The Biochemistry of Silicon.
With a Quantitative Method for its Determination in Tissue.

A Thesis
Submitted to the Faculty of the
Graduate School
in part fulfillment
of the requirement for the
degree of Doctor of Philosophy

by

Moses Legis Isaacs
Merrell Fellow in Biochemistry.

May 4, 1923.

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I. Introduction.

It is remarkable that some of the problems of science, although most easily stated and apparently of the simplest nature, have thus far escaped solution. What, for example, are the absolutely essential inorganic constituents of protoplasm, in what quantity do they occur, and what role do they play? At present we can answer these questions for very few elements. Manganese and selenium have just recently been reported as present in the human body, while lately some data on copper, and zinc, and tin has been published. The facts concerning such an element as silicon have never been clearly established, but notwithstanding have furnished much material for speculation.

The necessity of knowing more of the biochemistry of these elements is constantly growing more imperative and is fortunately appreciated at the present time, but progress is, at best, very slow. We may keep in mind the story of iodine in connection with the human body as an example of how important an almost insignificant

quantity (statistically) of an element may prove.

Attention has recently been turned toward silicon, an element which, in the older textbooks on physiological chemistry was considered equally as important as iron or sulphur. Thus, Gorup-Besanez in his textbook of 1862 (1), says (page 38):

"Der Tierkörper enthalt aber noch anorganische Stoffe die zu seiner Entwicklung ebenso wesentlich sind wie die organischen; die wichtigsten davon sind: Phosphorsauren Alkalien, und phosphorsaure Erden (Kalk und Bittererde) kohlensaure Erden (in dem Knochen) Chlorkalium und Chlornatrium, schwefelsaure Alkalien, Eisen und Kieselerde."

The recent return of interest has come chiefly with the appearance of some quantitative data, although the latter has been quite meagre and contradictory. In the following part will be described what work has been reported on the biochemistry of silicon, both in plants and animals.

II. Silicon in Plants.

That the ash of many plants consists largely of silica has been known a very long time, much of the quantitative data having been reported by Liebig. A list of analyses up to 1880 has been collected by Wolff (2).

Table I.

		Potassium and Sodium Salts	Calcium and Magnesium Salts	Silica
Silica	Oat straw with ears	34.00	4.00	62.00
Plants	Wheat straw	22.50	7.20	61.50
	Barley straw with ears	19.00	25.70	55.30
	Rye straw	18.65	16.52	63.89
	Meadow hay	6.00	34.00	60.00
Calcium	Tobacco, Havana	24.34	67.44	8.30
Plants	Tobacco in artificial earth	29.00	59.00	12.00
	Pea stems	27.82	63.74	7.81
	Potato leaves	4.20	59.40	36.40
Potassium	White turnips	81.60	18.40	-
Plants	Potato eyes	85.81	14.19	-
	Helianthus tuberoses	84.30	15.70	-

Table II.

Date	Total Ash % of dry weight	Amounts of various elements in ash calculated as oxides. % of total ash.			
		K_2O	CaO	P_2O_5	SiO_2
May 16	4.1	42.1	13.8	32.4	1.6
July 18	4.7	17.1	42.3	8.2	21.3
Oct. 15	7.1	7.1	50.6	5.1	30.5

Plants divide themselves into three groups according to which element is most abundant in the ash, viz. silicon plants, calcium (and magnesium) plants and potassium (and sodium) plants. This is illustrated by the following table after Liebig (3). The grasses, in general,

(Insert Table I)

are the silicon plants although the age of a plant has much to do with its silica content. The following table from Palladin (4) (page 83) illustrates this. It gives the analyses of beech leaves (Fagus sylvatica) at three stages of development.

(Insert Table II)

It must be remembered that while the percent of phosphorous becomes less the actual quantity present in a leaf may increase over, or be the same as in the immature leaf. It would be difficult to say from the data above to which class the beech belongs.

Wood ash contains 1 - 3% silica. This value increases with age to a maximum. The value for a 50 year old tree is about that of a 345 year tree.

It appears that silica serves as a protection to the plant, but in just what capacity it is hard to say. Whatever protection it affords to the beech leaf apparently is

not required by the very young leaf. The silica does not serve necessarily to form a tougher stem. Pierre (6) in 1886 showed that the laying of corn by the wind was not due to lack of silica in the stalk as had been supposed up to that time, but rather to faulty illumination of the plants.

On the other hand, in the Diatomea, the presence of silica is obviously to strengthen the cell wall. These plants are a kind of algae, found in most waters and moist earth,"forming largely the plankton of the ocean - the drifting mass of organisms floating on the surface". They are unicellular bivalve structures whose skeletons are strongly impregnated with silica. The extent to which these plants flourished in bygone ages is almost incredible. A deposit of diatomataceous ooze between Santa Ynez and Los Alamos in California reaches a thickness of 4700 feet! (7) (page 34).

Recently some very interesting work has been done in France on diatoms. Coupin (8) placed cultures of diatoms in a nutrient medium and scattered over the surface different substances which might serve as a source of silica. The results briefly are as follows:

A. Culture medium containing no silica - no growth.
B. Medium plus vitreous silica - no growth.
C. Medium plus gelatinous silica - no growth.

