kennedy procedure	127.23	0.022	0.044
"	121.70	0.022	0.045
"	87.27	0.015	0.044
"	74.23	0.013	0.045
"	68.41	0.014	0.047
"	49.38	0.009	0.045
"	38.31	0.007	0.046
"	27.00	0.007	0.046
"	18.65	0.004	0.050
Unmodified Kennedy procedure	316.00	—	0.041
Elvehjem ashing method [1930]	79.33	—	0.054
"	87.31	—	0.051
"	102.74	—	0.059

¹ This dilution permits duplicate colorimetric determinations to be made on each sample. In later analyses the digest was transferred directly to a 10 cc. measuring cylinder with the aid of three 1 cc. portions of water. 2 cc. of 40 % sodium hydroxide solution were carefully added from a burette, and after cooling the determination was completed as above.

The values obtained for the iron content of lecitho-vitellin were in extremely good agreement when the final colorimetric determination was made with amounts of iron from 0.007 to 0.022 mg. The analysis of a larger sample of the protein by the unmodified procedure of Kennedy gave a slightly lower value. The method of Elvehjem gave figures which were not only higher than those obtained by acid digestion but were also more variable. We are in complete agreement with Stugart [1931] that any method of determining iron, such as that of Elvehjem, which involves boiling with alkali for one hour in a glass container, invites iron contamination as a result of the solvent action of the hot alkali on the glass.

II. THE DETERMINATION OF SMALL AMOUNTS OF IRON IN AN ACID DIGEST OF AN ORGANIC SUBSTANCE BY POTENTIOMETRIC TITRATION WITH TITANOUS SULPHATE.

The rapid and accurate method of determining ferrous and ferric iron by titanium titration described by Knecht and Hibbert [1903] has been applied to biological materials by Jahn [1911], Peters [1912], Pincussen and Roman [1931] and Lásztó [1931]. In modifying the procedure Jahn used Neumann's wet incineration with nitric acid to destroy organic matter. The nitric acid was removed by boiling with sulphuric acid and the titration finally made with $N/250$ to $N/500$ titanous chloride at 10° , using potassium thiocyanate indicator. Peters applied the titration in essentially the same way to a solution of the ash of the organic substance. Barcroft [1914] considered the method to be highly accurate and simple, while Murray [1924], disagreeing so far as the simplicity is concerned, has recommended micro-titration with permanganate. According to Lásztó, the iron content of blood cannot be determined with sufficient accuracy by the titanium-salt process of Jahn.

Recently Pincussen and Roman [1931] have developed a method, based on titanium-salt titration, to determine $\text{Fe}^{\equiv}$, $\text{Fe}^=$ and organically bound iron in tissues and blood.

Some values recorded for the total inorganic iron content of whole blood, plasma and serum are:

Author	Inorganic iron content
Hausermann [1899]	1.0 mg./100 cc. calf-blood
Fontès and Thivolle [1925]	0.2 „ horse-serum
Heinriques and Roche [1927]	0.2 „ serum
Barkan [1927]	0.22 „ serum
„	1.34 „ whole blood
Starkenstein and Weder [1928]	1.9 „ dog-blood
„	1.1 „ protein-free plasma

Assuming the inorganic iron (ferrous and ferric) content of blood to be about 1.5 mg. per 100 cc. the 1 cc. sample taken by Pincussen and Roman to determine ferrous and ferric iron should contain about 0.015 mg. total inorganic iron. The iron equivalent of the titanous solution used by them was such that 1 cc. reduced 0.7 mg. $\text{Fe}^{\equiv}$. While one of the objects of the present paper is to

point out how extremely accurate and sensitive the titanium titration of iron is, we would not care to suggest its use to determine the $\text{Fe}^=$ and $\text{Fe}^{\equiv}$ content of a solution the total inorganic iron content of which is approximately 0.015 mg.

