Proc. Nat. Acad. Sci. USA
Vol. 70, No. 5, pp. 1608–1612, May 1973

A Bound Form of Silicon in Glycosaminoglycans and Polyuronides

(polysaccharide matrix/connective tissue)

KLAUS SCHWARZ

Laboratory of Experimental Metabolic Diseases, Veterans Administration Hospital, Long Beach, California 90801; and Department of Biological Chemistry, School of Medicine, University of California at Los Angeles, Calif. 90024

Communicated by P. D. Boyer, March 19, 1973

ABSTRACT **Silicon was found to be a constituent of certain glycosaminoglycans and polyuronides, where it occurs firmly bound to the polysaccharide matrix. 330–554 ppm of bound Si were detected in purified hyaluronic acid from umbilical cord, chondroitin 4-sulfate, dermatan sulfate, and heparan sulfate. These amounts correspond to 1 atom of Si per 50,000–85,000 molecular weight or 130–280 repeating units. 57–191 ppm occur in chondroitin 6-sulfate, heparin, and keratan sulfate-2 from cartilage, while hyaluronic acids from vitreous humor and keratan sulfate-1 from cornea were Si-free. Large amounts of bound Si are also present in pectin (2580 ppm) and alginic acid (451 ppm). The bound Si is not dialyzable, does not react with ammonium molybdate, is not liberated by autoclaving or 8 M urea, and is stable against weak alkali and acid. Strong alkali and acid hydrolyze the Si-polysaccharide bond. Free, direct-reacting, dialyzable silicate is obtained. Enzymatic hydrolysis of hyaluronic acid or pectin does not liberate silicic acid, but leads to products of low molecular weight still containing Si in bound form. It is concluded that Si is present as a silanolate, i.e., an ether (or ester-like) derivative of silicic acid, and that $R_1—O—Si—O—R_2$ or $R_1—O—Si—O—Si—O—R_2$ bridges play a role in the structural organization of glycosaminoglycans and polyuronides. Thus, Si may function as a biological cross-linking agent and contribute to architecture and resilience of connective tissue.**

Silicon (Si) is essential for growth and general development (1). In all-plastic isolators that exclude the element from the environment (2, 3), growth of rats is reduced by 30–35% when Si-deficient aminoacid diets are fed, and bone deformations develop. Dietary supplements of silicate prevent these symptoms. Similar findings have been reported for chicks (4). Si, moreover, is needed in rats for normal pigment formation in the enamel of incisors (5).

Even though it has been known for years that Si occurs in small, varying amounts in all animals, very little concrete evidence exists about its functional significance and biochemical behavior (6, 7). In mammals, Si occurs most abundantly in connective tissue and related structures. While amounts in blood and parenchymal organs are relatively low, the Si content of skin, cartilage, ligaments, and other tissues of mainly mesodermal origin frequently exceeds 100 μg/g dry wt (8)*.

In extensive experiments with cartilage and bovine-nasal septum we found Si to be strongly bound to the organic matrix of these tissues. During attempts to identify the binding site of Si we discovered that certain glycosaminoglycans, notably hyaluronic acid, chondroitin 4-sulfate, dermatan sulfate, and heparan sulfate, contain relatively large amounts of the element, not as free silicate ions or silicic acid but as a firmly bound component of the polysaccharide matrix. High amounts of bound Si were also detected in two polyuronides, pectin and alginic acid. Stability tests and enzymatic studies provide evidence for the assumption that the Si is linked covalently to the polysaccharides in question, possibly as an ether (or ester-like) derivative of silicic acid, i.e., a silanolate.

* Reported Si amounts vary greatly, depending on the analytical methods used. A large number of results (9, 10) obtained before development of suitable methods (11, 12) and the advent of plastic laboratory ware are much higher than those found more recently (8, 13). Many of these older data must be discarded.

