CXVI. THE BIOCHEMISTRY OF SILICIC ACID

VIII. THE DETERMINATION OF SILICA

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THE method employed for the determination of silica in tissues and body fluids must depend on the amount and nature of the silica present. For tissues such as the lungs of workers in dusty atmospheres, where a great deal of mineral matter may be present, the ordinary gravimetric procedures are applicable. In the case of normal tissues of low silica content a micro-gravimetric method is suitable [Morgan & King, 1932], but a colorimetric method may be preferred because of the absence of the technical difficulties inherent in the micro-gravimetric procedure. The quantities of silica normally encountered in urine and blood are so small as usually to demand a colorimetric method.

The silica of lungs and lymph glands consists largely of particles of mineral dust which have gained access to the tissues through the air passages. Much of it, being derived from refractory silicate rocks, is insoluble in chemical reagents (e.g. quartz, mica). This material must be decomposed before it can be estimated. For this reason it is essential to fuse the tissue, or its ash, with sodium carbonate. The endogenous "silica of constitution" of other tissues is more easily brought into solution. But it is found most convenient to fuse these also with sodium carbonate, when there can be no question that all of the silica in the tissue is being determined. In blood and urine all the silica appears to be in a soluble state. This makes possible a direct determination through a chemical reaction leading to the production of a colour without the necessity of preliminary decomposition with sodium carbonate.

Several alterations and improvements have been made in the methods of silica analysis employed in this investigation since the publication of the first paper of the series [King & Stantial, 1933] on the "micro-determination of silica". These are outlined in the present communication, together with directions for the application of the method to a variety of biological materials, both normal and pathological, and to mineral dusts. The description of a gravimetric procedure is likewise given.

Methods

Preparation of tissue for analysis. There is a large degree of variation in the silica content of different parts of some tissues. This is notably the case with lungs, where the inhaled siliceous dust settles in largest amount in the region of the hilum, and becomes concentrated in the root glands. With these tissues it is essential to obtain as representative samples as is possible, so that the analysis may represent the composition of the whole organ; wherever possible the entire organ should be taken [cf. Collins & Dible, 1935]. Tissues like liver and muscle are much more uniform in composition, and need not be sampled so carefully. The analysis is preferably done on dried and powdered tissue rather than on fresh tissue. A block of tissue or the whole organ is trimmed free from adherent material, and is washed first with tap water and then with distilled water. It is

cut in small pieces and placed on metal trays in an oven at 105° to dry. After 24 hr. in the oven it will usually have reached constant weight. The hard lumps of dried tissue are pounded to a powder in an iron mortar and again left in the oven overnight. If a power-driven mortar and pestle are available, the reduction of the sample to a fine powder is an easy matter. If it has to be done by hand, the process is facilitated by screening off the finest material several times during the grinding. The powdered dry tissue is finally thoroughly mixed on a sheet of paper, a portion is taken for analysis and the remainder stored in a closely stoppered bottle or in envelopes in a desiccator.

Gravimetric analysis—semi-micro-method (for tissues having a high silica content). The lungs of workers who have been exposed to the inhalation of silica dust may contain 0.5–5 % or more silica on the dry weight. An amount of the dry powdered tissue is taken which may be expected to yield 5–50 mg. SiO_2 . The sample is intimately mixed with 4 times its weight of anhydrous Na_2CO_3 in a platinum crucible of 10–20 ml. capacity. The mixture is carefully “smoked off” and heating continued until a clear liquid melt is obtained. The cooled contents of the crucible are dissolved in water on the steam bath. Conc. HCl is carefully added until the mixture is acid to methyl orange, and 3 or 4 ml. extra added. The mixture is evaporated to dryness on the steam bath, and heated for 1 hr. at 105° in the oven to dehydrate the silica. The residue is moistened with a few drops of water and HCl , and again heated for 1 hr. at 105° .

The dehydrated silica is separated from the other constituents of the residue by bringing the latter into solution with dil. HCl . The residue is moistened with conc. HCl and the crucible three-quarters filled with water. The mixture is warmed on the steam bath and stirred with a glass rod. When solution appears to be complete, the crucible is removed from the bath and allowed to stand for a few minutes for the silica precipitate to settle.

