CXXIX. THE BIOCHEMISTRY OF SILICIC ACID.

I. MICRO-DETERMINATION OF SILICA.

BY EARL JUDSON KING AND HELEN STANTIAL.

*From the Department of Medical Research, Banting Institute,
University of Toronto.*

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OF the several colorimetric methods which have been described for the estimation of small amounts of silica, probably that of Dienert and Wandenbulche [1923] has met with most favour. The principle of this method is the production of a yellow silicomolybdic acid complex; it was first studied by Schreiner [1903] and Lincoln and Barker [1904]. Winkler [1914] used a similar method for studying the silicic acid content of natural waters, but the procedure of Dienert and Wandenbulche, variously modified by Atkins [1923], King and Lucas [1928], King [1928], Steffens [1930], Thayer [1930], and Liebknecht *et al.* [1931], has been most generally used during recent years.

Reduction of the yellow silicomolybdic acid complex to give a blue colour has been made the basis of other colorimetric methods proposed by Isaacs [1924] who used sodium sulphite at the temperature of boiling water to effect the reduction, by Oberhauser and Schormüller [1929] who used stannous chloride, by Nemec, Lanic and Koppova [1931] who used sodium thiosulphate, by King [1930] and Pincussen and Roman [1931] who used quinol, and by Berg and Teitelbaum [1930] who used pyrrole as the reducing agent.

Most of these methods have been developed with a view to determining the silica content of various natural waters. Rather large amounts of water (usually 100 cc.) are used for the test solutions and the colour comparisons are made in Nessler or Hehner tubes. While yellow-coloured solutions are difficult to compare accurately in a Duboscq colorimeter, blue colours can be matched relatively easily and very much more accurately. An attempt has been made to develop a blue colour method, which would be accurate in practice though simple in principle and in detail, and which could readily be applied to a variety of biological materials, particularly blood, urine and tissue.

EXPERIMENTAL.

Optimum acidity. The formation of the yellow silicomolybdic acid complex is best accomplished at a weak acidity corresponding to about 0.08 *N* (4 drops 50 % H_2SO_4 (vol.) in 50 cc.), and a corresponding acidity has been found most favourable for the production of the blue colour in the presence of several reducing agents. Thus 2 cc. of *N* H_2SO_4 in a total volume of 25 cc. gave the maximum intensity of blue colour when test solutions of sodium silicate and ammonium molybdate were treated with various reducing agents in the presence of varying amounts of acid. Half this amount of acid gave almost as much colour, but it was less permanent, tending to fade after the course of half an

hour. With more acid the blue colour took on a greenish tint and was not so intense.

In Fig. 1 the results of several experiments are presented in which the relative blue colour production with different reducing agents was studied. 1:2:4-Aminonaphtholsulphonic acid was definitely superior to all other reducing agents tried, including several—stannous chloride, monomethyl-*p*-aminophenol, benzidine, *etc.*—not shown in the figure. It has been shown by Fiske and Subbarow [1925] and by Vasarhelyi [1929] to have advantages over other reducing agents in the colorimetric estimation of phosphate and has now come into common use for this purpose.

Fig. 1. Relative intensity of colour in test solutions containing 0.1 mg. of SiO_2 as silicic acid, 1 cc. 10 % ammonium molybdate and varying amounts of $N H_2SO_4$ in a volume of 25 cc. with A, 1 cc. 20 % sodium sulphite, colour allowed to develop 30 mins. at room temperature; B, 1 cc. 20 % sodium sulphite, heated 10 mins. at 100° and cooled before reading; C, 1 cc. of 0.5 % quinol in 20 % sodium sulphite, colour allowed to develop 30 mins. at room temperature; D, 0.5 cc. of 0.2 % 1:2:4-aminonaphtholsulphonic acid, colour allowed to develop 10 mins. at room temperature.

Concentration of molybdate. The amount of ammonium molybdate first tried in the test, i.e. 1 cc. of 10 % in 25 cc., appeared to be the optimum for the production of the blue colour. With half this amount of molybdate only about three-quarters as much colour developed. More than 2 cc. in the test caused the production of a slight "blank" colour and the proportionality of colour produced was not as good. Sodium molybdate appeared to have no advantage over ammonium molybdate.