D. Medium plus washed kaolin - abundant growth.

E. Medium plus powdered orthose - abundant growth.

F. Medium plus powdered argile - growth depended on sample used.

G. Medium plus sodium or potassium silicate - no growth.

H. Medium plus glass - no growth.

Vernadsky (9) discusses the question as to whether the diatoms themselves render the silica soluble or whether this is done by symbiotic bacteria and inclines to the former belief.

The important conclusion from the work of these writers is that diatoms require aluminum silicates as the source of their silica, i.e., compounds containing the nucleus $H_2 Al_2 Si_2 O_2$. Decomposition by chemical means requires strong acid and a high temperature. Perhaps we are dealing here with an enzyme which acts on an inorganic substance - a truly remarkable phenomenon.

An evident case of protection is afforded by Roehea falcata, (4) (page 253) a plant growing in South Africa, which is covered with a layer of bladder-like cells containing water, the walls of the cells being rich in silica. These cells act as water reservoirs.

Marshall Lundie (10) using a special medium was able to grow wheat with a very low silica content, and therefore concluded that silica was not essential to laboratory raised

Table III.

Ash: % of dry material	SiO_2: % of ash	Soil grown plants Av. SiO_2: % of ash
Leaves of Wheat Plants		
6.38	1.088	
7.53	1.369	
8.75	1.18	
Av. 7.55	1.212	42.2
Stems of Wheat Plants		
7.20	.46	
7.66	.143	
7.27	.744	
Av. 7.34	.449	

plants. His results were as follows:

(Insert Table III)

It will be noted that the plants were not silica free; the average value being about that reported for some animal tissues.

Sachs,(11) in 1862 reported practically the same results.

Dr. Hahn (reported in Sundie's paper) showed that plants poor in silica were very subject to certain rusts. It has been shown that fungus hyphae cannot easily penetrate cell walls containing silica. "Wheat, rye, etc. grown in nutrient solutions deficient in silicic acid often suffer so severely from rust that only great care can prevent their complete destruction. The hardness of silicated cell walls is also a very good protection against animal attack. Thus, for instance, one plant of Lithospermum arvense, grown in a nutrient solution without silicic acid, suffered severely from plant lice, even though these were removed daily, while two similar plants, standing nearby and grown in similar solutions, but not tended so carefully, were completely killed by the insects.

"The distribution of silicic acid in different parts of seeds is another indication of its protective action. Millet seeds without seed coats contain only from 4.8 to 7.1 per cent of the total silicic acid of the seed, all

the remainder (from 92.6 to 95.1 per cent) being deposited in the seed coats. Such a marked accumulation in the seed coats suggests the importance of this substance to plants growing under natural conditions. The investigations of Sabanin upon ripening seeds of millet, shows that this plant hastens as it were, to accumulate enough silicic acid in the peripheral parts of the grain (as in the palea) to protect the increasing reserve material from unfavorable external conditions." (4) (page 80).

We are not certain that silica is present merely for the protection of the plant. The following statement appears in Duggar's, "Plant Physiology" (12) (page 153). "Wolff regarded silicon as important in furthering the migration of phosphoric acid compounds from maturing leaves and stems to the forming seeds." In Thatcher's "Chemistry of Plant Life" (13) (page 12) the following remarkable statement is made. "Silicon appears to take the place of phosphorus in phosphorus starved plants." Unfortunately in neither case is the original reference given. These remarks, if true, cast a powerful and suggestive light upon the role of silicon in protoplasm.

One more remarkable occurrence of silica in plants must be mentioned. This is what is known as "Tabasheer" and is found on the nodes of bamboos. It consists, it is said, largely of colloidal silica with some organic mater and appears to be a pathological growth (14).

As to the nature of the silicon compound or compounds in plants very little can be said, although attempts at the solution of this interesting problem were made as early as 1872 by Ladenberg (15), and somewhat later by Lange (16). Their researches led to no definite conclusions but indicated that the silicon was present as colloidal silica. The latter made the observation, that the silica, although colloidal, was diffusable - an apparent exception to Graham's rule, then considered rigidly true, on the diffusibility of colloids and crystalloids.

Somewhat before these investigations, Brewster had stated that silica in the cuticle of equisetum was crystalline and polarised light, a result which Bailey (17) could not substantiate.

P. B. Wilson (18) working with wheat grown in earth containing diatoms, found these incorporated in the plant wall and concluded that no silica compounds were necessary in the ground - only silica particles. Molish (31) also found silica particles in plant walls, but the whole matter becomes clear if we consider the work of Berthelot and Andre (37) who found that one portion of the silica in plants dissolves in water, another in dilute potassium hydroxide, while a third is soluble only in hot alkali.

III. Silicon in the Animal Kingdom.

Silica is found in the organisms of some of the lowest Protozoa (19). Some of the first order of Class I, Orobeae, have a covering of square siliceous plates (Euglypha). Another form, Paulinella, has hexagonal filose plates. Others as well as certain of the Heliozoa secrete a sticky substance which collects grains of sands

and thus forms a skeleton. In other forms of the order Heliozoa there is a truly secreted skeleton of colloidal silica. Thus in Clathrulina the animal is surrounded by a perforated sphere of silica. Raphidiophrys, belonging to the same order, has a skeleton of siliceous needles. In the order Radiolaria many genera have siliceous skeletons in the form of loosely woven spines. In one genera of this order, acanthin (strontium sulphate) is found in place of silica. The Radiolaria are marine organisms which float on the water. After death their bodies fall to the bottom of the ocean forming the well known "Radiolarian Ooze". Likewise some of the Flagellata have siliceous skeletons.