MATERIALS AND METHODS

Eight of the mucopolysaccharides studied were standard reference samples obtained from Dr. M. B. Mathews, Department of Pediatrics, University of Chicago, Ill. These samples were prepared under a grant from the National Heart Institute to serve as reference standards for research on connective tissue polysaccharides. (For details see ref. 14.) The following materials were analyzed (see Table 1): hyaluronic acid (standard reference sample), from human umbilical cords, molecular weight 230,000; hyaluronic acid, grade 1, from human umbilical cord, Sigma Chemical Corp.; hyaluronic acid from bovine-vitreous humor, P. L. Biochemicals, Inc.; chondroitin 4-sulfate (standard reference sample) from notocord of rock sturgeon (*Acipenser fulvescens*) 98% C-4 sulfated disaccharide, molecular weight 12,000; chondroitin 4-sulfate from rat costal cartilage, courtesy of Dr. M. B. Mathews; chondroitin 6-sulfate (standard reference sample) from human umbilical cord (15), 80% C-6 sulfated, 10% C-4 sulfated, and 10% unsulfated disaccharide, molecular weight 40,000; chondroitin 6-sulfate from human cartilage, courtesy of Dr. M. B. Mathews; chondroitin 6-sulfate from sturgeon cartilage, courtesy of Dr. M. B. Mathews; dermatan sulfate (standard reference sample) from hog mucosal tissue byproducts of heparin isolation, molecular weight 27,000; heparan sulfate (standard reference sample) from beef lung byproducts, calcium salt, assumed molecular weight 15,000–17,000; heparin (standard reference sample) from hog mucosal tissues, anticoagulant activity 180 U.S.P. international units/mg, molecular weight 11,000; keratan sulfate-1 (standard reference sample) from bovine-corneal tissue, molecular weight 16,000; keratan sulfate-2 (standard reference sample) from human costal cartilage, heterogenous, containing portions of material incompletely substituted by sulfate; pectin (pectinic acid), purified, from citrus fruit, Nutritional Biochemicals Corp., molecular weight 100,000–200,000; alginate from *Laminaria digitata*, courtesy of Dr. Arne Haug, Institute for Marine Biochemistry, Trondheim, Norway, containing about 60% D-mannuronic and 40% *Laminaria*-guluronic acid; alginate, prepared from *Laminaria digitata*, courtesy of Dr. Arne Haug, containing about 40% D-mannuronic acid and

60% *Laminaria*-guluronic acid, average molecular weight 200,000; glycogen, purified, from rabbit liver, Nutritional Biochemicals Corp., average molecular weight 1,000,000, total ash 0.13%; starch, purified, from corn, General Biochemicals, Inc.; Dextran (Clinical Grade), Nutritional Biochemicals Corp., molecular weight 200,000–300,000 (median 254,000), prepared from culture medium of *Leuconostoc mesenteroides* (NRRL-B512), 1,6-linked with slight trace of 1,3-linked glucoside, 0.02% ash; inulin, purified, from Dahlia tubers, Sigma.

Plastic laboratory ware was used in all procedures, except for pH measurements done with a miniature combination glass electrode. Samples were kept covered during practically all manipulations to avoid Si contamination from dust. Si was determined by the technique devised by King and collaborators (11), as modified by McGavac *et al.* (12, 13). The method is based on the formation of silicomolybdate ($SiO_2 \cdot 12MoO_3$) and the production of a blue color upon reduction. Interference by phosphate ions was not a problem in these analyses. In addition to colorimetric Si determinations, several materials were subjected to emission spectroscopy, by Mr. George Alexander and staff, Laboratory of Nuclear Medicine and Radiation Biology, University of California at Los Angeles.

In the colorimetric assay, 1 μg of Si produced an absorbance of 0.167; the detection limit was 0.03 μg of Si. Complete reagent blanks were applied side-by-side with the analyzed specimen. Whenever samples were subjected to procedures such as dialysis, autoclaving, or hydrolysis, blanks were simultaneously treated in identical fashion. The following chemicals were used: ammonium molybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, sodium sulfite, ferric chloride, sodium acetate, anhydrous powder (all analyzed reagents); and 1,2,4-aminonaphthol-sulfuric acid (all from J. T. Baker Chemical Co.); sulfuric acid, 95–97%, A.C.S. reagent (Dupont); sodium metabisulfite, reagent (Matheson, Coleman & Bell); sodium carbonate, anhydrous powder, A.C.S. analytical reagent, and hydrochloric acid, analyzed reagent, U.S.P. (Mallinckrodt); congo red, certified (Allied Chemical).