Amount of reducing agent. As in the case of molybdate, lowering the amount of reducing agent present lessened the colour production, and with more than 1 cc. of 0.2 % aminonaphtholsulphonic acid a "blank" colour was introduced and the proportionality of colour production was less good.

"Blank" colour production with reagents. Different amounts of sulphuric acid were tried in tests containing 1 cc. of 10 % ammonium molybdate, 0.5 cc. of sulphonic acid and no added silica. There was only a slight blue coloration even after several hours when 1 or 2 cc. of $N H_2SO_4$ were present; 3 cc. gave a little more, but 4 cc. or over soon brought up a considerable blue colour. After 8 hours a test containing 6 cc. of acid had almost as much colour as one containing 0.1 mg. SiO_2 . It is necessary, therefore, to develop the colour at the

lower acidity of 2 cc. of $N H_2SO_4$, corresponding as it does with the maximum production of blue colour in the presence of silicic acid in short periods of time.

Proportionality of colour production. When different amounts of a standard sodium silicate solution are treated with the reagents the depth of blue colour produced is not directly proportional to the concentration of silicate present. If the apparent concentrations calculated from the colours produced in a series of tests of different strength be plotted against the actual concentrations, however, the result is a straight line, which, although it does not go through the origin, crosses the vertical axis only a slight distance above the zero point (see Fig. 2). The slope of this line is numerically thus not far from 1 and results

Fig. 2. Colour production with different amounts of sodium silicate: x mg. SiO_2 (as a solution of $Na_2SiO_3 \cdot 9H_2O$) + 2 cc. 5 % ammonium molybdate in $N H_2SO_4$ + 0.5 cc. aminonaphtholsulphonic acid in 25 cc.; read after 10 mins. against a 0.1 mg. SiO_2 standard. The slope of the line, m , is 0.94 and the actual concentration x at any point can be calculated by means of the equation

$$y = mx + b, \text{ where } y \text{ is the apparent and } x \text{ the actual concentration.}$$

$$y = 0.94x + 0.0066 \text{ and } x = 1.06y - 0.007.$$

calculated without the introduction of an equation to allow for the slope are approximately correct. In practice it is sufficient to use the standard most nearly approximating the test in colour.

Interfering substances. Several substances might interfere with the reaction giving the silicomolybdic blue colour. Tungstate is unlikely to be present. Arsenate does not appear to give any colour within 10 minutes at the acidity used when present in amounts up to 2 mg., although a colour appears on longer standing; when this amount of arsenate is added to a 0.1 mg. SiO_2 standard it matches a similar standard without the added arsenate after 10 minutes, but after 20 minutes is definitely darker. However, as arsenate, like tungstate, is very rarely present in biological material, it has not been further considered. Iron, which interferes with the production of both the yellow and blue colours, will be referred to later. Phosphate gives a blue colour in the presence of molybdic acid and aminonaphtholsulphonic acid at almost all acidities. As phosphorus is an invariable constituent of all tissues its removal is necessary before the silica can be estimated.

Precipitation of phosphate. Of the several reagents which are used for precipitating phosphate from solution probably magnesia mixture, calcium chloride and iron chloride, all used in the presence of ammonium hydroxide, are the most common. Ferric chloride was suggested by Atkins and Wilson [1926] to remove phosphate from silicate solutions, but it has been shown by Thayer [1930] that variable amounts of ferric silicate are precipitated along with the phosphate. Magnesia mixture has been successfully used by several workers, particularly with the yellow colour method, where it appears to give complete precipitation of the phosphate without removing any of the silicate. Its use with the blue colour method has not, however, been a complete success. The phosphate can be quantitatively removed, and the silicate appears to pass quantitatively into the filtrate, but the high concentration of ammonium hydroxide necessary to bring about complete precipitation of phosphate in the presence of magnesium seems to interfere with the production of the blue colour with aminonaphtholsulphonic acid.

Calcium chloride was used in the carefully controlled experiments of Thayer [1930] and of Liebknecht, Gerb and Bauer [1931], and found to be satisfactory for the complete removal of phosphate without the simultaneous removal of any silicate. Its use has been adopted in the present method and with good results. The calcium does not appear to affect the colour production in any way, and the amount of ammonia necessary to bring about precipitation of calcium phosphate is not great enough to interfere seriously with the development of the blue colour¹.