Concerning silica in the next Phylum, Porifera, there is more to be said (20). Here the skeleton consists of interlocking spicules, giving rise often to such very beautiful forms as the Venus Flower Basket (Euplectella aspergillum) (Picture on page 11 of the "Treatise on Zoology" Volume II, edited by E. R. Lankester. The spicules owe their hardness to colloidal silica or to the calcium carbonate. Each arm of a siliceous spicule has an organic axis which are laid alternate layers of organic matter and colloidal silica, the outer coat being organic. In calcareous sponges the calcium carbonate is crystalline, the organic axis is very slender, and the layer structure is absent. There is an outer sheath, however, of organic material. The spicules

are shaped like those of the siliceous sponges.

Some sponges have a skeleton of spongin, a silk like organic substance which incorporates grains of sand and other hard substances into itself forming a horny rigid sheath. To the spongin type belongs the ordinary bath sponge.

Schulze (20) pointed out that in the Hexactinellida, or siliceous sponges, the spicules have axes in the same symmetry as crystals of the cubic system, - although the silica itself is colloidal. Minchin (20) suggests that this is the culmination in the evolution of a supporting framework mechanism.

The spicule arises from an intracellular deposit, but usually greatly outgrows the mother cell. Just how this growth takes place is not well known. In the case of the Calcarea, the deposit of calcium carbonate grows by the mother cell breaking up into daughter cells which spread themselves over the spicule. When the latter has attained full growth, the formative cells, in the case of the siliceous sponges disappear, but probably remain in the Calcarea.

The spicules are either disconnected (Lyssacine) or are cemented together in the skeleton (Dictyonine). The cement is colloidal silica. In the genus Monorhaphis there is a single spicule two to three feet in length as thick as a pencil "transfixing the body like a skewer from above downwards". Besides the external spicules in the

skeleton (megascleres) there are smaller ones (microscleres) which lie throughout the tissues supporting for example such parts as the gastral membrane.

The question as to where the sponge obtains the quantities of silica it uses, (sponges grow remarkably fast), how it converts it into the colloidal condition etc., have barely called forth a guess. The exact form, i.e. the degree of hydration of the silica is unknown. Apparently it is the source of the flint and chert nodules found in chalk beds.

There is a gap in our knowledge of silicon at this point, for nothing is mentioned in the literature concerning this element in the lower animals save one determination on the ash of the skin of a Holothuroidea (Phylum Echinodermata) which contains .5% silica (21).

Turning to the vertebrates we find some quantitative data on silicon in the tissues of birds, cattle, humans and a few other animals. Unfortunately, little can be said for the accuracy of these determinations. The whole subject will be taken up later. Suffice it to say here that the silicon has in all cases been weighed as silica and reported as such. We might mention that, by the methods used about 100 grams of wet tissue are required to give a determination with an accuracy of something like 1 - 5%, many special precautions having been observed.

Historically, it is impossible to say, who first

reported that silica was in tissue. Fourqueuy and Vauquelin (22) in 1803 found silica in a urinary calculus. W. Henneberg (23) in 1847 found 1.24% silica in the ash of hen's blood. He also analysed some hen feathers.

The first extensive work was carried on by Gerup-Besanez in the same year. In his book of 1862 he reports that traces of silica have been found in the ash of blood, bile, urine and eggs. It was found in large quantities however, in the ash of hair, feathers, excrements (as sand) and in the protective coating of Infusoria and Bacteria. Silica he reports, has also been found in concretions. He reports the work of earlier investigators, especially the work of Oidtman.

Gerup-Besanez analysed the feathers of many birds with the following general results:

Table IV.

Average values	% SiO_2
Ash of feathers of grain-eating birds	40.
Ash of feathers of meat-eating birds	27.
Ash of feathers of insect & berry-eating birds	27.
Ash of feathers of fish-eating birds	10.5

The age has much to do with the silica content of the feathers.

Hoppe Seyler raised the objection (24) that the

silica content of the feathers depended largely on the dust contamination, and in truth so it seems. It may be mentioned here that Miss Winogradow working under Drechsel (25) in 1897 thought she isolated a silicic acid ester containing a cholesterol like alcohol ($Si(C_{34}H_{59}O)_4$) and suggested that this ester may exist in sponges. A further report was promised, but was never published due perhaps to the death of Drechsel. The matter was re-investigated by Cerný (26) in 1909 but he could not substantiate the former work. His work however is not absolutely convincing and it should be repeated. He also tried to find silicon compounds in various organs but without certain results. Gennermann (27) believes the silica makes far a greater resistance in the feathers. He believes there is no organic silicon present. In one analysis (Columba palumbus) silica made 77% of the ash of the feathers.

Lersch in his "Einleitung in die Mineralquellenlehre", 1855 thought that silica perhaps played a part in bone building.

Kunkell (28) in 1898 reported some results on silica in the pancreas. These (two in number) led to the statement by Rohden that the pancreas is the organ which controls silicon metabolism and others have compared it to the thyroid handling iodine. This impression has lasted with some writers up to the present day. More will be said about this later.