The colorimetric method was used to determine the amount of *free*, directly reacting silicate and silicic acid, and to determine the amount of *total* Si after sodium carbonate fusion in platinum crucibles. In some cases, indirect reacting Si was measured after treatment of various polysaccharides with dilute sodium hydroxide at 100°.

Details of experiments to liberate bound Si by dialysis, autoclaving, 8 M urea, alkali, or acid are described in Table 2. For enzymatic hydrolysis of hyaluronic acid, hyaluronidase from bovine testes was used (Sigma, 348 units/mg). Depolymerization of hyaluronic acid was measured by the turbidimetric assay (16) with fraction V bovine albumin (Sigma). For the enzymatic breakdown of pectin, pectinase from *Aspergillus niger* was used (Sigma, approximately 1 unit/mg solid). Pectinase activity was followed by iodometric titration of reducing end groups (17, 18).

RESULTS

Determination of Free, Unbound Silicic Acid. Free, unbound silicic acid in mucopolysaccharides can be directly determined by colorimetric analysis since, within reasonable limits, the presence of mucopolysaccharides and polyuronides does not interfere with the colorimetric Si determination. On numerous occasions free silicate was added in microgram quantities to glycosaminoglycan or polyuronide solutions. In experiments done under various conditions over extended periods of time, no evidence could be found for spontaneous formation of bound Si.

TABLE 1. *Free, total, and bound Si in glycosaminoglycans, polyuronides, and some glycans*

Substance and source	Si (μg/g) Free	Total	Bound (total minus free)
Glycosaminoglycans			
Hyaluronic acid			
(*a*) Human umbilical cord	25	354	329
(*b*) Human umbilical cord	1533	1892	359
(*c*) Bovine vitreous humor	980	949	—
Chondroitin 4-sulfate			
(*d*) Notocord of rock sturgeon	44	598	554
(*e*) Rat costal cartilage	30	361	331
Chondroitin 6-sulfate			
(*f*) Human umbilical cord	45	123	78
(*g*) Human cartilage	36	227	191
(*h*) Sturgeon cartilage	64	121	57
Dermatan sulfate			
(*i*) Hog mucosal tissue	46	548	502
Heparan sulfate			
(*j*) Beef lung	39	466	427
Heparin			
(*k*) Hog mucosal tissue	33	175	142
Keratan sulfate-1			
(*l*) Bovine cornea	31	37	—
Keratan sulfate-2			
(*m*) Human costal cartilage	37	105	68
Polyuronides			
Pectin			
(*n*) Citrus fruit	5	2586	2581
Alginic acid			
(*o*) Horsetail kelp	—	43	—
(*p*) Horsetail kelp	5	456	451
Polyglycans			
Glycogen			
(*q*) Rabbit liver	8	34	26
Starch			
(*r*) Corn	—	22	—
Dextran			
(*s*) *Leuconostoc mesenteroides*	19	22	—
Inulin			
(*t*) Dahlia tubers	15	29	14

Bound Si in Glycosaminoglycans and Polyuronides. Comparison of the free, directly reacting silicate to total Si determined after carbonate fusion reveals that large amounts of bound Si occur in certain acid mucopolysaccharides and polyuronides (Table 1). 13 Samples of acid mucopolysaccharides and three polyuronides were studied. In addition, four commonly occurring polyglycans were analyzed. Of all 20 samples, only two contained large amounts of free, directly reacting silicate ions (1533 and 980 μg/g); both were commercial preparations of hyaluronic acid. All other substances showed negligible amounts of free silicate. 330–554 ppm of bound Si were detected in hyaluronic acid prepared from umbilical cord, chondroitin 4-sulfate, dermatan sulfate, and heparan sulfate.

Smaller amounts of bound Si (57–191 ppm) occurred in chondroitin 6-sulfate, heparin, and keratan sulfate-2 from cartilage. Hyaluronic acid from vitreous humor and keratan sulfate-1 from cornea were practically free of bound Si.

