[Chem. Pharm. Bull. 27(8)1733—1739(1979)]

UDC 615.453.2.011.3.014.2 : 546.284-35.03.09

Effect of Degree of Polymerization of Silicic Acid on the Gastrointestinal Absorption of Silicate in Rats[1]

Hideharu Yokoi and Saburo Enomoto

Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University[2]

(Received October 23, 1978)

Three varieties of sodium aluminosilicate gels with known distributions of molecular forms of silicic acid were orally administered to rats. One preparation was composed chiefly of low molecular weight silicic acids, another preparation of soluble polysilicic acids, and the third preparation of insoluble polysilicic acids. Urinary silicic acid excretion was regarded as corresponding to silicic acid absorption, after oral administration of these silicate preparations, and the relation between silicic acid absorption and degree of polymerization was studied. Among various silicic acids formed upon acid hydrolysis of silicates in the stomach, orthosilicic acid in particular was absorbed, while polysilicic acids, regardless of solubility, were hardly absorbable. It seems likely that silicic acids are absorbed from the digestive tract through the lipoid membrane pore route, the mechanism being common in the permeation of hydrophilic molecules. The possible mechanism of formation of calculi composed of silicic acid is discussed.

Keywords——sodium aluminosilicate gel; silicate antacid; absorption of silicic acid; excretion of silicic acid; monosilicic acid; gastrointestinal absorption; trimethylsilylation; siliceous calculi; pore route absorption; rat

The breakdown of magnesium silicate in contact with gastric juice after oral administration and the gastrointestinal absorption of the silicic acids thus formed have been studied both clinically[3] and experimentally with various species of animals.[4–6] Herman *et al.*[7] and Lagergren[8] have reported cases with urinary calculus consisting chiefly of silicic acids. All the cases had received silicate antacid preparations regularly for several years. In addition, Newberne *et al.*[9] reported renal damage in dogs after ingestion of various silicates for 4 weeks. Trace amounts of silicon were also detected in the tissues of the liver and the kidney.[10] Nothing is known, however, about the forms of silicic acids or silicon existing in the tissues, or the relation between the molecular forms of silicic acids and the gastrointestinal absorption. In the previous report, the trimethylsilylation technique was used to demonstrate the presence of silicic acid ions such as SiO_4^{4-} and $Si_2O_7^{6-}$, soluble polysilicic acid ions given by the general formula $Si_{2n}O_{5n+2}^{2(n+2)-}$, and polysilicic acids insoluble in acids in studies of the molecular forms of silicic acids in silicate gels such as magnesium silicate,[11] aluminium silicate[12] and sodium aluminosilicate gels.[1] In the present report, silicates with known

1) This paper forms Part IV of "Polymeric Forms of Silicic Acid in Various Silicates." Part III: H. Yokoi and S. Enomoto, *Yakugaku Zasshi*, **98**, 1651 (1978).
2) Location: *3190 Gofuku, Toyama-shi, Toyama, 930, Japan.*
3) R.R. Heffner, R.C. Page, and A. Frey, *Am. J. Digest. Diseas.*, **8**, 219 (1941).
4) F. Sauer, D.H. Laughland, and W.M. Davidson, *Can. J. Biochem. Physiol.*, **37**, 183 (1959); *idem, ibid.*, **37**, 1173 (1959).
5) W.R. Settle and F. Sauer, *Am. J. Vet. Res.*, **21**, 709 (1960).
6) G. Mohn, *Beitr. Silikase. Forsch.*, **94**, 25 (1968).
7) J.R. Herman and A.S. Goldberg, *J. Am. M. A.*, **174**, 1206 (1960).
8) C. Lagergren, *J. Urology*, **87**, 994 (1962).
9) P.M. Newberne and P.B. Wilson, *Proc. N. A. S.*, **65**, 872 (1970).
10) S. Akiya and A. Tanimura, *Seikagaku*, **26**, 430 (1954).
11) H. Yokoi and S. Enomoto, *Chem. Pharm. Bull.* (Tokyo), **26**, 1846 (1978).
12) H. Yokoi and S. Enomoto, *Yakugaku Zasshi*, **98**, 1460 (1978).

distributions of silicic acids were orally administered to rats and urinary silicic acid excretion was determined in order to estimate the gastrointestinal absorption of silicic acid. The effect of the degree of polymerization of silicic acids on the absorption was studied to investigate the mechanism of their absorption and metabolism in connection with the molecular structure.