The first systematic work, however, on silicon determination after Gorup-Besanez was that of Schulz. His first article appeared in 1901 (29) and contained analyses on various tissues. His method of determination was as follows:

The tissue is ground and thoroughly dried on a water bath. The dry material is extracted with ether to remove fat. It is then charred in a nickel dish over a gas flame. The charred matter is then gradually ashed in a platinum dish in a muffle at a dull red heat. The ash is ground up in an agate mortar by pressing the lumps with the pestle. It is then dried for 12 hours at 110°- 115° and finally over sulphuric acid over night and weighed. A portion of the ash is weighed out into a platinum dish which is covered by a watch glass with a hole bored in its center. Through the hole, the ash is first treated with a little distilled water and then with concentrated hydrochloric acid. When bubbling has ceased, the cover is removed and its underside washed, the water being added to the platinum dish. The contents of the dish are evaporated almost to dryness and the hard particles broken up. Then the evaporation is continued to dryness. Concentrated hydrochloric acid is added and once more evaporated off on a water bath. On cooling concentrated hydrochloric acid is added with hot distilled water. The solution is filtered through a tared filter, dried at 110° and weighed. This weight is that of the silica, the sand and of carbon particles.

In order to separate the sand from the silica, the dried filter paper is cut up into small pieces and placed in a platinum dish containing some C.P. sodium carbonate and some distilled water. The contents of the dish are kept at a slow boil for one half hour. The dissolved matter is filtered. The filtrate is evaporated in a platinum dish. The dry matter is treated just as the original ash in order to render the silica insoluble. The insoluble matter is well washed and ignited and weighed. Ammonium fluoride is finally added, the crucible reheated and weighed. The difference is the silica. The average of several blanks was .00029 gms. The amount of carbon and sand found is subtracted from the original weight of ash taken.

The method above is open to serious criticism in that much silica may be lost or gained in the many steps of the determination. The solubility of silica in wash water is

considerable (30). Noticing therefore the amount of silica actually weighed, his results in many cases cannot be considered accurate. Where more than .01 grams are weighed, the results are probably fairly accurate (20%).

Schulz drew two conclusions from his work. The first is that the silica content of an organ was directly proportional to its connective tissue content. Some of his results may be summarized as follows:

Table V.

	Mgs. SiO_2 per 100 gms. tissue.
Dry Muscle contains	2
Skin contains	4
Tendon contains	6
Dura mater contains	9

This statement must be reinvestigated using freed connective tissue and many more organs must be examined.

Schulz concluded in the second place that older individuals have less silica than young individuals. The foetus, he states, is very rich in silica. He gives no data to support the last statement.

Table VI.

Comparison of silica content of young and old tissue.

		Mgs. per 100 gms. dry tissue.
Muscle	Young	2.6
	Old	1.9
Skin	Young	5.1
	Old	3.9
Tendon	Young	8.7
	Old	4.1

Here again is work which needs substantiation.

Schulz in 1915 (29) attacked the problem of the silica in the pancreas. Kahle (32) in the year previous had reported that in cases of tuberculosis, the pancreas was low in silica; in cases of carcinoma, high. He also reported that in tuberculosis and carcinoma the amount excreted in the urine was very low. His data were as follows:

Table VII.

	Mgs. SiO_2 per 100 gms. dry tissue
Normal pancreas	14 - 15
Tuberculosis	8
Carcinoma	33

In operated cases of carcinoma, the silica would return to normal.

Schulz analysed seventy-three pancreas. His method was similar to that already described although he does not mention the sodium carbonate treatment. His results may be summarized briefly:

Table VIII.

	Mgs. per 100 gms. dry tissue
Grand average of all pancreas examined	12
Tuberculosis and cancer excluded	13
11 Tuberculosis	14
9 Carcinoma	16

In one or two cases he found none. He concluded

therefore that the silica content of pancreas had not much to do with either disease.

Kahle (33) repeated his own statements and reported some new experiments recently, concluding to his own satisfaction that there is a definite relation between the silica of the pancreas and tuberculosis.

Schulz also investigated the silica content of the thyroid (34). He found:

Greifswald material (Dry) normal .0084% mgp SiO_2 per 100 gms dry.

pathological .0175% "

Average of 8 analyses

Zurich material pathological .0434% "

Since 1917 M. Gonnermann has reported many silica determinations. His method is somewhat different from Schulz's (35).

The tissue is hardened under alcohol, ground up to a powder and extracted in a Soxhlet flask with ether. The ashing is carried out in a porcelain dish, previously boiled with hydrochloric acid. The ash is evaporated three times with concentrated hydrochloric acid and extracted with concentrated nitric acid to remove ferric phosphate. This is repeated until the liquid on filtering no longer gives a test with ammonium sulphide. The silica is ignited and finally volatilized with ammonium fluoride.

Unfortunately this method also has sources of error. For example, in ashing considerable quantities of the compound $P_2O_5.SiO_2$ must be formed. Phosphoric acid also attacks porcelain. This compound mentioned is unaffected by acids.

Upon treatment with ammonium fluoride much of the phosphoric anhydride can be volatilized. The only mention of $P_2O_5.SiO_2$ interfering with an analysis is made by Hasenbaumer (36) in the determination of phosphorus in Thomas Meal. Gonnermann's results would therefore be expected to be too high, barring errors due to the considerable solubility of silica in concentrated acids.

A few of the analyses of Schulz and Gonnermann and some others may be compared.