Filtration of the silica is accomplished without removing it from the crucible by means of the filter stick illustrated in Fig. 1. In this way it is possible to complete all the operations involved in the analysis in the original vessel without

Fig. 1. Special filter stick for silica analysis. A, sintered glass filter stick. B, jacket. C, assembled filtering apparatus. (Obtainable from Messrs Gallenkamp.)

transferring the precipitate. This makes for greater accuracy in the method by avoiding the possibilities of error involved in transference. Filtration by sucking off the filtrate and washing is, moreover, faster than filtration by gravity. As a filtering medium, filter paper pulp shaken up with water, or powdered anthracene is suitable. The latter is easily eliminated by mild heating, whereas paper must be destroyed by ignition. The filtering medium is packed tightly into the cavity formed at the flared end of the filtering apparatus. A piece of fine rubber

suction tubing connects the filter stick to a small suction flask. Suction may be applied by means of a water pump, or, preferably, by mouth with a piece of rubber tubing carrying a pinch-cock attached to the side arm of the flask.

The filter stick is held so that its filtering surface is just below the level of the liquid in the crucible, and the filtrate sucked off under mild suction. The crucible is filled again with warm dil. HCl and the precipitate stirred, allowed to settle, and the supernatant again sucked off. Washing is repeated once with dil. HCl and twice with water. The precipitate is finally sucked dry in the bottom of the crucible.

The plug of filtering medium is dropped out of the filtering apparatus into the crucible by pushing the inner tube of the apparatus (the filter stick) a few mm. through the rubber collar which attaches it to the outer tube or jacket. Particles of silica adhering to the glass are washed into the crucible with alcohol or water from a wash bottle. Any liquid in the crucible is now evaporated on the water bath. The crucible is dried in an oven, or carefully with a flame, and then slowly ignited to red heat, cooled in a desiccator and weighed. HF (5 ml.) is added and a few drops of H_2SO_4 . The crucible is gently heated to evaporate off the HF and SiF_4 , and then more strongly to drive off the H_2SO_4 . It is then ignited, cooled and weighed. The loss in weight on treatment with HF is taken as the weight of silica. Some typical results obtained by this method are given in Table I.

Table I. *Gravimetric analyses of silicotic tissues*

Tissue	Wt. of dry sample g.	mg. SiO_2 found	% SiO_2
Spl��en 108 (1)	2.000	10.2	0.51
(2)	2.000	10.9	0.54
(3)	2.000	10.6	0.53
Lung 109 (1)	0.250	2.2	0.88
(2)	1.000	9.2	0.92
(3)	2.000	18.3	0.91
Lung 110 (1)	2.000	16.0	0.80
(2)	2.000	16.2	0.81
Lung 111 (1)	1.000	13.4	1.34
(2)	2.000	28.2	1.41
Lung 116	2.127	142.7	6.71
Lung 121 (1)	1.030	17.2	1.67
(2)	1.103	17.6	1.60

The colorimetric determination of silica

The colorimetric method adopted for the determination of silica in tissues consists in the production of a yellow silicomolybdic acid complex. This colour may be utilized as such, or it may be reduced by 1:2:4-aminonaphtholsulphonic acid to give a blue colour. Both colours are proportional to the amount of silica present [King, 1926; King & Stantial, 1933] and can be compared colorimetrically with those produced in standard solutions containing a known amount of silica.

The method is applicable to much smaller amounts of silica-containing material and can be executed in a fraction of the time that is necessary for gravimetric determinations. If the procedure is rigidly followed and due precautions are taken to insure the purity of the reagents used and to eliminate interfering substances, the results obtained are nearly as accurate as those given by the gravimetric procedure.

The yellow colour method is much less sensitive than the blue colour method. It may be used with any materials having a high silica content, such as silicotic tissue, lungs and lymph glands. The sensitive blue colour method may be used for any material, but is especially suitable for those of low silica content, normal tissues, blood, urine etc. The yellow silicomolybdic acid colour is preferably read in a photoelectric colorimeter, a photometer, or in Nessler or Hehner tubes. It is difficult to match in a Duboscq colorimeter. The reduced silicomolybdic acid blue colour can be assessed in any type of colorimeter; it is easily read in a Duboscq instrument. The comparison is greatly facilitated and made much more accurate if made in the light of a sodium lamp, such as those supplied with the Hellige and Klett instruments.