Where titanium titration has been employed to determine the iron content of organic substances, the end-point in the titration has heretofore been detected by potassium thiocyanate indicator. With hydrochloric acid solutions of ferric salts or of the ash of biological materials, this end-point is extremely sharp, and no advantage is to be gained by substituting any other method of end-point detection. We have found, however, that when titrating the ferric iron in a sulphuric-perchloric acid digest of an organic substance the indicator end-point is quite unsatisfactory.

In sulphuric acid solution the colour of the ferric thiocyanate fades slowly to a reddish-brown during the titration, and the exact end-point cannot be accurately detected¹. Again there are occasions when it is desired to determine the iron content of solutions which are coloured. We have found that a much wider, and often more accurate, application of titanium titration can be made when the end-point is detected by measuring the change in potential of a suitable electrode during the titration.

Experimental

The following experiments are mainly concerned with the determination of iron in sulphuric-perchloric acid digests of organic materials by potentiometric titration with titanous sulphate.

I. *Apparatus*. A highly sensitive Leeds and Northrup "type P" galvanometer was used, and the difference in the potential of the platinum electrode was followed with a Leeds and Northrup "type K" potentiometer. The reference electrode was a saturated potassium chloride-calomel-platinum cell, which was connected to the titration vessel by a salt-bridge consisting of a capillary tube of 1 mm. inside diameter, filled with agar-agar potassium chloride gel. The electrode in the titration vessel was a $\frac{1}{2}$ -inch spiral coil of bright platinum wire.

An accurately calibrated 10 cc. gas burette (Haldane gas analysis apparatus), the constricted 3 cc. portion of which was graduated in 1/100 cc. and a 500 cc. gas-washing bottle with ground-in stopper were used to construct the burette shown in Fig. 3. By means of this burette, the titanous sulphate solution is protected from air, does not come in contact with rubber during storage and can be accurately measured to 0.01 cc.

The titanous sulphate solution is poured into the bottle and the entire apparatus flushed with hydrogen through stopcocks A and B by adjusting stopcock C. The titanous sulphate solution is forced up into the burette by hydrogen pressure through A, with C adjusted and B open to the air. A and B are three-way stopcocks which are finally closed to the air but adjusted to give

¹ For a probable explanation, see Part I, The effect of acidity (p. 1035).

an open circuit within the burette. When stored in this manner the strength of the titanous sulphate solution undergoes relatively little change in two weeks. The solution should, however, be standardised on the day on which it is used.

The titration vessel has a capacity of 50 cc. and is arranged so that the titration can be carried out at 50° to 55° under carbon dioxide. The carbon dioxide is bubbled through a series of wash-bottles containing, in the following order, concentrated sulphuric acid, dilute titanous sulphate in sulphuric acid solution, pyrogallol and aqueous permanganate solution. The titration is best

Fig. 3. Titration apparatus.

carried on at 50° to 60° as the last traces of Fe^{III} are only slowly reduced at room temperatures.

II. *The titanous sulphate solution.* This solution was prepared by suitable dilution of a 20 % titanous sulphate solution (La Motte Chemical Co.) after adding the requisite amount of sulphuric acid to prevent hydrolysis with the formation of metatitanic acid. The solution was standardised against ferrous ammonium sulphate by the method recommended by Thornton and Chapman [1921] as follows.

A weighed portion of ferrous ammonium sulphate was dissolved in water acidified with 10 cc. 5 *N* sulphuric acid and titrated with *N*/100 permanganate solution, which was standardised against pure anhydrous sodium oxalate as the

primary standard. The titration was carried out with a micro-burette, and with carbon dioxide bubbling through the solution. At the completion of the oxidation the solution was boiled, cooled to 50° , 1 cc. 10 % potassium thiocyanate solution added, and titrated at 50° with the titanous sulphate solution. The end-points were detected by both the indicator and the voltage method. The titanous sulphate solution was also standardised in the same manner against a standard iron solution (1 cc. = 0.174 mg. Fe) prepared from "analytical" iron wire. This standard iron solution was used to determine the titre of the titanous solution each day it was used.