(Insert Table)

Table IX.

Some Silica Values Reported for Human Tissues.

Material	SiO_2 mgs. per 100 gms. dry tissue (fat free)	SiO_2 % of ash	Approximate amount weighed gms.	Reported by
Adrenal	290 - 540	7.4 - 16.1		Gonnermann (35)
Amion covering		20		Gonnermann (35)
Blood		2.26	.002	Gonnermann (38)
Blood after ingestion of SiO_2		2.65	.003	Gonnermann (38)
Fibrin	130	16 - 30	.015	Gonnermann (35)
Liver	3 - 10	.12 - .27		Oidtman (1)
Lung (newborn)	none			Kussmaul (35)
Lung (8 months)	trace			Kussmaul (35)
Lung	70 - 90	2.4 - 3.1	.006	Gonnermann (38)
Lung	40 - 72	1 - 14.5		Hirsh (52)
Muscle (breast)	2.6	.056	.001	Schulz (24)
Muscle (heart	8.	4.2	.020	Gonnermann (35)
Pancreas	30.	.67 - 3.7	.020	Gonnermann (38)
Pancreas	10.	.2	.001	Schulz (29)
Spleen	1 - 30	.17 - 1.0		Oidtman (1)
Spleen	17	.35		Schulz (39)
Spleen	28			Symmers (40)
Thymus	380	8.0		Gonnermann (35)

Silicon in Pathology.

Something has been said already regarding silicon and tuberculosis. Rossle, (48), Kahle (49) and Kobert (50) maintain that silica has much to do with this disease. They picture the state of affairs thus. In a lung provided with plenty of silica, bacteria find it very difficult to dissolve the tissue; if the tissue has had little silica and has been dissolved by the bacteria, then the administration of silica will help and hasten encapsulation. Kahle, working on guinea pigs, found marked encapsulation of tuberculous lesions after administration of colloidal silica. This writer further suggests that the bad course of tuberculosis in pregnancy is due to the withdrawal of large quantities of silica from the maternal pancreas by the foetus. The foetus is conceded by all, without analysis, to be very rich in silica. Robin from a few analyses, ~~reported in the last table~~, concluded that the tubercular lung had much less silica than a normal lung. This conclusion, in view of the quantity of silica dust inhaled constantly, is not justified.

In regard to encapsulation, Weaver and Wells (51) have recently pointed out that this step follows recovery.

Turning to another phase of silica in the lungs, we find some interesting material. It is well known that quartz workers are very susceptible to tuberculosis, while coal miners are not. According to Haldane (53) coal dust causes a loosening of the epithelial tissue while quartz dust does not, thereby remaining and causing injury to the lungs. He suggested

that coal dust be introduced into places where quarts was worked for a mixture acted as coal dust. McCrae (54) found silica particles in miners' lungs of indefinite shape and less than 1 μ in diameter. The bronchial lymph glands in some cases contained as much as 6 to 30% of the ash of silica. In normal average adults these glands contain more coal pigment than silica; in children the reverse is true. In stone workers there is more silica in the lungs than in the glands. More information may be found in Bulletin no. 293 on "The Problem of Dust Phthisis in the Granite-stone Industry", and also several articles in the "American Review of Tuberculosis" for November, 1922.

Mayer and Wells (51) have carefully studied the subject of silica in lung calcifications, and reach the following conclusions:

"Calcified peribronchial lymph nodes and pulmonary lesions contain a small proportion of silica but not more than uncalcified peribronchial lymph node on lung tissues contain in adults. The silica is derived chiefly if not entirely from inhaled siliceous dust, for calcified tuberculous mesenteric lymph nodes do not contain appreciable amounts of silica, and only a trace was found in a calcified tuberculous pleural exudate."

Gonnermann (38) (35), analysed a great many concretions and found silica in most of them, (lowest value .2%; highest, 33% of the ash).

Deposits in a syphilitic spleen were found by Symmers, Gettler and Johnson to contain quantities of silicates. They employed a method of silica determination which would probably

give low results. They found that

Normal spleen contains 28 mgs. SiO_2 per 100 gms. dry tissue.

Enlarged spleen contains 240 mgs.

Normal spleen contains 5 mgs. SiO_2.

Enlarged spleen contains 1488 mgs. SiO_2.

Golden granules in the diseased spleens were thought to consist of iron or calcium silicate.

Gonnermann (35) gives an historical account of the subject of silica in bladder stones based on the review by Klimmer (55). Koningle and Wurzer in 1822 first observed silica in bladder stones of plant eating animals. The observation of Fourcroy and Vanquellin has already been mentioned.

Liebner found 71% silica in a sheep stone. A similar case was recently reported by Schlick (56). Dammann found almost 98% silica in a cow stone. The deposition of such stones in cows is correlated with heavy potato feeding. In horse stones, silica is rarely found, and never in the stones of the dog, cat and pig.

As we can readily see, silicon in pathology is a remarkably suggestive theme, but much work needs to be done before more can be said.

Silicon Compounds in Therapy and Their Physiological Action.

Schulz (39) gives a very interesting historical account of the history of silica in therapy. Briefly, the important points are as follows:

Middle ages: Avicenna regarded tabasheer as a very fine

remedy and Gerardus of Cremona gives a long list of diseases where tabasheer can be used. Paracelsus used sodium silicate ("Ludus") for treatment of "tartaric" diseases, i.e., diseases where concretions are formed in the body or deposits are laid down as in arthritis or cholelithiasis.