Several substances interfere with the reaction giving the silicomolybdic blue colour. Of these phosphate and iron are the most important in biological material. Phosphate gives a blue colour in the presence of molybdic acid and aminonaphtholsulphonic acid at all acidities. As phosphorus is an invariable constituent of animal tissues, its removal is necessary before the silica can be estimated. The presence of iron in the solution leads to the development of a greenish tint, and the colour can neither be matched against a simple silicate standard, nor is it proportional to the amount of silica present. In the original method the phosphate and iron were removed in two stages, the iron by precipitation with excess ammonium dihydrogen phosphate and the phosphate by precipitation with calcium chloride and ammonia.

The simultaneous elimination of phosphate and iron from a silicate-containing solution is accomplished by the basic ferric acetate procedure of Jacobs [1931], which was previously used for the preparation of a protein- and phosphate-free filtrate of blood. By this means the alkaline precipitation with ammonia is avoided and the precipitation of phosphate can therefore be carried out in glass vessels. Moreover, both phosphate and iron are precipitated in one step instead of the two which were previously necessary. The method removes phosphates as ferric phosphate, excess iron as basic ferric acetate and any colloidal or suspended material which may be present, such as protein, fat and debris (and in the case of urine, part of the pigment).

Solutions and reagents

Silica standard. Sodium silicofluoride. The most satisfactory standard used thus far is a solution of sodium silicofluoride. This salt is a light anhydrous non-hygroscopic powder which hydrolyses in solution to give silicic acid. 314 mg. of the solid in 1 l. of water give a concentration equivalent to 1 mg. SiO_2 in 10 ml. ("strong standard"). A "weak standard" (0.1 mg. SiO_2 /10 ml.) is made by diluting the "strong standard" 1 in 10. It has been found advisable to make these fresh each week.

10 N H_2SO_4 . 278 ml. of conc. acid to 1 l. with water.

N H_2SO_4 . 1 in 10 dilution of 10 N.

N NaOH . The NaOH , to be silica-free, must be prepared from the metal. 2.3 g. metallic sodium are dissolved in 100 ml. of water in a nickle crucible, and preserved in a wax bottle. B.D.H. "sodium hydroxide pure (from sodium)" is satisfactory. Glass pipettes should be brought into the NaOH solution only for the briefest possible times.

Ferric chloride in hydrochloric acid. 1 % FeCl_3 , 6 H_2O in 0.02 N HCl .

Sodium acetate in sodium hydroxide. 1.5% $\text{C}_2\text{H}_3\text{O}_2\text{Na}$, 3 H_2O in 0.028 N NaOH . The ferric chloride and sodium acetate solutions, when mixed in equal amount, diluted with water, heated and filtered, yield a fluid of approximately pH 6.

Acid molybdate. 5 % ammonium molybdate in $N H_2SO_4$.

Reducing agent. 0.2 % 1:2:4-aminonaphtholsulphonic acid in 2.4 % Na_2SO_3 , 7 H_2O and 12 % $NaHSO_3$.

Ammonium phosphate solution. 2 % $(NH_4)H_2PO_4$.

Calcium chloride. 2 % $CaCl_2$.

Perchloric acid. 60 % $HClO_4$.

Acetic acid. Glacial and 10 %.

Potassium ferrocyanide. 5 % aqueous solution.

Pyramidon. 1 % aqueous solution.

The original article should be consulted for details of the preparation of solutions, and for the precautions which must be observed in the use of the method. Frequent "blank" determinations should be carried out on the several solutions to determine that they are silica-free. Only chemical reagents of analytical grade should be used.

The preservation of fluids for analysis should always be in non-glass vessels. Urine and blood which are kept in glass increase in silica content. The dissolution of silica from the glass can be retarded by the addition of acid to the fluid; this is only possible in the case of urine. The Lusteroid test-tubes made of heavy cellophane by the International Equipment Co. of Boston, U.S.A. (and sold by Messrs Gallenkamp) are satisfactory for the collection and preservation of fluids for silica analyses.