Silicate preparations used were amorphous sodium aluminosilicate gels ($Na_2O \cdot Al_2O_3 \cdot xSiO_2 \cdot yH_2O$) composed chiefly of low molecular weight silicic acids (smaller than tetramer; S-1), a preparation composed chiefly of soluble polysilicic acids (average degree of polymerization, about 12; S-2), and a preparation consisting chiefly of insoluble polysilicic acids (S-3).

Experimental[13]

Preparation of Silicates——S-1: Sodium aluminate solution (0.5 M, 100 ml) was added to 800 ml of 0.25 M sodium orthosilicate solution at about 20° with stirring. The mixture was allowed to stand for about 30 min in order to complete the reaction, then neutralized with 159 ml of 10% HCl, and stirred for another 30 min. The precipitate was washed and dried in the usual way, and passed through a sieve (100—200 mesh).

S-2: Sodium aluminate solution (0.5 M, 400 ml) was added to 800 ml of 0.5 M JIS No. 3 sodium silicate solution at 20° with stirring. The mixture was allowed to stand for about 30 min in order to complete the reaction, then neutralized with 159 ml of 10% HCl and stirred for another 30 min. The precipitate formed was treated as described for the preparation of S-1.

S-3: Sodium aluminate solution (0.5 M, 80 ml) was added to 800 ml of 0.5 M JIS No. 3 sodium silicate solution at about 20° with stirring. The mixture was allowed to stand for about 30 min in order to complete the reaction, then neutralized with 112 ml of 10% HCl and stirred for another 30 min. The precipitate was treated as described for the preparation of S-1.

The results of chemical analysis of the three silicate preparations are summarized in Table I.

Table I. Chemical Analyses of Sodium Aluminosilicate Gels

Samples[a]	S-1	S-2	S-3
Component (%)			
SiO_2	45.93	45.50	63.76
Al_2O_3	26.17	23.92	13.42
Na_2O	10.47	10.57	6.39
Ignition loss (%) (800°, 1 hr)	12.69	15.55	12.37
Acid consuming capacity (ml/g)[b]	173.8	162.0	92.3
Slurry pH (4% aqueous suspension)	10.1	10.5	9.7

a) Samples S-1, S-2, and S-3 corresponded to SAS 4-0.25, SAS 2, and SAS 8, respectively, of the previous report.[1]
b) J.P. IX, The Ministry of Health and Welfare, 1976, p. 279.

Trimethylsilylation——The method was described in the previous report.[11,12]

Absorption Experiments——Animals: Male Wistar rats weighing 200—300 g were separated into groups of 4 animals each and kept on distilled water and solid feed (F-1, produced by Funabashi Farm) in metabolic cages. Only distilled water was fed during 24 hr following administration of the silicate preparations.

Administration of the Silicate Preparations: The silicate preparations were suspended in 1 ml of 0.5% carboxymethyl cellulose solution and given at a dose of 20, 50, 100, 250, and 500 mg of SiO_2/rat kg·day by means of a stomach tube. Each rat received silicate several times at intervals of over 2 weeks using the cross-over method; a background level of urinary silicic acid being recovered in 2 days.

Collection and Analysis of Urine Samples: Twenty-four hr urine samples were collected prior to and after silicate administration. The volume and pH were measured and silicic acid content was determined.

Determination of Urinary Silicic Acids: The silicomolybdate method of Akiya *et al.*[14] was applied. An aliquot of urine, 5 ml, was diluted with 20 ml of distilled water and then 2 ml of 5% calcium chloride

13) All chemicals, except JIS No. 3 sodium silicate, were analytical grade commercial materials and were used without further purification.
14) S. Akiya and A. Tanimura, *Seikagaku*, **28**, 635 (1956).

solution and 3 ml of 1 N NH_4OH was added to the urine. After mixing, activated charcoal, about 0.2 g, was added and the mixture was shaken and filtered by suction. The filtrate was adjusted to pH 1.5 with 1 N HCl then diluted with distilled water to a volume of 50 ml. Silicic acids were determined colorimetrically at 400 nm, 10 to 20 min after addition of 2 ml of 10% ammonium molybdate. A calibration curve was prepared in the same manner with rat urine containing known amounts of sodium orthosilicate.

