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Silicon in Biological Material. I. Determinations Eliminating Silicon as a Contaminant. (27469)

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Silicon has been found to be present in all biological material(1-7). Since the content of silicon is small, a micro-method is required for the determination and extreme care must be taken to avoid contamination from outside sources of silicon, such as glassware and dust of the air. King(1) has shown that blood samples stored in glass tubes have a higher silicon content than those stored in cellophane tubes. Therefore, previously reported values for silicon in biological materials, obtained with the use of glassware, are probably too high. Inasmuch as nearly all laboratory wares are now available in materials other than glass, contamination of biological samples by extraneous silicon can be eliminated.

The present study is concerned with 1) determination of silicon content in biological material by a modification of King's method with the use of the least amount of glassware; and 2) absorption and excretion of silicon in the rat.

Equipment and reagents. All laboratory wares were made of either polyethylene or polypropylene plastics or stainless steel. They were washed separately from laboratory glassware in plastic dish pans with haemosol, rinsed with tap water, then distilled water and drained and re-rinsed with redistilled water, and dried in a 50° oven prior to use.

All water used was redistilled in a pyrex distilling set; this was the only place in the entire procedure that glass containers were employed. Such water was promptly stored in large polyethylene bottles. For ashing and subsequent fusion of the samples with Na_2CO_3, 8 ml platinum crucibles were used. Measurements of absorbancy were made in a Beckman DU spectrophotometer. Except for items 1 to 4 below, all reagents are as described in the original method of King *et al.*(2). All chemicals used were "reagent grade".

1. Silicon Standard Solution—a stock solution was made from sodium meta-silicate, $Na_2SiO_3 \cdot 9H_2O$ (10.15057 g/liter), representing an equivalent of 1 g of silicon/liter. A working standard solution containing 0.5 μg Si/ml was prepared from the stock solution. The $Na_2SiO_3 \cdot 9H_2O$ used is completely soluble. 2. Phenolphthalein Indicator—1% in ethanol. 3. Activated Carbon

—Darco, grade S-51 (Atlas Powder Co., Wilmington, Del.). 4. Congo Red Indicator—0.1% in water. 5. 10N H_2SO_4—278 ml of concentrated acid diluted to 1 liter with redistilled water. 6. N H_2SO_4—a 1:10 dilution of 10N. 7. Acid Ammonium Molybdate—a 5% solution of ammonium molybdate, $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$, in N H_2SO_4. 8. Reducing Solution—a 0.2% solution of 1:2:4 aminonaphtholsulfonic acid in a solution of 2.4% of sodium sulphite, Na_2SO_3, and 12% of sodium metabisulphite in water. 9. Sodium Carbonate—anhydrous (Mallinckrodt). 10. Ferric Chloride in HCl—a 1% $FeCl_3 \cdot 6\ H_2O$ solution in 0.02N HCl. 11. Sodium Acetate in NaOH—a 1.5% $C_2H_3O_2Na \cdot 3\ H_2O$ solution in 0.028N NaOH. 12. Calcium Chloride—a 0.5% solution of anhydrous $CaCl_2$ in water. 13. Calcium Hydroxide—a saturated solution in water. 14. Trichloroacetic Acid—a 5% solution in water.

Preparation of sample. All tissues were dried in a stainless steel pan in an oven at 100° overnight. Venous blood was obtained from normal individuals through stainless steel needles directly into plastic centrifuge tubes. The procedure for the precipitation of serum protein was that described by King *et al.*(2). Twenty-four hr samples of urine were collected in plastic bottles. Phosphate was removed by King's procedure(2).

In the intake and excretion experiment an 8-month-old male rat was isolated in a metabolic cage. Urine and feces were collected every 24 hr, and the amount of food consumed determined each day. The rat was fed only redistilled water and powdered Purina chow, on which the silicon content had been determined. Each 24 hr fecal sample was homogenized with redistilled water, in a stainless steel cup, with a VirTis "45"* homogenizer, at a setting of 25 for 5 minutes. Aliquots were taken and dried for determination. Twenty-four-hour urinary samples were diluted to 20-25 ml. Aliquots were taken for determination.