Tissue analysis: 0.1–0.5 g. (depending on its silica content) of dry tissue is weighed into a platinum crucible or dish (preferably of large size, 25–50 ml.). Anhydrous Na_2CO_3 equal to about four times the weight of tissue is added and the mixture carefully heated. After the sample has been "smoked off" destruction of the remaining organic matter takes place smoothly and rapidly in the melting mass. The heating is continued until a clear melt is obtained. Water (about 10 ml.) is added to the cooled crucible, and the fused material dissolved by heating on the steam bath. When solution is complete the crucible is cooled, and its contents washed through a funnel into a 100 ml. volumetric flask. The solution is carefully neutralized with $N H_2SO_4$, using Congo red¹ as indicator, and shaken vigorously the while to rid the mixture of CO_2 . The titration is facilitated by first neutralizing to phenolphthalein with 10 $N H_2SO_4$ and then finishing the titration with Congo red and $N H_2SO_4$. Approximately the same amount of acid is necessary for the second part of the titration as for the first. 20 ml. each of ferric chloride and sodium acetate solutions are now added. The volume is adjusted to 100 ml., the mixture shaken and poured into a 250 ml. conical flask. It is brought rapidly to the boiling point with vigorous shaking. By this means the iron and phosphate are completely removed as ferric phosphate and basic ferric acetate. The hot mixture is poured on a folded 30 or 32 Whatman filter paper. A water-clear filtrate is obtained which is suitable for the colorimetric silica estimation.

In order to determine that the solution is now phosphate-free, 1 ml. of the cooled filtrate should be tested for $PO_4^{=}$ ion by the addition of 2 drops of 10 $N H_2SO_4$, 2 drops of molybdate and 1 of reducing agent. The development of any blue colour indicates the presence of phosphate, and the basic ferric acetate precipitation should be repeated by heating 50 ml. of the filtrate with 10 ml. of ferric chloride and of sodium acetate.

Congo red is preferred as indicator because it is completely removed in the subsequent precipitation with basic ferric acetate. Other indicators, e.g. methyl orange, are not so removed, and make the final colorimetry difficult or impossible.

A second 1 ml. portion of the filtrate is tested for iron by the addition of 2 drops of potassium ferrocyanide solution and a drop of dilute acetic acid. Careful neutralization of the solution before the ferric acetate treatment usually ensures that no phosphate or iron will come through in the filtrate.

Yellow colour method

An aliquot portion of the filtrate—10, 25 or 50 ml. according to the amount of silica expected—is placed in a 50 ml. flask or cylinder. For standards 2, 5 and 10 ml. of “strong standard” (0.2, 0.5 and 1.0 mg. SiO_2) are transferred to 50 ml. flasks. The volumes of tests and standards are adjusted to 50 ml., and 4 ml. of acid molybdate are added to each. The yellow colours are compared after 10 min. in Nessler or Hehner tubes, or in a photometer or photoelectric colorimeter.

Blue colour method

An amount of the filtrate which may be expected to contain from 0.02 to 0.12 mg. SiO_2 is transferred to a 25 ml. volumetric flask. 2, 5 and 10 ml. portions of the “weak standard” (equivalent to 0.02, 0.05 and 0.1 mg. SiO_2) are pipetted into similar flasks. Water is added to about 20 ml., and then 2 ml. of acid molybdate and 0.5 ml. of reducing agent. The flasks are shaken, and kept for 10 min. for the colours to develop. 10N H_2SO_4 (1 ml.) is then added to each flask to stabilize the blue colours, the volumes are adjusted to 25 ml. and the contents of the flasks thoroughly mixed. Comparison of the test is then made against the appropriate standard.

Notes on tissue analysis. Lung and thoracic and mesenteric lymph nodes of adults are usually much richer in silica than other tissues and small samples should be used for analysis. 100 mg. (or less) of a silicotic lung powder or of peribronchial and mediastinal lymph nodes give sufficient silica for colorimetric determinations (lung tissue and lymph glands from young persons or animals however require the larger amounts).

Brain and nerve tissues have higher phosphate contents than most other tissues and here 25 ml. of the precipitating reagents should be used. (Sufficient of the reagents should be added before boiling to provide an excess of iron which is indicated by the formation of the brown precipitate of basic ferric acetate. If insufficient iron is present only white ferric phosphate is formed and the filtrate will contain at least a trace of phosphate when tested.)