The highest amount of bound Si was encountered in purified pectin from citrus fruit, which contained over 2500 ppm by colorimetric analysis. Two samples of alginic acid from horsetail kelp differed in their Si content. Where one contained 456 ppm, an amount comparable to that found in acid mucopolysaccharides, the other was low in Si. A direct estimation of free silicate in the latter material was not possible since a precipitate formed upon addition of the ammonium molybdate–sulfuric acid reagent. No such problem existed in direct analyses of the other samples tested, except for cornstarch.

The four polyglycans, glycogen, starch, dextran, and inulin, contained only minute amounts of Si, most probably as silicate. All had been manufactured under conditions that avoided exposure to strong alkali or acid. This consideration is important since the bond between Si and the carbohydrate matrix is sensitive to alkali and acid.

Comparison of Results of Colorimetric and Emission Spectroscopic Si Analysis. The results of Si determinations by dc arc emission spectroscopy in general confirmed those of the colorimetric method. In some cases, the two methods produced practically identical data, for example in samples *d*, *k*, *q*, and *r* of Table 1 (600 compared to 598, 180 compared to 175, 33 compared to 34, and 24 compared to 22 μg/g). In other instances larger differences appeared, e.g., in samples *a*, *j*, *m*, *p*, and *t* (482 compared to 354, 580 compared to 466, 80 compared to 105, 360 compared to 456, and 12 compared to 29 μg/g).

TABLE 2. *Stability of Si binding in hyaluronic acid (HA)[a] and pectin[b]*

Treatment	Free Si (μg/g) HA	Free Si (μg/g) Pectin	Liberated Si (%) HA	Liberated Si (%) Pectin
Control	25	20	—	—
(I) Dialysis, 0.1 N NaOH[c,d]				
Dialysate, 70 hr, at 4°	42	25	5	0
(II) Autoclaving[e]				
15 lb pressure, 1 hr	49	41	7	<1
(III)Urea, 8 M[f]				
20 hr at 4°	22	43	0	<1
1 hr at 60°	23	36	0	<1
(IV)Alkali at room temperature (24°)				
pH 12.4, 0.1 N NaOH, 60 hr	60	28	12	<1
pH 13.5, 1 N NaOH, 2 hr	316	40	88	<1
pH 13.5, 1 N NaOH, 18 hr	399	392	100	15
(V)Alkali at 100°				
pH 12.5, 0.1 N NaOH, 2 hr	313	460	95	29
pH 13.4, 1 N NaOH, 2 hr	—	1960	—	77
(VI)Acid at room temperature (24°)				
pH 3.0, 18 hr	20	22	0	0
pH 1.2, 0.2 N HCl, 2 hr	147	32	45	<1
pH 1.2, 0.2 N HCl, 18 hr	190[g]	242	58	9

[a] Tests performed on solutions containing, per ml, 1 mg of hyaluronic acid, sample *a* (329 μg bound Si per g). [b] Tests performed on solutions containing, per ml, 5 mg of pectin, sample *n* (2581 μg bound Si per g). [c] 10 ml of solution dialyzed against 100 ml of 0.1 N NaOH for 5 hr, followed by a second dialysis for 65 hr. [d] Experiments with nearly identical results done with heparan sulfate (sample *j*) and heparin (*k*). [e] Hyaluronic acid autoclaved at pH 5.7, pectin at pH 7.0. [f] 5 ml of hyaluronic acid solution, 2.4 g of urea. Measurements of Si in the 8 M urea read against a standard curve done in 8 M urea. [g] Partially separated as $SiO_2 \cdot xH_2O$, dissolved in 1 N NaOH for analysis. Determination of Si in the supernatant showed 157 μg still in bound form.

Experiments to Liberate Bound Si were done mainly with hyaluronic acid (sample *a*, standard reference sample) and pectin (sample *n*). The results obtained with these two materials were analogous, but the Si in pectin was less readily liberated by alkali or acid (Table 2). While silicic acid ions were dialyzable against water or 0.1 N NaOH when added to solutions of mucopolysaccharides or polyuronides, only a small fraction of the bound Si was removed by extended dialysis (Exp. *I*, Table 2). The amount dialyzable corresponded to that liberated by prolonged standing in 0.1 N NaOH at room temperature (24°). Neither autoclaving (Exp. *II*) nor treatment with 8 M urea (Exp. *III*), which could break hydrogen bonds, liberated the bound Si. A small quantity of free silicic acid appearing after autoclaving of hyaluronic acid may have been due to hydrolysis, since the experiment was performed at pH 5.7.