Fig. 4 Showing typical permanganate and titanium titration curves with 0.83 mg. iron.

To determine with what accuracy small amounts of iron could be estimated, and to get some idea of the sensitivity of the potentiometric method of detecting the end-point, a number of analyses of small quantities of ferrous ammonium sulphate, weighed on a micro-balance, were made.

Weight of ferrous ammonium sulphate mg.	Theoretical iron content mg.	Titanous sulphate titre in cc.	Iron value of 1 cc. titanous sulphate mg.
5.73	0.81	1.42	0.57
4.72	0.67	1.28	0.52
3.95	0.56	1.00	0.56
4.60	0.65	1.25	0.52
5.82	0.83	1.53	0.54

Typical titration curves with permanganate, and back titration with titanous sulphate, are reproduced in Fig. 4. In both titrations the change in potential in the vicinity of the end-point is always very large, enabling one to determine it without difficulty. The actual values of the potentials are highly

reproducible. The complete data of the titration curves are recorded for illustrative purposes only. It will be noted that in both titrations the indicator end-point just precedes the potentiometric end-point. In Fig. 5 are shown the results obtained on titrating, at 50°, 3 cc. of a standard iron solution (1 cc. = 0.174 mg. Fe) with the same titanous sulphate solution when 1 cc. 10 % potassium thiocyanate solution was added and the titration carried out in an atmosphere of carbon dioxide. The iron value of the titanous sulphate

Fig. 5. Titanous sulphate titration of 0.522 mg. iron with 1 cc. 10 % potassium thiocyanate present.

solution was calculated from this titre. In this particular determination we have then:

Wt. of ferrous ammonium sulphate	5.82 mg.
Theoretical iron content, of ferrous ammonium sulphate	0.83 mg. Fe
1.62 cc. 0.00928 <i>N</i> KMnO ₄ (Fig. 4)	0.84 "
1.52 cc. Ti ₂ (SO ₄) ₃ solution (Fig. 5)	0.80 "

According to Kolthoff and Furman [1926] the oxidation-reduction potential of titanic-titanous solutions depends to a large extent upon the p_H . The reducing action of titanous salts increases with increasing p_H . These workers have recommended titrating ferric iron in the presence of a large excess of Rochelle salt.

We have recorded in Fig. 6 the results of some of the experiments which we have made to determine the conditions for the maximum change in potential at the end-point. The first, second and third titration curves in each experiment are for 1, 2 and 3 cc. respectively of a standard iron solution (1 cc. = 0.174 mg. Fe). It will be seen that in experiment A (unbuffered) the end-point is ill-defined, while in experiments B and C the end-point is marked by an inflection

potential of at least 0.2 volts (against saturated calomel electrode). On repeating experiment B with widely varying amounts of Rochelle salt, the most satisfactory conditions were obtained when the solution was buffered with 0.1 g. Rochelle salt. 1 cc. of 10 % potassium thiocyanate (exp. C), while a very unsatisfactory indicator, was found to give the most favourable conditions for accurate end-point detection by electrometric means. Under the conditions of exp. C, each addition of the reducing agent is accompanied by a rapid establishment of a steady potential. At the completion of iron reduction, however, the E.M.F. drifts slowly (2 minutes) to zero. The inflection potential is not affected by pronounced changes in the sulphuric acid concentration.

Whilst potassium thiocyanate evidently acts as an excellent buffer in these titrations, it is difficult to explain the difference in the results obtained with potassium thiocyanate and Rochelle salt from the standpoint of buffer action

Fig. 6.

alone. The iron values of 1 cc. of the titanous sulphate solution, calculated from the potentiometric end-points (exp. C, Fig. 6) are 0.58, 0.57 and 0.57 mg. respectively.