Bone, which is largely calcium phosphate, is difficult to ash, and more sodium carbonate is necessary, the melt never becoming clear. After the fusion mixture has been taken up in water and neutralized, the precipitate of calcium phosphate should be removed by filtration, the precipitate and filter paper being washed thoroughly to remove all silica. The filtrate still contains much phosphate and 25 ml. each of the ferric chloride and sodium acetate precipitating reagents may be required.

Calcified lymph nodes present the same difficulties as bone and should be treated in a similar manner.

A comparison of results obtained by the yellow and blue colour methods and by gravimetric analysis is given in Tables II and III.

Table II. *Comparison of silica analyses by different methods*

Tissue	Blue colorimetric % SiO_2	Gravimetric % SiO_2
Spleen 108, abrasive soap powder worker	0.54	0.53
Lung 109, silica brick grinder	0.85	0.90
Lung 110, coal miner	0.82	0.80
Lung 112, coal miner	1.45	1.45
Lung 116, fuller's earth worker	6.70	6.71
Lung 121, coal miner	1.34	1.53

Table III. *Comparison of results of tissue analyses by yellow and blue colorimetric procedures*

Lymph glands	Age in years	Yellow colour method	Blue colour method
228, housewife	43	0.98	0.98
257, traveller	60	1.33	1.34
259, labourer	41	1.23	1.26
271, housewife	73	1.37	1.37
275, housewife	86	1.78	1.75
286, pensioner	79	0.64	0.63
289, housewife	46	1.07	1.03
290, taxi-driver	61	0.93	0.95
293, laundress	50	1.05	1.00
394, policeman	80	0.64	0.64
582, motor driver	67	0.61	0.62
586, housewife	73	2.38	2.41
597, housewife	87	1.58	1.57
601, dairyworker	44	1.33	1.35
606, housewife	62	3.27	3.33
643, pensioner	74	1.37	1.35
663, hairdresser	62	0.53	0.54

Urine analysis

5 ml. of human urine (or of the urine of carnivorous animals) are treated with 5 ml. of water and 20 ml. each of ferric chloride and sodium acetate solutions. 1 or 2 ml. of the urine of herbivorous animals, which contains a much higher concentration of silica, are sufficient. The mixture is heated rapidly to boiling in a conical flask, and poured hot on the filter paper.

The filtrate should be clear and practically colourless. It is tested for phosphate and iron. If either be found, a fresh specimen of urine should be carefully acidified with acetic acid (Congo red indicator) and the precipitation repeated. 10 or 20 ml. of filtrate are transferred to 25 ml. volumetric flasks, and 2, 5 and 10 ml. portions of "weak standard". The solutions are treated as previously described to develop the blue colours.

Table IV. *Colorimetric analysis of urine—recovery of added silica*

	mg. SiO ₂ per 100 ml.	SiO ₂ added, mg. per 100 ml.	Total SiO ₂ found, mg. per 100 ml.	mg. SiO ₂ recovered
Synthetic urine	0	0.5	0.5	—
" "	0	3.0	2.98	—
Dog urine	0.53	1.0	1.47	0.94
Sheep urine	17.2	5.0	22.1	4.9
Human urine	0.85	1.0	1.87	1.02
" "	1.56	1.0	2.63	1.07
" "	2.35	1.0	3.32	0.97
" "	2.35	5.0	7.40	5.05
Human urine after	6.85	1.0	7.87	1.02
ingestion of silicic acid	6.85	10.0	17.0	10.15

Blood analysis—soluble silica

Perchloric acid—basic ferric acetate deproteinization. "Direct" method. The deproteinization of blood with basic ferric acetate, which was previously described [King & Stantial, 1933], has not in practice always yielded a completely protein-free filtrate. For this reason a preliminary partial deproteinization with perchloric acid is now recommended. The phosphate in the filtrate is then removed by the basic ferric acetate method. The resulting filtrate contains all the

silica originally present in the blood, as is shown by comparison of the values obtained by this method with those for ashed samples of blood. Measured amounts of silicic acid, added to the blood, have been quantitatively recovered in the filtrate by the colorimetric procedure.