Alkaline hydrolysis broke the bond between Si and the polysaccharide molecule (Exps. *IV* and *V*). Free silicate was formed that reacted directly with ammonium molybdate and was dialyzable. At room temperature, hyaluronic acid lost only 12% of the Si after 60 hr in 0.1 N NaOH, but in 1 N NaOH 88% was liberated within 2 hr (Exp. *IV*). The Si in pectin remained bound under these conditions, and even after 18 hr at pH 13.5, only 15% of the bound Si had been released. At 100° (Exp. *V*), 0.1 N NaOH liberated almost all of the Si from hyaluronic acid within 2 hr, but only 29% from pectin. 2-hr treatment with 1 N NaOH at 100° liberated 77%. Prolonged alkaline hydrolysis was needed to completely recover the bound Si from pectin as silicate ion.

At pH 3, the bond between Si and polysaccharide matrix was stable over extended periods of time, but treatment with 0.2 N HCl (pH 1.2) liberated a large portion of the bound Si (Exp. *VI*). In acid, as in alkali, hyaluronic acid released its bound Si more readily than pectin. Within 2 hr, 45% of the Si from hyaluronic acid was found as free, directly-reacting silicic acid, and after 18 hr, 58% had been removed. Much of it precipitated out as a sediment of silica. Tests for Si in the supernatant by sodium carbonate fusion showed that the conversion was incomplete. Under these conditions, pectin released only 9% of its bound Si; heating was required for complete liberation (details to be published elsewhere).

Effect of Enzymatic Hydrolysis on Bound Si. Studies with hyaluronidase and pectinase showed that the enzymatic breakdown of the polysaccharide structure does not convert the bound Si into free, directly-reacting silicic acid (Table 3). Instead, breakdown products of low molecular weight were obtained to which the Si is firmly attached. Under the conditions applied (Exp. *I*), hyaluronidase rapidly depolymerized the hyaluronic acid. After 60 min, turbidity readings amounted to only 0.7% of those of the initial reaction mixture at zero time. No release of silicate ions was detected. Hydrolysis of the incubation mixture at pH 12.5 for 2 hr at 100° was used to release bound Si (indirect Si). The equivalent of 300 μg of indirect Si was found per g in the reaction mixture.

Untreated hyaluronic acid produced 319 μg of indirect Si under the same conditions. In a subsequent experiment (Exp. *II*), an attempt was made to break the hyaluronic acid down to tetrasaccharide units, as described by Ludowieg *et al.* (19). Again, free silicate was not detectable in the incubation mixture, but a large amount of bound, indirect Si was found. It was dialyzable against water without decomposition.

After nearly complete enzymatic hydrolysis of pectin by pectinase (Exp. *III*) only 6% of the Si initially bound to the pectin matrix was found in free form. The main portion was present as bound, indirect Si. As shown above, however, the standard treatment for 2 hr at pH 12.5 was inadequate to completely liberate the bound silicic acid present in pectin. The enzymatic breakdown products of pectin containing the bound, indirect reacting form of Si were dialyzable.

Thus, the results of the enzymatic hydrolysis of hyaluronic acid and pectin provide further evidence that Si si covalently bound. It appears to be an integral component of these substances in their natural state.

TABLE 3. *Effect of enzymatic hydrolysis on bound Si*

	Free Si (μg/g)	Indirect Si[a] (μg/g)	Indirect Liberated (%)
(I)Hyaluronic acid, 60 min incubation[b]			
Before	33	329	—
After[c]	33	300	0
(II)Hyaluronic acid, 45 hr incubation[d]			
Before	32	322	—
After	—[e]	321	0
(III)Pectin, 20 hr incubation[f]			
Before	20	2560	—
After[g]	179	1279[h]	6

[a] Si liberated at pH 12.5 after heating to 100° for 1 hr. [b] Mixture containing 0.6 mg of hyaluronic acid, sample *a*, and 0.1 mg of hyaluronidase per ml 0.1 M sodium acetate buffer pH 5.3, kept for 60 min at 37°. [c] Turbidity readings in depolymerization assay dropped from 1.489 for the initial reaction mixture to 0.009 after 60 min. [d] Conditions as in *b*. Fresh enzyme added after 3 and 24 hr. [e] Slightly turbid. No blue color. [f] Mixture containing 5 mg of pectin, sample *n*, and 4 mg of crude pectinase per ml at pH 4.0, kept for 20 hr at 25°. [g] Iodometric titration revealed that about 93% of galacturonic acid had been liberated. [h] Incomplete "indirect" analysis (see *text*).