Tissue analysis. The dried tissues were ground in a Wiley mill.† Approximately 0.2 g of the dried ground tissue was weighed into a platinum crucible, ignited over a Fisher burner and ashed with a platinum lid on the crucible. 1.2 g of anhydrous Na_2CO_3 was added and the mixture was kept fused for exactly 20 minutes. After cooling, the melt in the crucible was dissolved in redistilled water over a small electric water bath (Renwal).‡ The solution was transferred to a 100-ml Nalgene beaker by means of a plastic dropper, then neutralized to congo red with 10N H_2SO_4. 0.2 ml of acid in excess was added. The solution was transferred to a 50-ml plastic volumetric cylinder and diluted to volume with redistilled water. Correction for any silicon which may have been contributed by the reagents used was made possible by carrying blanks through the entire procedure. In the case of bone, the melt was first neutralized to phenolphthalein with 10N H_2SO_4 and then neutralized to congo red. Phosphorus was removed by King's method(1). Phosphate-free filtrate was used for colorimetry.

Colorimetry. Duplicate 10-ml samples of water blank, standard solutions containing 5, 10 and 20 μg of silicon, reagent blank and tissue samples were each pipetted into 40-ml plastic centrifuge tubes. All samples were diluted to 15 ml with redistilled water and 2 ml of 5% acid ammonium molybdate reagent was added, and the whole mixed thoroughly. After 10 minutes, 5 ml of 10N H_2SO_4 and 0.5 ml of aminonaphtholsulfonic reducing agent were added. The solutions were mixed and, after 10 minutes, the absorbancies of silicomolybdic acid were measured in matched 1.0 cm corex cells at a wavelength of 810 mμ with a Beckman DU spectrophotometer. At the same time when a sample was measured, readings were also taken for the reagent blank and for H_2O in the same cuvette. This method, which served as a check for possible small errors arising from surface changes of the cuvette, reagent composition, etc., was deemed necessary in view of the low values for the absorbancy (frequently below 0.003) of the samples. Silicon was calculated as μg Si/g of dry tissue.

* Clay-Adams, New York.

† A. H. Thomas Co., Philadelphia, Pa.

‡ American Sundries Co., Brooklyn, N. Y.

TABLE I. Recovery of Added Silicon.

Samples	μg Si in tissue studied*	μg Si added	Total Si found	μg Si recovered	% recovery
Rat liver	.755	1.03	1.63	.88	85
" "	.755	1.13	1.61	.85	76
" "	3.225	5.34	9.03	5.81	109
" "	3.225	5.12	8.60	5.38	105
" "	1.470	5.59	7.14	5.67	101
" "	1.470	10.43	12.60	11.13	108
Rat lung	.440	3.71	3.58	3.14	85
" "	.440	5.67	5.39	4.95	87
" "	.440	8.12	7.32	6.88	85
Rat skin	1.44	3.55	4.38	2.94	83
" "	1.26	5.73	5.73	4.47	78
Femur	7.05	20.00	27.85	20.80	104
Blank	—	3.57	—	3.03	85
"	—	4.98	—	4.73	95
"	—	5.21	—	4.73	91
"	—	5.47	—	4.83	88
"	—	5.95	—	5.12	86
"	—	10.29	—	10.11	98
"	—	20.00	—	20.60	103
"	—	20.00	—	20.15	101

* In each instance 200 mg of tissue were used.

Results. The recoveries of silicon added to tissues and blanks are shown in Table I. From 76-109% of the added silicon was recovered by the present method. In Table II are shown the silicon content of several biological materials obtained by our method. These are compared with those reported previously. In the study of rat tissue, our results on muscle and lung were close to those reported by Holt *et al.*(3), but our result for kidney tissue was appreciably lower, and, in femur, much higher than that reported by them. For human blood, our values were lower than those of King *et al.*(2). Our data on the 24-hr urinary excretion of silicon in man were lower than those reported by Fregert(5), and, in human lung, lower than those reported by King(8) and by Fregert(5). An illustration of the results of intake and excretion studies of silicon in the rat for a 3-day period are shown in Table III.