Removal of iron. The presence of iron may interfere with the colorimetric determination of silica in two ways. If the iron remains in the solution in which the blue colour is developed from the silicomolybdic acid complex, a greenish tint is produced and the colour can neither be matched against a simple silicate standard, nor is it proportional to the amount of silica present. It is necessary, therefore, to remove the iron previous to bringing up the colour. As has already been pointed out, if there be insufficient phosphate present, it is impossible to precipitate iron from solution in an alkaline medium without loss of some of the silica as ferric silicate. It is thus not practicable to remove the iron along with the phosphate by precipitation with ammonia and calcium chloride.

Liebknecht *et al.* [1931] accomplished the simultaneous precipitation of phosphate and iron by heating the solution to which had been added calcium chloride and calcium carbonate, together with a slight excess of phosphate over that necessary to precipitate all the iron. With the present blue colour method, it was found that this procedure was not sufficient to remove all the phosphate. The yellow colour method which was used by Liebknecht is not sufficiently sensitive to phosphate to be interfered with by traces coming through in the filtrate. Unfortunately, such is not the case with the present method. After filtering off the iron precipitate, together with the calcium carbonate, however, the small amount of phosphate remaining can easily be removed by making the filtrate alkaline with ammonia and filtering off the calcium phosphate.

¹ For routine analyses it has been found expedient to neglect the slight depression of the blue colour due to the presence of ammonia in the test (as well as of chloride in the case of urine). In "recovery" experiments the interfering substance has been added to the standard in amount sufficient to equal its concentration in the test. While it is recognised that this is not in conformity with the best practice, it seems justified until some other means is devised of eliminating the effect of interfering substances.

Reagents required.

Solutions of a neutral or acid reaction are best preserved in 100 cc. or 250 cc. glass-stoppered bottles of Jena or other good glass of low solubility. Alkaline solutions must be preserved in wax.

Silica standard. Sodium silicate ($\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$) can be obtained in very pure condition on the market. The crystals are readily soluble in water and solutions made from them serve as a good standard for silica determinations. 47.3 mg. dissolved in 100 cc. of distilled water give a solution containing the equivalent of 0.1 mg. SiO_2 per cc. This solution must be standardised for its silica content, however, as the water content of the sodium silicate is not constant. Standardisation can be accomplished by evaporating successive 5 cc. portions in a small platinum crucible until a total of 10 or 20 cc. has been used. By treating the residue with sulphuric acid and igniting, the sodium silicate is transformed into sodium sulphate and silica. The loss in weight after treatment with hydrofluoric and sulphuric acids is taken as the weight of silica. For this procedure a micro-balance is necessary and very pure reagents must be used [Morgan and King, 1932].

A more convenient method of standardisation is to compare the yellow colour developed on treatment with ammonium molybdate and sulphuric acid with that of the picric acid standard of King and Lucas [1928]¹. Yellow colours are best matched in Hohner tubes. The comparison can conveniently be made with 2 cc. of the picric acid standard diluted to 100 cc. with water and equivalent to 0.1 mg. of SiO_2 . 1.0 cc. of the silica standard (or 10 cc. of the 1 in 10 dilution used for routine work) is treated with 4 cc. of 5 % molybdate in $N \text{H}_2\text{SO}_4$ and water to 100 cc. If it is desired to make the comparison in a colorimeter a more intense colour is needed. 10 cc. of the silicate solution may then be treated with 1 cc. of the molybdate and water to 25 cc., and 20 cc. of the picric acid standard diluted with water to 25 cc. to give a colour equivalent to 1.0 mg. SiO_2 .

Finally, a silicate solution of known strength can be made by fusing a weighed amount of pure silica with sodium carbonate in a platinum dish and dissolving the melt in a definite volume of water.

A dilute standard for routine use may be prepared by dilution of the strong standard 1 in 10 with water. This corresponds to a concentration of 0.01 mg. SiO_2 per cc. Both standards should be preserved from bacterial growth by saturation with chloroform (0.5 cc. of chloroform for each 100 cc. of solution, shaken until dissolved). These standards will remain unaltered for months, if kept in a cool place in glass-stoppered bottles of resistant glass—Jena glassware has been found particularly suitable. It has been considered advisable, however, to prepare new standards at least once a month.