DISCUSSION

The data show that firmly bound Si is a constituent of certain mucopolysaccharides and polyuronides, notably hyaluronic acid, chondroitin 4-sulfate, dermatan sulfate, heparan sulfate, and pectinic and alginic acids. We were unable to find any published reference to previous findings on the occurrence of Si in such compounds.† The element appears to be present as a derivative of silicic acid. Bound Si does not react with ammonium molybdate and is not dialyzable. Since it is not liberated by autoclaving or treatment with 8 M urea and is stable against dilute alkali and acid at room temperature, hydrogen bonding does not seem to play a decisive role in Si binding.

The findings thus lead to the conclusion that Si in glycosaminoglycans and polyuronides is covalently bound to the carbohydrate matrix, most likely as a silanolate, i.e., an ether (or ester-like) derivative of silicic acid and hydroxyl groups. The bridge between the Si atom and the carbohydrate polymer chain would consist of a Si—O—C group. This concept is strengthened by the results of the enzymatic studies.

Acid mucopolysaccharides with large amounts of bound Si are similar to each other in Si content, even though they are very dissimilar with respect to molecular weights. The Si-containing acid mucopolysaccharides bind 1 atom of Si per 50,000–85,000 molecular weight; this corresponds to 1 atom of Si per 130–280 repeating units. Calculation of the molar ratios shows that there are, per atom of Si, about 0.3 molecule of hyaluronic acid from umbilical cord (molecular weight 230,000), 2 molecules of dermatan sulfate (molecular weight 27,000), 4 mol of chondroitin 4-sulfate (molecular weight 12,000), 4 mol of heparan sulfate (if average molecular weight is assumed to be about 16,000), and 8 mol of chondroitin 6-sulfate. It remains to be seen whether these figures are coincidental or whether they express an innate regularity of mucopolysaccharide chemistry. The pectin contains 10–20 atoms of Si per mol (molecular weight 100,000–200,000), i.e., 1 atom of Si per 10,000 molecular weight. The minimum molecular weight of pectin is about 10,000 (21). The Si could be attached to a suitable hydroxyl group of the uronic acid moiety. It is also possible, however, that Si is specifically connected to certain minority sugars that may occur at interspersed intervals in the polymeric glycan chain and act as tie-up sites.

Bound Si may play an important role in the structural organizaiion of acid mucopolysaccharides and polyuronides. In principle, Si could link two binding sites through a R_1—O—Si—O—R_2 group; the Si atom would carry two additional radicals. Orthosilicic acid, $Si(OH)_4$, could connect up to four binding sites directly. An alternative exists in which the Si atoms of two compounds would be linked by an oxygen-bridge, forming a R_1—O—Si—O—Si—O—R_2 group; the two Si atoms could bind up to four additional radicals by way of oxygen. Si has a strong tendency to form such groups (7, 22). This tendency could lead to attachment of further Si atoms and formation of Si—O ring systems or polymers. Thus, firm structural arrangements between bundles of mucopolysaccharides, and also proteins, are possible. Such interlacing structures could hold acid mucopolysaccharides and also proteins together in an organized fashion, contributing to the architecture and resilience of connective tissues, e.g., cartilage (23). Si functions as a biological crosslinking agent in connective tissue. Stereochemical questions arise from these considerations since the substituents of the Si atom, like those of the carbon atom, are arranged in tetrahedral geometry that is much more rigid than that in carbon chemistry. "Minimization of motion and angle distortion for nonreacting groups is an important factor in organosilicon mechanisms" (24).

With respect to individual polysaccharide molecules, bound Si could function in several ways: (*a*) It could connect different portions of the same polymeric saccharide chain, establishing a secondary structure and controlling molecular shape. (*b*) It could link different polysaccharide chains to each other.