About 1650 Glauber used the same treatment as Paracelsus.

In 1850, Socquet, a Frenchman, used water glass, internally for rheumatism, arthritis, etc.

1859, Kuchenmeister (Vol. II of Gravell's "Notizen") used sodium silicate for insect stings and bites and for erysipelis.

1872, Dubreuil (Gazette des hospitaux) reported using sodium silicate for a case of bladder trouble.

Picto used a 2% solution for cases of blenhorrhagia and diabetes. Three cases of the former were cured in 5, 7 and 11 days. (Comptes Rendu 1872, Vol. 75)

Rabuteaux and Papillon (Comptes Rendu, Vol. 75) stated that sodium silicate (1 - 2% solution) had an antiputrefying and antifermenting action. (Schuls could not substantiate this.)

1875, Champouillon (Comptes Rendu, Vol. 76) used water glass in treatment of blenhorrhagia and fluor albus. He had patients inhale the same for bronchial catarrh. He also found it useful in treatment of chronic cystitis. "Aucune medication, je l'afferine ne reussit aussi bien que les injections du silicat de soude contre la cystite chronique, catarrhale, purulente ou hemorrhageque." Doyan (Annales de dermatologie et

de syphiligraphie) used sodium silicate in syphylitic infections.

1875, A. Wolff found in the treatment of blenorrhagia with sodium silicate that the disease grew worse for 56 days and then regressed.

1889, Löwenhaupt stated that sodium silicate had an antiseptic action. (Cf. with Rabuteaux)

The folk medicines often contain silica. According to Söderberg a knife point of powdered flint is given for boils.

Of silica and tuberculosis, something has already been said. Silicon teas, lemonades, "compounds" for injection, etc. have become very popular, especially in Germany. One method has recently been advocated of spraying calcium silicate into the lungs. This recalls the method of Champouillon in 1873.

Administration by mouth seems to give most satisfactory results as injections are said to be painful. Of the various forms used, colloidal silica is said to be the best (57). Calcium salts do not hinder absorption(58).

Kuhn, although enthusiastic in his earlier articles (59) (60) (47), as to the therapeutic effect of silica has recently stated (49) that silica therapy in tuberculosis can not be used alone. Roth (58), does not believe that the course of tuberculosis is much influenced by silica. Zuckmayer (61), states that silica has a typical non-specific effect and therefore is no better than other non-specific agents. However, administration can be made per os, giving rise apparently to an increase in temperature and a leucocytosis (64). The leucocytosis

caused by taking silica by mouth is said to be small (58). The effect of sodium silicate taken by mouth on the white count in 23 cases has been reported by Ziekgraf (62). In three cases there was no increase, in ten a 40% and in 10 others a 200% increase. After giving a silica tea Kesseler (63) found a drop in white count in four cases, no increase in one and a rise in eleven cases. The average increase was 12.8% mostly in neutrophiles. The effects are transitory.

It may be suggested that silica found in milk may be for this stimulative action. According to Pfyl (35) cows' milk contains 1.9 mg. SiO_2 per liter. Oidtman for human milk reported 30 mgs. per 100 grams of dry milk or .72% of the ash. Gonnermann found the ash of woman's milk to be twice as rich in silica as cow's milk. These results, the latter is certain can not come from the glass vessels used. On the other hand Schulz found that on sterilization there was an increase of .03 gms. of SiO_2 per liter. He asks whether this may not be important in the production of ostipation in children suffering from Barlow's disease.

It has been recommended that small quantities of fluosilicates be added to milk to make good that precipitated on sterilization (66). The following is made in this article.

"Some practitioners have had success with this treatment, particularly during the months of August and September, when the lack of silicon and fluorine in the food of children causes all the disturbances now attributed to the lack of vitamines."

Gonnermann (35) gives a good deal of data on the silica

content of plants and drugs.

There has been much contradiction as to the effects of colloidal silica. Some say it is poisonous; others say it is without affect. Large and repeated doses are certainly detrimental producing a fibrosis, especially in the liver. Also, as Schuhbauer (68) has shown some effects attributed to silica are due to the sodium hydroxide produced when sodium silicate is used. However the anomalous effects often obtained may be in part explained by the work of Kopaczewski and Gruszewski (69). They prepared three types of silica gel, positive, negative and neutral. When incubated with serum, which is then injected into an animal, the negative colloid, produces symptoms of an anaphylactic shock. Neutral or positive Sols were without affect.

Silica has been recommended as a therapeutic agent for a host of diseases (see especially Gonnermann (35)) but the bases for these recommendations are so slim that they merit little attention.

Silicic acid can produce hemolysis, but the work done need hardly be discussed (70) (71) (72) (73).

Silica also absorbs enzymes toxins (74), lysins and bacteria. It is said to be a good agent for use in dermatology because of this absorptive property.

In What Form Does Silicon Occur in Tissue?

Very few writers have even attempted to guess as to the nature of the silicon compounds in tissue. The works of Lange, Ladenberg, Drechsel and Gerny have already been reported.

Gonnermann says that Gorup-Besanez reports sodium fluosilicate in the brain, but it is hard to see how he determined this.