2 ml. of blood (fresh or oxalated—1 drop of 10 % potassium oxalate for 3 ml. blood) are laked with 10 ml. of distilled water. 1 ml. of calcium chloride solution and 0.3 ml. of perchloric acid (60 %) are added. The mixture is shaken and centrifuged. 10 ml. of the supernatant are transferred to a 15 ml. volumetric flask. A drop of phenolphthalein is added, and *N* NaOH to neutralize the perchloric acid. The pink colour is discharged and the solution made faintly acid by a small drop of glacial acetic acid. 1 ml. each of ferric chloride and sodium acetate are added and the volume adjusted to 15 ml. The mixture (transferred to a 50 ml. conical flask) is brought rapidly to the boil with vigorous shaking over a free flame, and is poured, while still hot, on a folded filter.

10 ml. of cooled filtrate (equivalent to 1 ml. of blood) with 1.2 ml. of molybdate and 0.3 ml. of aminonaphtholsulphonic acid are transferred to a 15 ml. volumetric flask for the colorimetric estimation. A 0.01 mg. SiO_2 standard is used.

“*Indirect*” method by precipitation with pyramidon. King & Watson [1936] found that silica could be completely precipitated from solution as the pyramidon salt of silicomolybdic acid. Advantage of this fact may be taken to concentrate the blue colour in a smaller volume, thereby rendering it more easily assessable in the colorimeter. 10 ml. of filtrate are treated in a conical 15 ml. centrifuge tube with 1 ml. of acid molybdate and 1 ml. of pyramidon solution. The precipitate of pyramidon silicomolybdate is allowed to flocculate during 10 min. in a beaker of water at about 60° , and is then centrifuged down at 3000–4000 r.p.m. for 10 min. The supernatant is carefully poured off and the tube inverted to drain on filter paper for 5 min. The precipitate is dissolved in 0.5 ml. of reducing agent, and diluted with 3 ml. of water and 0.5 ml. of acid molybdate. Standards consisting of 1 ml. of “weak standard” and of 0.5 ml. with 0.5 ml. of water are precipitated with 2 drops each of sodium acetate, ammonium molybdate and pyramidon.

The final 4 ml. of coloured solution obtained by this procedure are of a much more intense blue than the 15 ml. of coloured solution obtained from the same amount of blood in the “direct” method. Because of the small volume of coloured solution it is necessary to use a colorimeter equipped with micro or semi-micro attachments.

Table V. *Colorimetric analyses of blood, recovery of added silica*

	mg. SiO_2 per 100 ml. of blood	mg. SiO_2 added per 100 ml.	Total mg. found per 100 ml.	mg. SiO_2 recovered
Horse serum	1.18	2.0	3.23	2.05
“ “	1.18	3.0	4.11	2.93
Defibrinated horse blood	1.27	3.0	4.16	2.89
“ “ “	1.31	3.0	4.16	2.85
Mixed, oxalated human blood (1)	1.66	1.0	2.60	0.94
(2)	1.47	1.0	2.50	1.03
(3)	{ 1.39 1.38	{ 1.0 1.0	{ 2.22 2.25	{ 0.83 0.87
(4)	{ 1.14 1.14	{ 2.0 3.0	{ 2.91 4.28	{ 1.77 3.14
(5)	1.30	3.0	3.96	2.66
(6)	1.39	3.0	4.42	3.03

Blood serum may be treated as in the perchloric acid-basic ferric acetate method for whole blood.

The bloods used in the recovery experiments (Table V) and for comparison of methods (Table VI) were preserved with the ordinary grade of potassium oxalate in the soft glass containers used for the routine collection of blood in the hospital. It will be noted that the figures are higher than those shown in Table VII for samples of blood preserved with analytical grade potassium oxalate, or with heparin, in non-glass vessels.