Ammonium molybdate. If a few g. of the molybdate dissolved in water and treated with a little sulphuric acid show any yellow colour when viewed in a cylinder against a white background, it should be discarded as containing silicic acid in quantity sufficient to give a considerable blank. Most brands tested have been silicate-free. The solution for use should be prepared by dissolving 5 g. of ammonium molybdate of the best quality in enough N sulphuric acid to give 100 cc. The small amount of insoluble sediment should be removed by allowing the solution to stand until clear and by filtering through a silica-free paper (Whatman's 32, 40 or 42). 2 cc. of this solution diluted to 25 cc. with water and 0.5 cc. of the reducing agent should give no blue colour in 1 hour. On longer standing a colour develops which may become very intense after a day or two.

Aminonaphtholsulphonic acid. 0.5 g. of the 1:2:4-acid is dissolved in water with 30 g. of sodium bisulphite and 6 g. of crystalline sodium sulphite and diluted to 250 cc. After standing overnight the solution is filtered clear.

The reducing agent should be preserved in a brown glass bottle and kept in a cool place.

$N \text{H}_2\text{SO}_4$. 28 cc. of concentrated sulphuric acid diluted to 1 litre with distilled water.

Calcium chloride. 5 g. of the pure anhydrous salt made to 100 cc. with distilled water and filtered if not perfectly clear.

Ammonium hydroxide. All samples of concentrated ammonia coming to this laboratory have contained too much silicate in solution to be directly usable when diluted. It is necessary, there-

¹ 25.6 mg. of pure vacuum-dried picric acid in 1 litre of water is of the same intensity of yellow colour as a solution of 50 mg. of silica per litre when treated with the reagents.

fore, to drive the ammonia gas over through a condenser into a well-cooled paraffined bottle of distilled water until the desired strength of N is reached. This can most readily be done by dropping strong sodium hydroxide into a flask containing the concentrated ammonia solution, or by gently heating the latter. If a tank of compressed ammonia is available, it may be bubbled directly into the distilled water. The ammonium hydroxide solution should always be kept in a paraffined or wax bottle.

Sodium hydroxide. 2.3 g. of metallic sodium dissolved by dropping, in small pieces, into 100 cc. of water in a nickel crucible give N NaOH. This solution must be kept in a wax bottle and glass pipettes brought into it only for the briefest possible time.

Ammonium phosphate. 2 g. of ammonium dihydrogen phosphate dissolved in 100 cc. of water. The acid ammonium salt of phosphoric acid seldom gives any test for silica.

All these solutions must be tested periodically to determine that they are silicate-free. 2 or more cc. (in the case of acid or alkali after neutralising to phenolphthalein) should be treated with water, 2 cc. of molybdate and 0.5 cc. of sulphonic acid to 25 cc. Only a very faint blue colour is allowable.

Ferric chloride (for blood-analysis)—10 cc. of a stock solution of 10 % ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) in 0.2 N HCl diluted to 100 cc. with water.

Sodium acetate (for blood-analysis)—10 cc. of a stock 15 % solution of crystalline sodium acetate ($\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$) and 2.8 cc. of N NaOH made to 100 cc. with water. This amount of alkali insures that the protein-free filtrate will be of about p_H 6.

Anhydrous sodium carbonate. Kalbaum's "*pro analyse*" Na_2CO_3 is particularly suitable for fusing the ash of tissues in silica determinations, as it is free from silica, granular and easy to use.

Activated charcoal. Kalbaum's finely powdered "Carbo Activ" is almost silica-free and is very effective in removing the yellow pigment from urine and the colour from other silicate-containing solutions.

Distilled water prepared by means of a copper and tin still and kept in glass will generally remain silica-free.

The method.

All operations involving the use of alkaline solutions must be carried out in vessels of non-siliceous material, and since nearly all determinations of silica in biological material involve the removal of phosphate at an alkaline reaction, glass must be avoided. Where it is necessary to destroy organic material, platinum crucibles are almost essential; their resistance to the action of acids and alkali and to intense heat make them the most suitable vessels in which to carry out the preliminary manipulations.