There is one statement which has been gaining prominence, namely that silicon takes the place of carbon in certain compounds in protoplasm. It was first stated by J. Emerson Reynolds (14) in a speech delivered in 1909. He said that since in tissue phosphorus took the place of nitrogen to some extent, likewise sulphur that of oxygen, it would then be reasonable to suppose silicon would replace carbon. He says in one place: "In view of our newer knowledge, there is therefore nothing very far fetched in supposing that under suitable conditions a plant or an animal may be able to construct from silicon compounds, ultimately derived from the soil, something akin to silicon protoplasm for use in its structures."

H. Charlton Bastian, in his "Origin of Life" (75), described experiments in which he took dilute solutions of silicic acid and allowed them to stand in sealed tubes. After several months deposits formed which he claimed consisted of bacteria whose structure was consisted of a protoplasm containing no carbon - a silicon protoplasm. He gives many microphotographs. Needless to say his work has been refuted several times (76).

Very recently an article entitled "A Biochemical Explanation of the Silica Molecule" appeared in one of the medical journals (77). The writer says:

"That bacteria shatter the neutral salts in the molecule is conceded. As proof Klett ascertained, when testing many products in the presence of bacteria, that bacterial enzymes had the chemical power of reducing sodium silicate. However, no biochemic interpretation was attached to this finding by Klett or any other investigator."

In the article referred to , Klett (79) speaks of the reduction of sodium selenate not silicate.

The writer says farther on:

"Since silica is a preponderant neutral salt in the connective tissue cell, bacteria may dissipate it, causing a famine of the element at the site of bombardment. x x x
In the event of suppuration, a defensive barrier of connective tissue, loaded with silica molecules, is formed around the nucleus of the pus organisms to resist their systematic advance."

Silica forms only a fraction of the ash of connective tissue.

There has thus far been presented not a particle of evidence in favor of silicon replacing carbon in any compound in tissue. The chemical properties of the silicates resemble very closely those of the phosphates and not those of the carbonates.

The silicon probably exists in the fully oxidized state and probably chiefly as colloidal silica. Since the silicates and the phosphates resemble each other so closely, silicolipins are perfectly possible.

The Function of Silicon in Tissue.

What can be the function of silicon or silica in tissue? The following are some of the possibilities:

1st. Silica may have no function. It may be taken with food, absorbed into the blood stream, some deposited here and there where it can do no harm or sometimes where it does do harm as in concretions, while the majority passes out in the urine. Against this may be brought the statement, if true, that the foetus is particularly rich in silica.

2nd. Silica may be present as colloidal silica to absorb certain poisons, toxins, etc.

3rd. Silica may be present as an actual defense measure, strengthening certain cells - acting as a micro-skeleton for them. The supposed larger quantity of silica in connective tissue suggests this.

4th. Silica may be present as a catalytic agent. It is well known that silica is an excellent catalyst for certain reactions.

5th. Silicon may have some connection with phosphorus in tissue. We may recall what was said with regard to phosphorus and silicon in plants. Again, if the work of Drechsel is substantiated, we may find silicolipins to be of impertance.

6th. Silica may be a necessary stimulant of the hematopoetic system, possessing as it does the advantage over many other non-specific agents in being effective even when taken per os.

All the above suggestions are mere guesses as to the role of silica in tissue. Which one or ones will prove correct, only future work can say.

A Quantitative Method for the Determination of Silica in Tissue.

Since the gravimetric method for silica determination presents such difficulties both as to accuracy and convenience, a micro method was of extreme necessity. After trying to adapt many of the known methods to this purpose the following colorimetric determination was worked out.

I. Principle. Silicates and phosphates form with ammonium molybdate in acid solution the yellow silico- and phosphomolybdates respectively, and these on reduction with sodium sulphite give blue reduction compounds. However, in the presence of the proper concentration of acetic acid only the silicomolybdate is formed and reduced.

II. Reagents.

(a) Boric acid, saturated.

(b) Calcium nitrate $4H_2O$ - 5% solution.

(c) Nitric acid, concentrated.

(d) Sodium hydroxide. Made by the reaction of 2 gms. of metallic sodium on 50 c.c. of distilled water in a nickel dish.

(e) Acetic acid 10%.

(f) Standard silica solution. This may be conveniently prepared by diluting 1 c.c. of ordinary water glass to 1 liter with distilled water. The exact concentration of the solution may be found by colorimetric standardisation against a known weight of ignited silica

which has been fused with sodium carbonate and dissolved in a given amount of water. The standard made from water glass lasts two weeks, although sometimes after a few days the silica precipitates. 1 c.c. of the standard should contain.1 mg. of silica.

(g) Ammonium molybdate 10%. This solution may be kept for 1 week.

(h) Sodium sulphite, saturated.

III. Method. One half gram of dry tissue is weighed in a platinum dish or crucible and treated with 1 c.c. of boric acid solution, 1 c.c. of calcium nitrate, and about 2 c.c. of nitric acid. The dish is placed on a steam bath until all the tissue has dissolved, then placed on a triangle or in another crucible and heated until the material just chars. More nitric acid is added and evaporated off until there remains a white ash. The calcium nitrate aids in the ashing, and the boric acid prevents loss of silicon as silicon tetrafluoride. The material in the crucible is wet with a few drops of concentrated nitric acid and placed on a steam bath until all excess of the acid is evaporated. This, to convert the calcium oxide to nitrate. 2 or 3 c.c. of water and 3 c.c. of sodium hydroxide solution are added to the crucible, which is heated and rotated until its contents boil and touch all parts of the interior. Next 3 c.c. more than sufficient acetic acid is added than is necessary to neutralise the hydroxide, then 10 c.c. of water and 5 c.c. of molybdate

solution. The solution is transferred to a test tube graduated at 25 c.c.