In applying the voltage method of detecting the end-point to determinations of the iron content of sulphuric-perchloric acid digests of organic substances, we have added 1 cc. 10 % potassium thiocyanate before making the titration. An inflection potential of the same magnitude as that obtained in exp. C (Fig. 6) has always been obtained. As little as 0.10 mg. Fe^{III} can thus be accurately determined. The change in colour of the ferric thiocyanate also affords a preliminary indication that the titration is nearing completion.

The iron content of milk.

In a previous paper the author [1932, 1] has described a method of estimating the copper content of whole milk (cows) in which the copper in a hydrochloric acid solution of the milk ash is precipitated as cupric sulphide. The iron content of the filtrate has been determined by titanium titration as follows.

The filtrate from the cupric sulphide precipitate was transferred to a 250 cc. pyrex Erlenmeyer flask and evaporated almost to dryness. A small filter-funnel was placed in the neck of the flask to prevent loss by bumping. To oxidise the iron, 2 cc. conc. nitric acid were added and the solution was boiled for a few minutes, cooled, 2 cc. conc. sulphuric acid added and the solution and calcium sulphate precipitate again boiled until the nitric acid and SO_3 fumes were completely evolved. Thorough boiling was necessary to decompose completely the nitrates of sodium and potassium, the presence of which interfere with the titanium titration. The solution was finally diluted with 10 cc. hot water and filtered into the titration vessel. The calcium sulphate precipitate was washed

Fig. 7.

with two or three small portions of boiling water, and the washings were added to the filtrate. The potentiometric titration was made after adding 1 cc. 10 % potassium thiocyanate solution.

Complete decomposition of the alkali nitrates may be more thoroughly assured by evaporating the filtrate from the calcium sulphate precipitate almost to dryness in a beaker covered by a watch glass, taking up with water and carrying out the titration.

A steady "initial" potential should be obtained after bubbling carbon dioxide through the solution for a few minutes. If alkali nitrates are still present, side-reactions take place and a drifting potential results. Until the end-point is reached, a steady potential is almost instantaneously obtained after each addition of titanous sulphate. At the end-point when the E.M.F. is about 0.35 v.

the potential slowly (2 minutes) drifts to zero. In such titrations the indicator end-point is extremely indistinct.

The iron contents of a number of samples of milk have been determined by the above procedure. The titration curves are shown in Fig. 7. The results may be summarised as follows:

	Sample of milk	Amount of milk analysed cc.	Ti ₂ (SO ₄) ₃ (voltage end-point) cc.	Iron content of milk mg./litre
A	{ 1	250	0.18	0.53
	{ 2	150	0.15	0.57
	{ 3*	150	0.14	0.55
B	{ 1	100	0.12	0.68
	{ 2*	150	0.16	0.64
C	1	300	0.25	0.48

* Results not plotted in Fig. 7. Note: 1 cc. Ti₂(SO₄)₃ solution reduced 0.57 mg. Fe.

The results of duplicate and triplicate analyses are in good agreement. The most recent iron determinations on milk are those recorded by Stugart [1931] using a modification of Kennedy's method. He records values from 0.44 to 0.53 mg. iron per kg.

Iron content of lecitho-vitellin.

The iron content of two samples of lecitho-vitellin [for method of preparation, *etc.* see McFarlane, 1932, 2] was determined. The samples were digested with sulphuric-perchloric acid as described in Part I of this paper¹. The digest was diluted with water, transferred to the titration vessel and, after adding 1 cc. 10 % potassium thiocyanate solution, titrated with titanous sulphate. The end-point was determined electrometrically.

Sample of lecitho-vitellin		% iron		Iron titrated mg.
No.	Weight g.	Titanium titration	Micro-colorimetry (See Part I)	
1.	0.3050	0.047	0.045	0.14
2.	0.4272	0.043	0.042	0.18

Iron content of crystalline haemoglobin.