For routine analysis of urine it is obviously impossible, however, for most laboratories to possess sufficient platinum to do any large number of determinations at one time. Recourse has been had to painting glassware with a variety of coatings, by far the most suitable of which is an air-drying varnish of the synthetic formaldehyde-phenol resin bakelite¹. This material will adhere firmly to glass surfaces and will not peel off on exposure to liquids if the glass surface is thoroughly clean and dry before the coat of bakelite is applied. After being allowed to dry in the air the bakelite is best baked for a few hours in an oven at about 100°. Test-tubes treated in this way and fitted with rubber stoppers are suitable for collecting samples of urine and blood.

Even this material, however, is not completely satisfactory, as it tends to flake and scratch off glass beakers and funnels after it has been used for some time. Lately we have been fortunate in obtaining a quantity of funnels and 2-oz. drinking tumblers², crucible shaped, of a non-siliceous synthetic material

¹ Kindly supplied to us by Mr J. E. Buchan, of the Toronto branch of the Bakelite Company, Limited.

² These vessels sell under the name of "Bonnyware" and are manufactured by the Reynolds Spring Company, Jackson, Michigan, U.S.A.

stated to be a urea-formaldehyde resin. Used either uncoated or painted over with bakelite they have proved highly satisfactory.

Jena and monax laboratory glassware have been found suitable for solutions and manipulations at neutral and acid reactions.

Urine. It has been found that phosphate can be completely precipitated, the colour removed and the silica quantitatively recovered at a 1 in 5 dilution of urine with distilled water. 5 cc. of urine are treated with 15 cc. of water and neutralised by stirring in a little solid calcium carbonate. 2 cc. of 5 % calcium chloride and 3 cc. of N NH_4OH are added, making a total of 25 cc. A small spoonful of charcoal (about 0.25 g.) is added, the mixture stirred with a bakelite stirring rod or iron wire and the vessel covered. After 1 hour precipitation of the phosphorus is usually complete. No. 32 Whatman or other silica-free papers are used for filtration. The filtrate is usually water clear and shows no test for phosphorus. A "blank" consisting of 20 cc. water, a little CaCO_3 , 2 cc. CaCl_2 , 3 cc. NH_4OH and a spoonful of charcoal is always set up at the same time as the urine specimens, and its silica value must be subtracted from those found for the samples of urine.

15 cc. portions of the filtrates are measured into 25 cc. volumetric flasks, and a drop of phenolphthalein is added, followed immediately by N H_2SO_4 , until the pink colour is discharged. Standards are prepared by measuring 2, 5 and 10 cc. portions of the dilute standard solution into 25 cc. flasks and adding water to about 15 cc. The ammonium molybdate in sulphuric acid (2 cc.) is now added to each flask and 0.5 cc. of the aminonaphtholsulphonic acid, the flasks being gently shaken after each addition. After diluting to the mark with distilled water, the flasks are inverted and the solutions mixed. The blue colours are allowed to develop for 5 minutes before reading against the nearest standard in a Duboscq colorimeter.

Each filtrate must be tested for the presence of phosphate, which, if found in more than the faintest traces, will render the silica determination useless. 1 cc. of filtrate is made acid in a small test-tube with 2 cc. of N H_2SO_4 and 4 drops of molybdate and 2 of aminonaphtholsulphonic acid are added. At this high acidity the silico-molybdic acid does not react and any blue colour will indicate the presence of phosphate. Occasionally urines are encountered in which the 2 cc. of CaCl_2 are insufficient to remove all the phosphate. This has been the case with the urine of some dogs which seemed to have a very high phosphorus content.

Results are best expressed in mg. SiO_2 per 100 cc. of urine. The calculated mg. of SiO_2 in each test will, when the blank is deducted, represent the SiO_2 content of 3 cc. of urine, since each 5 cc. of filtrate represents 1 cc. of urine. This value multiplied by 100/3 will, then, give the silica content in mg. SiO_2 per 100 cc.