† However, Holt postulated in 1953 that in silicosis, silicic acid from the inhaled silica dust *replaces* normal mucopolysaccharides in the interfibrillary spaces, thus causing the pathological changes in the lung (20).

Si may contribute to the very high molecular size of glycosaminoglycans and polyuronides in their natural state. (c) Si could also link acid mucopolysaccharides to proteins. Almost all glycosaminoglycans, and many other polysaccharides, occur in nature primarily as protein–polysaccharide compounds (25). Si could establish a bridge between glycosaminoglycans and collagen, or the globular protein found in the ground substance of connective tissue (26). Such a link could exist aside from the well-documented polysaccharide–xylosyl–serine–protein bridge and similar links found in proteoglycan molecules (27).

A consistent difference is seen between samples of chondroitin 4-sulfate and those of chondroitin 6-sulfate, the latter containing only a fraction of the quantity of bound Si found in the former. Low amounts of Si could either be characteristic of chondroitin 6-sulfate, or they could be due to impurities. In view of the great difficulties in obtaining mucopolysaccharides in pure form, other samples in which we found low amounts of bound Si may also not have been homogeneous.

The data on pectin and alginic acid show that the occurrence of bound Si is not limited to acid mucopolysaccharides of animal origin.‡ However, the preparation of these products normally involves treatment with strong alkali or acid, often at elevated temperatures, conditions under which Si may be lost.

Numerous attempts have been made to extract and identify organic Si compounds from tissues, but in no case has a pure substance been isolated and chemically characterized (6, 7). Si "esters" of lipids such as cholesterol, lethicin, and choline have been described after extraction of tissues by ethanol–ether. Holt and Yates demonstrated that such products are artifacts, obtainable *in vitro* by treating these compounds with soluble silicic acid. They arise from micelle formation of inorganic oligo- or polysilicate ions with organic compounds (10). Similar derivatives have been described for carbohydrates. A critical review of these studies, and of Si in plant growth, is available (28). Because of the stability of the bound form of Si in glycosaminoglycans and polyuronides presented here, micelle formation can be excluded from the interpretation of our findings.

The metabolic processes by which Si is assimilated are little understood, even though large amounts of the element are used for structural stability by diatoms, many higher plants, and two groups of animals, the *Radiolaria* (protozoa) and sponges. Limpets (*Patellacea*) assimilate Si to make opal baseplates for their teeth (29). In diatoms, Si supplementation stimulates numerous enzyme systems and leads to an immediate increase in amounts of DNA. Inhibitors of energy metabolism interfere with the uptake of silicate.

Since Si is essential for higher animals and since it is apparently an integral constituent of acid mucopolysaccharides and polyuronides, the question arises whether there are common pathways of Si metabolism in bacteria, plants, and animals. With respect to mucopolysaccharides, it is theoretically possible that inorganic Si is bound after the macromolecular structure has been formed. An alternative, more plausible from stereochemical considerations, would consist of the incorporation of preformed mono- or disaccharide Si derivatives during synthesis of the polysaccharide chain. Investigations on the enzymatic formation of biological Si derivatives and on Si-containing intermediates appear indicated. The finding of Si as a constituent of glycosaminoglycans and polyuronides introduces a new aspect into the discussions surrounding the chemistry of these compounds.

‡ In cotton, considered the purest form of naturally occurring cellulose, 116 μg of Si were found per g.

I thank Dr. M. B. Mathews, Department of Pediatrics, University of Chicago, Ill., for generous gifts of acid mucopolysaccharides; Dr. Arne Haug, Institute for Marine Biochemistry, Trondheim, Norway, for alginic acids; and Mr. George Alexander, Laboratory of Nuclear Medicine and Radiation Biology, UCLA, for emission spectroscopic analyses. The excellent technical assistance of Mrs. Billie Ricci, Mrs. Martha Duckworth, and Mr. Dennis Kim is gratefully acknowledged. This work was supported by USPHS Grant AM 08669.