TABLE II. Silicon Content in Various Biological Materials.

Biological material	Author's data	Previous report†
	Range	
	μg Si/g dried tissue	μg Si/g wet tissue
Rat kidney	2.6– 7.7 (7)*	172 ±43 (4) [3]
" muscle	2.6– 6.3 (7)	3 ± .5 (2) [3]
" lung	5.5– 9.7 (8)	12 ± 2 (4) [3]
" femur	87 –134 (6)	12 [3]
	μg Si/g wet tissue	μg Si/g dried tissue
Human lung	21–166 (4)	178–1365 (19) [8]
		635 (15) [5]
	μg Si/100 ml	μg Si/100 ml
Human blood serum	88 ± 34 (11)	140 (4) [2]
	mg Si/24 hr	mg Si/24 hr
Human urine	23.10 ± 9.13 (9)	37.10 (10) [5]

* No. of determinations corresponds to No. of animals.
† Reference numbers in brackets.

TABLE III. Intake and Excretion of Silicon in a 3-Day Balance Study in the Rat.*

	Day 1	Day 2	Day 3	Total
Rat chow intake (g)	12.95	10.12	15.03	
Silicon intake (mg)	20.91	16.34	24.27	61.52
Feces excreted (g)	5.70	6.10	4.34	
Fecal Si (mg)	22.75	18.31	16.15	57.21
Urine excreted (ml) †	25.0	20.0	20.0	
Urinary Si (mg)	.174	.112	.176	.462
		Total Si excretion		57.67

* Wt of animal was 250 g.
† Urine + wash.

Discussion. The colorimetric method of King(1), as later modified by him and his associates(2), for determining silicon may still be the best procedure for the microdetermination, except for the 2 following problems: (i) the step of diluting and neutralizing

the fused sample in glass volumetric flasks may cause contamination of the silicon from glass to the sample; (ii) the impossibility of making a 0.1% congo red solution in alcohol. In regard to the latter, we substituted an aqueous solution of congo red. The present procedure eliminates the first problem by affording no opportunity for contamination by silicon ordinarily present in glassware. Therefore, the present data represent the actual amounts of silicon in the tissues. In view of our handling of these problems, no valid comparisons can be made between the methods of King(1,2) and the modifications thereof, which have been used here.

Values for silicon in the present study are lower than those found in serum by King(2) an in urine by Fregert(5). This is probably due to the complete elimination of silicon contamination from our samples. Our data for the silicon content of tissues differ from those reported by Holt *et al.*(3). These workers did not mention the method they used for determination of silicon, but we presume it was that of King *et al.*(2).

Recently, Sauer *et al.*(4) determined the silicon content of the tissues of guinea pig. Their values were: 59 μg Si/g of dried tissue of lung; 33 μg Si/g of muscle; 25 μg Si/g of kidney. Since our data on silicon content of human brain and rat brain(9) are similar, it is probable that the silicon content of the brains of guinea pigs and rats are also similar. Their higher values for the content for guinea pig brain may be due to contamination with extraneous silicon.

In the illustration of balance data used here (Table III), more than 90% of food silicon appeared in the feces. Appreciable amounts of silicon were excreted in the urine. All tissues, including blood were found to contain silicon. Of all tissues studied, bone has been found to have the highest content of silicon.

Summary. 1. The silicon content of the tissues of man and rat were reinvestigated by a modified King's method, in which contact with glassware was avoided. 2. All tissues studied, except bone, contained between 2-10 μg silicon/g of dried tissue. Bone contained approximately 100 μg silicon/g of dried tissue. 3. In a single balance study, 90% of the silicon in rat food was excreted in the feces; some silicon was excreted in the urine.

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Silicon in Biological Material. II. Variations in Silicon Contents in Tissues of Rat at Different Ages. (27470)

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An increase in collagen content of animal tissues with increasing age has been reported by several workers(1-7). Silicic acid has been observed to have both a stimulating and an inhibiting effect upon synthesis of collagen in tissue culture(8). Saline precipitation of acid soluble collagen of rat tendon in a fibrinous state is said to have been prevented by