The source of this particular preparation was unknown. The sample was digested as described for lecitho-vitellin. The "initial" potential before adding titanous sulphate was extremely unsteady. This difficulty was overcome by adding an excess of bromine water (1 to 2 cc.) at the completion of digestion. The digest was boiled until the excess bromine was driven off. It was then cooled, and titrated as before with 1 cc. 10 % potassium thiocyanate solution present.

¹ 2 cc. concentrated sulphuric acid and 1 cc. 60 % perchloric acid were, however, required for the digestion.

Wt. of crystalline haemoglobin g.	% iron	Iron titrated mg.
0.0903	0.328	0.29
0.1907	0.316	0.59
0.0427	0.322	0.14

Iron content of blood.

The iron content of oxalated human blood was determined by the same procedure as that described for crystalline haemoglobin. The final oxidation with bromine, already mentioned, greatly improved the results. The following values illustrate the accuracy of the method:

Amount of blood analysed cc.	% Iron	Iron titrated mg.
2.0	0.052	1.05
1.0	0.051	0.51
0.5	0.051	0.26

The haemoglobin content of this sample of blood was 14.62 % as determined by oxygen capacity [Sendroy, 1931]. Assuming that 1 g. haemoglobin contains approximately 0.0032 g. iron (see above), the iron content should be about 0.048 %. A determination of the iron content of blood can be made in less than 30 minutes.

Iron content of liver.

According to Starkenstein and Weder [1928], the inorganic iron, and none of the iron in organic combination, is extracted from blood or tissue by boiling with 5 *N* hydrochloric acid. The protein-free filtrate (trichloroacetic acid) contains the whole of the inorganic iron. We have found by applying these and our own findings already recorded in this paper that the inorganic and organic iron contents of liver tissue can be determined rapidly and accurately.

A weighed portion of rat-liver, about 1 g. is pulverised with a small amount of quartz sand¹ in a mortar, and then transferred to a 100 cc. pyrex centrifuge-tube with the aid of 10 cc. 5 *N* hydrochloric acid². After boiling about 10 minutes the solution is cooled and the protein precipitated with 20 % trichloroacetic acid. After centrifuging, the clear supernatant liquid is transferred to an Erlenmeyer flask and evaporated to a small volume. The iron is completely oxidised with saturated bromine water, and the excess bromine boiled off. The inorganic iron is determined as ferric iron by titrating with titanous sulphate, as already described. The protein residue in the centrifuge-tube is oxidised with sulphuric-perchloric acid and the "organic" iron determined by the procedure recommended for blood. The accuracy of the method is comparable with that for blood. The inorganic iron content of adult rat-liver (calculated on the fresh weight) was found to vary from 0.017 to 0.058 % and the "organic" iron from 0.72 to 1.13 %. Detailed observations in this regard will be recorded in a later paper.

¹ The quartz sand was freed from iron by extraction with hot concentrated hydrochloric acid.

² The hydrochloric acid must be freed from iron by redistilling.

SUMMARY.

Part I.

The method of Kennedy [1927] for the estimation of iron in organic materials has been studied with a view to applying this procedure to the micro-colorimetric estimation of minute amounts of iron in organic substances. Under the conditions described, and with the quantities of iron being determined (0.007 to 0.022 mg.) the intensity of the red colour of ferric thiocyanate is not proportional to the concentration of iron present. It is possible to correct for this deviation from Beer's Law so that the iron content of as little as 0.03 g. of lecitho-vitellin (0.014 mg. Fe) can be accurately estimated.

Part II.

Methods are described for the rapid and accurate estimation of as little as 0.15 mg. of iron in a sulphuric-perchloric acid digest of an organic substance by potentiometric titration with titanous sulphate. A modification of the procedure is outlined, whereby the total iron contents of milk and blood, and the total "inorganic" and "organic" iron contents of tissues may be determined.

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