Results

Molecular Forms of Polysilicic Acids and Their Distribution in the Preparations

Contents of individual silicic acids were determined in terms of SiO_2 by the trimethylsilylation method and the percentages were calculated as the proportions to total silicic acids.

Table II. Analyses of the Composition of Silicic Acids in Sodium Aluminosilicate Gels

Samples	SiO_4^{4-}	$Si_2O_7^{6-}$	$Si_3O_{10}^{8-}$	$Si_4O_{12}^{8-}$	Soluble polysilicic acids	Insoluble polysilicic acids
S-1	9.08	15.23	11.21	16.75	47.73	trace
S-2	7.75	5.03	2.19	2.21	82.04	0.78
S-3	0.71	0.27	0.11	0.11	1.26	97.54

Values are given as SiO_2/total recovered SiO_2 (%).

Trimethylsilylated SiO_4^{4-}, $Si_2O_7^{6-}$, $Si_3O_{10}^{8-}$, and $Si_4O_{12}^{8-}$ were determined quantitatively by gas-liquid chromatography. Besides these four compounds, trace amounts of volatile trimethylsilyl derivatives of $Si_4O_{13}^{10-}$, $Si_6O_{17}^{10-}$, and $Si_7O_{19}^{10-}$ were also detected and classified as soluble polysilicic acids in Table II. Major components of the non-volatile fraction of soluble silicic acids were identified as polymers corresponding to $Si_{2n}O_{5n+2}^{2(n+2)-}$ by elemental analysis.[1,11,12] The average degree of polymerization in terms of $2n$ was determined as shown in Table III. Insoluble polysilicic acids form three-dimensional network structures.[11]

Table III. Elemental Analyses of Soluble and Insoluble Trimethylsilylated Polysilicic Acids and Average Degree of Polymerization of Soluble Polysilicic Acids

Samples	Soluble polymer				Inosoluble polymer		
	Elemental analysis			Average D.P. ($2n$)			
	C (%)	H (%)	H/C ratio		C (%)	H (%)	H/C ratio
S-1	30.16	7.69	3.04	7.02	—	—	—
S-2	28.60	7.37	3.07	11.72	—	—	—
S-3	30.47	7.52	2.94	6.44	8.71	5.96	8.15

Urinary Excretion of Silicic Acids——The urinary excretion of silicic acids prior to and after admininstration of the silicate preparations is shown in Fig. 1. Silicic acids amounting to 2.07 ± 0.41 mg/rat kg·day were excreted in the urine prior to the administration.[15] When the S-1 preparation was given to rats at a dose of 250 mg/rat kg, 5.29 mg/rat kg·day of silicic acids was excreted in the urine during 24 hr following the administration. When silicates administered exceeded 250 mg/rat kg, the excretion rate appeared to be suppressed, although the background level was recovered in 48 hr. Namely, silicic acids were mostly excreted within 24 hr after administration, and excretion was slight after the second day. In this report,

15) The background level is considered to be due to ingestion of silicate contained in the feed.