Some results are shown in Table I. In each case samples of 5 cc. of urine were taken and to them a stated amount of silica, in the shape of a measured quantity of the silicate standard, was added to test the efficiency of recovery. The figures shown have been taken at random from a large series of determinations and illustrate the accuracy which may be expected with the method.

The presence of albumin in the urine may sometimes cause trouble because of the protein precipitate produced when the molybdate is added to the neutralised filtrate. If this should occur, the determination can be saved by immediately centrifuging off the precipitate. Very little of the silico-molybdic acid is carried down with the precipitate, as is shown by the fact that almost as good recovery of added silicate can be made in the presence of protein as in

Table I.

Urine	SiO ₂ per 100 cc. mg.	SiO ₂ added to 5 cc. urine mg.	SiO ₂ recovered mg.
Human	1.07	0.128	0.125
	0.93		0.123
	1.60	0.125	0.130
	1.80		0.120
	1.20	0.125	0.121
	1.26		
Rabbit	19.0	0.250	0.260
	19.5		0.260
	6.0	0.250	0.230
	5.5		0.235
Dog	0.90	0.125	0.115

its absence. That the "soluble silica" value represents the total silica content of the urine has been shown by the fact that the values have in all cases tested agreed with those obtained on ashed samples of the urine treated according to the method for tissues.

Blood. Two procedures are used for the determination of silica in blood, depending on whether it is desired to estimate the total or soluble silicate. In practice it has been found that the two are almost always equal. This appears to indicate the absence of any insoluble particulate silica and of any organic compound of silicon in the blood, unless such a compound either reacts directly with the molybdate, which is unlikely, or is extremely susceptible to hydrolysis.

Soluble silica may conveniently be determined on 2 cc. of oxalated whole blood, which is laked with 36 cc. of water in a 150 cc. conical flask. Blood is preferably run directly from a graduated syringe into the water without the use of a pipette. If it is not desired to do the determination immediately, the blood should be kept in a test-tube coated with bakelite. The same amount of anticoagulant (oxalate) should be used in the blank.

The procedure for deproteinising blood is based on a modification of the Hoffman [1931] ferric acetate method by Dr H. R. D. Jacobs of Chicago. Various other methods of deproteinising had been tried with indifferent success. Tungstic acid and molybdic acid could not be used, because of interference with the reaction; the zinc, iron and copper hydroxide precipitates removed the greater part of any added silica, and recovery was poor with trichloroacetic acid. The basic ferric acetate method has the advantage that the protein is precipitated in an almost neutral (slightly acid) medium, at which reaction none of the silica is removed. It has the further advantage, moreover, of removing the phosphate, together with the protein and of giving a filtrate very low in extraneous salt material. Small amounts of sodium chloride and acetate remain, but the iron is completely removed.

To the 2 cc. of blood laked with 36 cc. of water are added 1 cc. of the ferric chloride solution, 1 cc. of sodium acetate and about 50 mg. of charcoal ($\frac{1}{4}$ teaspoon). The flasks are heated directly over a Bunsen flame, with agitation, until the mixture has boiled for a few seconds. A dark brown coagulated mass of protein is formed which should be filtered hot through two pieces of folded No. 42 Whatman filter-paper. The filtrate is protein- and phosphate-free.

After the filtrate has cooled to room temperature, 20 cc. (representing 1 cc. of blood) are measured into a 25 cc. volumetric flask. Standards of 5 and 2 cc. of dilute silicate are prepared and 2 cc. of molybdate and 0.5 cc. sulphonic acid added to each flask. The colours are matched after 10 minutes.

Of the many determinations on normal blood which have been made using this procedure, only a very few have shown more than a faint blue colour corresponding to that given by about 2 cc. of dilute standard. The results have agreed, however, with determinations of total silica on larger samples of blood both by colorimetric and gravimetric procedures, and when extra silicate has been added to the laked blood before deproteinising it has always been recovered in the filtrate.

In Table II are the results of some representative experiments in which added silica was recovered in the protein-free filtrate. In each case 2 cc. samples of blood were added to each of 4 flasks, 2 of which contained 36 cc. of water and the other 2 a measured amount of the dilute silicate standard with water to 36 cc.