1. Schwarz, K. & Milne, D. B. (1972) *Nature* **239**, 333–334.
2. Smith, J. & Schwarz, K. (1967) *J. Nutr.* **93**, 182–188.
3. Schwarz, K. (1970) in *Trace Element Metabolism in Animals*, ed. Mills, C. F. (E&S Livingstone, Edinburgh), pp. 25–38.
4. Carlisle, E. M. (1972) *Science* **178**, 619–621..
5. Milne, D. B., Schwarz, K. & Sognnaes, R. (1972) *Fed. Proc.* **31**, 700.
6. Garson, L. & Kirchner, L. (1971) *J. Pharm. Sci.* **60**, 1113–1127.
7. Fessenden, R. & Fessenden, J. (1967) *Advan. Drug Res.* **4**, 95–132.
8. Fregert, S. (1959) *Acta Dermato-Venereol.* **39**, suppl. 42, 1–92.
9. King, E. & Belt, T. (1938) *Phys. Rev.* **18**, 329–365.
10. Holt, P. & Yates, D. (1953) *Biochem. J.* **54**, 300–305.
11. King, E., Stacy, B., Holt, P., Yates, D. & Pickles, D. (1955) *The Analyst* **80**, 441–453.
12. McGavack, T., Leslie, J. & Tang Kao, K. (1962) *Proc. Soc. Exp. Biol. Med.* **110**, 215–218.
13. Leslie, J., Tang Kao, K. & McGavack, T. (1962) *Proc. Soc. Exp. Biol. Med.* **110**, 218–220.
14. Roden, L., Baker, J. R., Cifonelli, J. A. & Mathews, M. B. (1972) in *Methods of Enzymology*, ed. Ginsburg, V. (Academic Press, New York), Vol. 28B, pp. 73–140.
15. Mathews, M. B. (1966) in *Methods of Enzymology*, eds. Neufeld, E. F. & Ginsburg, V. (Academic Press, New York), Vol. 8, pp. 654–662.
16. Kertesz, Z. I. (1955) in *Methods of Enzymology*, eds. Colowick, S. P. & Kaplan, N. O. (Academic Press, New York), Vol. 1, pp. 152–164.
17. Owens, H. S., McCready, R. M., Sheperd, A. D., Schultz, T. H., Pippen, E. L., Swenson, H. A., Miers, J. C., Erlandsen, R. F. & Maclay, W. D. (1952) *Methods used at Western Regional Research Laboratory for Extraction and Analysis of Pectic Materials* (U.S. Dept. Agriculture, AIC-340).
18. *Worthington Enzyme Manual* (1972) (Worthington Biochemical Corp., Freehold, New Jersey), p. 112.
19. Ludowieg, J., Vennesland, B. & Dorfman, A. (1961) *J. Biol. Chem.* **236**, 333–339.
20. Holt, P. F. & Osborne, S. G. (1953) *Brit. J. Ind. Med.* **10**, 152–156.
21. Kertesz, Z. I. (1963) in *Comprehensive Biochemistry*, eds. Florkin, M. & Stotz, E. H. (Elsevier, N. Y.), Vol. 5, pp. 233–245.
22. Cotton, F. A. & Wilkinson, G. (1966) *Advanced Inorganic Chemistry* (Interscience Publishers, New York), pp. 468–474.
23. Barrett, A. J. (1968) in *Comprehensive Biochemistry*, eds. Florkin, M. & Stotz, E. H. (Elsevier, New York), Vol. 26B, pp. 438–442.
24. Sommer, L. H. (1965) *Stereochemistry, Mechanism and Silicon* (McGraw-Hill, New York).
25. *The Chemical Physiology of Mucopolysaccharides* (1968) ed. Quintarelli, G. (Little, Brown & Co., Boston).
26. Partridge, S. M. (1968) in *The Chemical Physiology of Mucopolysaccharides*, ed. Quintarelli, G. (Little, Brown & Co., Boston), pp. 51–62.
27. Barrett, A. J. (1968) in *Comprehensive Biochemistry*, eds. Florkin, M. & Stotz, E. H. (Elsevier, New York), Vol. 26B, pp. 434–438.
28. Lewin, J. & Reimann, B. E. F. (1969) *Annu. Rev. Plant. Physiol.* **20**, 289–304.
29. Lowenstam, H. (1971) *Science* **171**, 487–490.