At this point the standard is to be prepared. Into a similar test tube is placed 1 c.c. of the standard silicate solution, 12 c.c. of water, 3 c.c. of acetic acid and 5 c.c. of ammonium molybdate solution.

Standard and unknown are placed in boiling water for 5 minutes, removed, and each treated at once with 2 c.c. of saturated sodium sulphite solution. A blue color develops in each.

These are compared in a colorimeter or by using Nessler tubes and diluted standard.

In case the tissue contained blood, the ferric phosphate present will cause the final color to be green. In order to obtain standard to match this it is best to proceed as follows. Into a test tube is put 1 c.c. of a 2% ammonium phosphate solution, about 15 c.c. water, 3 c.c. acetic acid, 5 c.c. ammonium molybdate, and a drop or two of a strong iron alum solution. The tube is placed in boiling water for 5 minutes, and the contents then treated with sodium sulphite. The result is a yellow solution which can be added to the standard until a shade similar to that of the unknown is produced. Note should be taken of the volume added and correction made accordingly.

A correction must be made for silica in reagents. The concentrated nitric acid, the 10% acetic and the ammonium molybdate as well as the sodium sulphite and distilled water

are not likely to contain silica, however, the boric acid and sodium hydroxide do. This correction may be determined by running a blank, carrying out each step of the analysis, only omitting the tissue. The nitric acid may be tested separately by adding to 500 c.c., 1 c.c. of 10% ammonium molybdate. If no yellow color develops, the acid is silica free.

Sometimes the unknown at the time of comparison appears turbid. It may then be filtered.

Using Nessler tubes determinations can be made to ± .003 to ± .005 mgs. of SiO_2.

This is essentially the same method as published elsewhere,(79) but is more easily carried out. In that article the order of procedure was incorrectly given, in that it stated that the molybdate was to be added before the sulphuric acid, whereas it should have been the reverse.

In the following determinations the tissue was dried in small aluminum or nickel dishes, first on a steam bath and finally in a drying oven at 105° C. In taking a sample, as much fat as possible was removed.

Dog Tissue.

The dog had just been used in an operation and died under ether.

	Mgs. SiO_2 per 100 gms. tissue.		Mgs. in whole organ.
	Fresh	Dry	
Pancreas	.49	2.1	.16
Kidney	.44	1.7	.12
Heart	.16	.5	.10
Liver	.20	.5	.40
Lung	1.4	5.7	.90

Nessler tubes were used; two determinations on each tissue were made and agreed in each case.

Human Tissue.

a. Organs of a one year old child. Case of congenital syphilis.

	Mgs. SiO_2 per 100 gms. tissue.		Mgs. in whole organ.
	Fresh	Dry	
Lung	.13	.6	.09
Liver			none
Brain Frontal lobe			none
Medulla & Cerebellum	.34	1.5	.3
Heart			none
Spleen (full of blood)	.22	1.1	.05
Kidney	.56	1.9	.16
Pancreas	.28	.9	.06
Stomach wall			none

All of these organs were markedly pathological.

b. Lung of week old infant (tuberculosis) contained no silica.

Boric acid was not used in these determinations.

These organs were given to me by Dr. George Guest.

Rabbit Tissue.

Rabbit 1. Male. 1,807 gms.

	Mgs. per 100 gms. tissue. Fresh	Dry	Mgs. in whole organ.
Brain I	3.8	20	.33
" II		17	
Liver		none	

Rabbit 2. Male. 1,849 gms.

From same litter as #1.

Brain I	4.0	17.0	.28
" II		19.0	
Heart I	1.8	9.0	.10
" II		9.1	
Liver	1.2	5.	1.0
Kidney I		none	
" II		none	

Rabbit 3. Male. About 2,000 gms.

Lung I	.8	3.2	.08
" II		3.4	
Kidney I	1.1	6.9	.13
" II		7.0	
Heart I	.49	3.3	.05
" II		2.3	
Brain I	1.9	11.2	.20
" II		13.1	

Rabbit 4. Female. 2,500 gms.

	Mgs. per 100 gms. tissue.		Mgs. in whole organ.
	Fresh	Dry	
Brain I	3.9	16.4	.35
" II		16.2	
Lung I	2.6	13.1	.44
" II		13.1	
Kidney	3.8	12.6	.34
Spleen	2.3	13.8	.06

In the case of rabbit 2, determination I showed no silica in the kidney. In determination II, .064 mgs. of silica were added before ashing and of this the colorimetric determination showed .062 mgs.

It is difficult to draw conclusions from these results except that in rabbit tissue, the brain contains more silica per 100 gms. of dry tissue than the other organs examined. Rabbit tissue is not very well suited to good check determinations since the average organ dried weighs but a few grams and it is very hard to pick homogeneous material.

Acknowledgment

Dr. A. P. Mathews suggested the finding of a method for silica determination in order to settle once and for all the matter of its importance. He also gave during the course of the work suggestions and encouragement. For all this the writer is deeply in debt to him.

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