Table II. *Soluble silica in blood.*

Blood species	SiO ₂ in 20 cc. filtrate (1 cc. blood) mg.	SiO ₂ added mg.	SiO ₂ recovered mg.
Rabbit	0.020	0.140	0.140
	0.015	0.140	0.145
Dog	0.023	0.100	0.097
	0.022	0.100	0.104
Human	0.015	0.070	0.076

Total silica must, of course, be determined in an ashed specimen of blood. 5 cc. of whole blood are measured into a platinum crucible and 0.5 g. of sodium carbonate added. After drying in an oven at 110° until the following day, the blood is carefully ashed in a muffle furnace or over a gas burner. (The determination may be completed on the same day, but great care is necessary in heating the crucible to drive off the water as the blood tends to froth badly.) When the ashing is complete, or when only a small amount of carbonaceous material remains, the crucible is held in a Meker burner until fusion is complete. The cooled melt is warmed with distilled water on the water-bath until it is free from the crucible and appears to be dissolved. Iron remains undissolved as a flaky brown precipitate. $N H_2SO_4$ is added to the mixture until a small drop from a fine wire gives a red colour on a piece of methyl orange paper used as outside indicator. Warming on the water-bath is continued until all the carbon dioxide appears to be driven off. A small spoonful of calcium carbonate is then added, together with 1 cc. of ammonium phosphate and 4 cc. of calcium chloride solutions. After warming on the water-bath for 10 minutes precipitation of the iron as $FePO_4$ will be complete.

The cooled mixture is rinsed into a 30 cc. graduated cylinder or flask, brought to the mark with distilled water and mixed. 25 cc. of filtrate are measured into a bakelite-coated dish, or into the platinum crucible, and 2.75 cc. of ammonia added. After 1 hour the precipitated calcium phosphate is filtered off and 20 cc.

Table III. *Total silica in blood.*

Blood species	SiO ₂ in 100 cc. blood mg.	SiO ₂ added mg.	SiO ₂ recovered mg.
Rabbit	0.75	0.140	0.129
	1.26	0.140	0.140
Human	0.90	0.140	0.118

of filtrate taken for the determination. The silica value for 20 cc. of filtrate represents the silica content of 3 cc. of blood. Some results for total silica obtained by the ashing method are contained in Table III. It will be seen that they are in substantial agreement with those obtained by the deproteinising method.

Tissues. Before a determination is made on a sample of tissue it is necessary to destroy the organic material by ashing either in the wet or in the dry way. About 1 g. of fresh tissue or 0.25 g. of pulverised dried tissue (at 105° until of constant weight) is weighed accurately into a platinum crucible. For wet ashing nitric acid with the addition of ammonium nitrate is the most satisfactory reagent. 5 cc. of concentrated nitric acid are added to the crucible, and the mixture is heated gently on a hot plate until frothing has ceased. A small amount of ammonium nitrate is added, the mixture taken carefully to dryness and the crucible held in a flame until red. If the organic matter is not completely burned off, the treatment with a little nitric acid and ammonium nitrate should be repeated.

Where an electric muffle furnace is available, the tissue is more conveniently ashed by heating at a dull red heat until destruction of organic matter is complete or until only a small amount of carbonaceous material remains. If the amount of tissue to be ashed is small (less than 200 mg.) it may be heated directly with sodium carbonate in a flame. Destruction of the organic material takes place quickly and evenly in the melting mass.

The silica in the ash remaining after either the wet or dry treatment may be determined in one of two ways. The first consists in rendering the silica insoluble by boiling the ash in the crucible with 2 cc. of perchloric acid for 15 minutes. As all the other constituents of the ash are rendered soluble by this treatment they may be filtered off, leaving the silica behind. Filtration is best carried out by means of a "filter-stick".¹ Water is added to the cooled crucible after boiling with perchloric acid and the soluble salts sucked away through the filter-paper plug. The residue of silica is washed with water and the washings sucked off. The plug of paper with adhering silica is dropped into the crucible by pushing the inner tube down through the outer, and the filter-stick is washed clean by means of the wash-bottle. After evaporating to dryness, the contents of the crucible are ignited and the silica determined either by weighing before and after treatment with hydrofluoric acid [Morgan and King, 1932] or by colorimetric estimation of its solution after fusion with 0.25 g. sodium carbonate. This procedure may also be used for blood.