Table VI. *Comparison of "direct" and "indirect" methods for blood*

	mg. of SiO ₂ found per 100 ml. of blood	
	"Direct" method (HClO ₄ -FeOAc precipitation of proteins)	"Indirect" method (pyramidon-molybdate precipitation of SiO ₂ from filtrate)
Oxalated human blood	1.30	1.39
	1.27	1.31
	0.93	1.00
	1.06	1.12
	2.63	2.63
	1.50	1.42
	1.42	1.47
	1.03	1.13
Blood to which sodium silicate has been added	2.10	2.17
	2.69	2.73
	4.16	4.16
	3.76	3.94
	3.48	3.61

Table VII. *Silica of human blood*

	mg. SiO ₂ per 100 ml. of blood	
	1.11	1.13
Blood preserved in glass	0.89	0.90
" "	0.84	0.84
Fresh blood (post-prandial)	0.47	0.45
" (1 hr. post-prandial)	0.45	0.46
" (3 hr. " ")	0.43	0.42
Heparinized blood, in cellophane tubes	0.62	0.63
" " "	0.53	0.55
" " "	0.30	0.33
Oxalated blood, in cellophane tubes	0.60	0.62
" " "	0.32	0.30
" " "	0.42	0.35

Ascitic fluid and C.S.F.

Fluids such as these, of low protein content, are best treated by a direct deproteinization (with simultaneous elimination of phosphate) with basic ferric acetate [King & McGeorge, 1938]. 3 ml. portions of the fluid are treated in 100 ml. conical flasks with 6 ml. of ferric chloride and 6 ml. of sodium acetate solution. The mixtures are brought quickly to the boil, with vigorous shaking, and filtered hot. 10 ml. of filtrate in 15 ml. flasks are treated as for blood filtrates.

Total silica of blood

5 ml. of blood with 1 ml. of ammonium phosphate solution¹ are carefully dried in a platinum crucible. 0.5 g. Na_2CO_3 is used for the ashing and the analysis carried out as in the case of "Tissue Analysis".

Sputum analysis

Because of the presence of mineral particles in the sputa of individuals who have inhaled siliceous dust [cf. Burke, 1935], the analysis of the sputum for silica may occasionally be desirable. The analysis is best carried out on a weighed sample; because of its viscous nature sputum is not conveniently measured with a pipette. The samples are collected in waxed paper containers, and poured into weighed platinum crucibles. The wet weight is recorded, and the dry weight after drying at 105° . Na_2CO_3 is added, the sample is ashed, and the analysis completed as for tissues. Results are probably better expressed as a percentage of the dry weight, since the water content of sputum varies greatly.

Determination of silica in mineral dusts

It is frequently necessary, in investigations related to silicosis, to analyse samples of rock or of rock-dust for their total silica content. The gravimetric analysis will usually be preferred, but in special circumstances, e.g. with small samples of air-borne dust, the colorimetric procedure may be necessary. If the mineral specimen is known to contain no phosphorus and no iron, it should be fused with Na_2CO_3 , dissolved in water, brought to an appropriate volume and a suitable amount taken for colorimetric analysis. Most industrial dusts, however, contain iron. Ferric silicate is insoluble in an alkaline medium, viz. the solution of the Na_2CO_3 melt, and although hydrolysed by acid it may not be recovered on neutralization of the Na_2CO_3 solution because of its sticking to the walls of the platinum crucible. In order to avoid loss of silica through precipitation as iron silicate it is necessary to add sufficient phosphate to insure the complete removal of the iron as insoluble ferric phosphate. The excess phosphate is removed in the subsequent treatment with ferric chloride and sodium acetate.

An amount of mineral dust sufficient to contain 1–10 mg. SiO_2 is fused with Na_2CO_3 . 1 ml. of phosphate solution is added and water to dissolve the melt. The subsequent procedure is the same as for tissues. Some results are shown in Table VIII.

Table VIII. *Analysis of mineral dusts. Colorimetric method*

	Weight of sample mg.	% SiO_2 found	% SiO_2 found by gravimetric analysis
Quartz	10	97.0	96.6
Kieselguhr	5	87.7	88.3
	50	88.2	—
Flint	10	90.1	89.0
Stone dust	5	52.3	54.9

¹ See the section on "Mineral Dusts", in connexion with the necessity of adding extra phosphate in the case of materials containing an excess of iron over the phosphorus present.

SUMMARY

Methods for the determination of silica in tissues and body fluids are described. Directions are given for a gravimetric procedure using a modified filter stick, and for colorimetric procedures which employ the silicomolybdic acid yellow colour and the reduced silicomolybdate blue colour.

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