The second method consists in rendering the silica soluble by fusing the ash with 0.25 g. sodium carbonate and taking up in warm water, or by warming with 2 cc. *N* NaOH and a little water. The solution is neutralised to methyl orange on the hot water-bath with *N* H₂SO₄ and warmed until no more carbon dioxide is evolved. While the solution is still hot 0.5 cc. of ammonium phosphate, 50 mg. (approx.) of calcium carbonate and 4 cc. of calcium chloride are added. After 10 minutes the mixture is cooled and transferred from the crucible to a cylinder or flask and diluted to 30 cc. After filtering through a No. 32 Whatman paper, 25 cc. are transferred to a crucible or bakelited vessel and 2.5 cc. of *N* NH₄OH are added to complete the precipitation of phosphate. This usually takes about 1 hour.

¹ This consists of two concentric glass tubes, the inner one being a sliding fit in the outer and having a fine bore. The joint between the tubes is covered with a rubber sleeve. The inner tube is tapered at one end for attachment to a suction pump and its other end is pushed almost to the orifice of the outer tube, leaving a small space which is filled with a filter-paper plug.

For the colorimetric determination an aliquot portion of filtrate (*e.g.* 20 cc.) is measured into a 25 cc. flask and neutralised to phenolphthalein. Standards are prepared corresponding to 0.02, 0.05 and 0.1 mg. of SiO_2 , and 2 cc. of molybdate and 0.5 cc. of sulphonic acid are added to each flask. Water is added to the mark, the flasks inverted and shaken and the colours matched after 5 minutes. The blank resulting from the reagents used in this determination will usually be too small to measure and may be neglected.

On the basis of the amount found in the aliquot portion of filtrate taken for the determination, the silica content of the sample of tissue is calculated. Results are best expressed in mg. per 100 g. of tissue. In this way the figures are kept in line with the method of recording results for blood and urine.

Representative results for determinations on several tissues with comparative figures for the different procedures and recovery of added silica are given in Table IV.

Table IV. *Silica content of tissues by colorimetric and gravimetric procedures.*

Tissue	Colorimetric method Ash treated with		Gravimetric method	SiO_2 added mg.	SiO_2 recovered mg.
	Na_2CO_3 mg. SiO_2 per 100 g. of tissue	NaOH mg. SiO_2 per 100 g. of tissue			
Lung:					
Ox	—	87.2	—	0.360	0.357
	—	81.2	—	0.360	0.339
Calf	4.8	4.2	—	0.360	0.333
	4.4	—	—	0.350	0.315
Rabbit	18.4	14.2	—	—	—
Rabbit exposed to SiO_2 dust	856	856	829	—	—
Silicotic	860	—	810	—	—
	7115	—	7310	—	—
	—	—	7682	—	—
Liver:					
Rabbit	20.8	—	—	0.120	0.131
Rabbit	12.4	—	—	0.125	0.121
Rabbit	—	13.0	13.3	—	—
	—	—	12.3	—	—
Rabbit	30.6	30.6	29.8	—	—
	—	—	28.5	—	—
Kidney:					
Rabbit	14.4	—	—	0.117	0.117
Rabbit	16.4	—	—	0.125	0.114
Dog	16.4	—	18.2	—	—
Dog	50.0	47.6	52.3	—	—
	Residue after HClO_4 treatment				
Lung:					
Rabbit exposed to SiO_2 dust	895	—	829	—	—
	785	—	790	—	—

SUMMARY.

1. Several procedures for determining silica in biological material have been considered.

2. A colorimetric method which makes use of the blue colour produced when silicomolybdic acid is reduced by 1:2:4-aminonaphtholsulphonic acid is described.

3. The potentially colour-producing phosphate is removed by precipitation with calcium chloride and ammonia and the silicate recovered in the filtrate.

4. Iron is removed as ferric phosphate in the presence of calcium carbonate on the acid side of neutrality.
5. Blood is deproteinised by the basic ferric acetate method, the silica remaining in solution.
6. The procedure has been adapted to urine, blood and a variety of other tissues, and figures have been presented to demonstrate the degree of accuracy which may be expected.

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