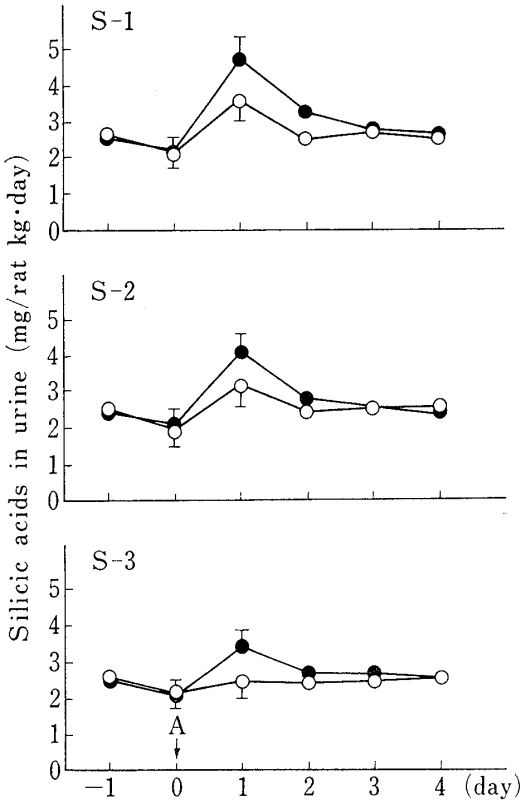

Fig. 1. Excretion of Silicic Acids in Urine

○: SiO_2 50 mg/rat kg·day.
●: SiO_2 250 mg/rat kg·day.
A: administration of silicate.
The points at 0 and 1 days are given as the means ±S.E. of at least eight rats.

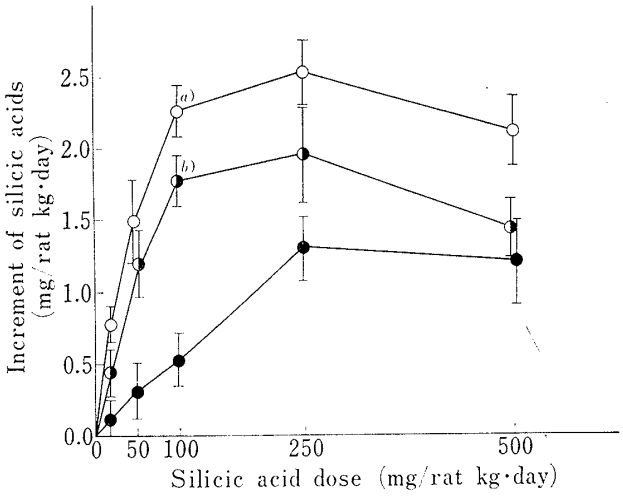

Fig. 2. Effect of Silicic Acid Dose on the Increment of Silicic Acids Excretion

○: S-1, ◑: S-2, ●: S-3.
Points are given as the means ±S.E. of at least eight rats.
a) Significantly different from S-2 ($p < 0.05$).
b) Significantly different from S-3 ($p < 0.01$).

the increment of silicic acid excretion due to silicate administration was calculated by subtracting the background level from the excretion during 24 hr after administration. The relation between the dose of silicic acids and the increment of SiO_2 excretion is shown in Fig. 2. The increment depended markedly on the preparation administered and the dose. In the case of S-1, the increment increased in proportion to the dose over the 20—100 mg/rat kg range. An increment ranging from 2.27 to 2.53 mg/rat kg·day was observed following administration of the silicates in a dose ranging from 100—250 mg/rat kg, but a slight decrease was noted after administration of 500 mg/rat kg. In the case of S-2, the increment pattern was essentially the same as for S-1. However, the average increment of 1.78 mg/rat kg·day after 100 mg/rat kg dose was slightly lower than that after S-1. In the case of S-3, on the other hand, the increment increased gradually in proportion to the dose over the 20—250 mg/rat kg range, but the amount was as low as 1.30 mg/rat kg·day even after the 250 mg/rat kg dose. Although the excretion varied widely with the individual animal or even within the same rat, the differences of increment among the different preparations were distinct.

Absorption of Silicic Acid

Regarding the increment described above as the amount of silicic acid absorbed, percentages of absorption for individual silicic acid molecular forms were estimated using equation (I).

$$\text{Absorption } (\%) = \frac{Sb - Sa}{S_N} \times 100 \qquad \cdots\cdots (I)$$

Sa and *Sb* are silicic acids excreted in the urine during 24 hr prior to and following administration, respectively, and S_N is one of SiO_4^{4-}, SiO_4^{4-}—$Si_3O_{10}^{8-}$, SiO_4^{4-}—soluble polysilicic

acids, and total silicic acids administered. The percentage for total silicic acid was only 0.2—4.0%, while the percentage for silicic acid monomer was very high.

Table IV. Absorption of Silicic Acids in Rats

Total SiO_2 dose (mg/rat kg·day)		Absorption[a] in comparison with dose of (S_N)			
		SiO_4^{4-}	SiO_4^{4-}—$Si_3O_{10}^{8-}$	SiO_4^{4-}—Soluble polymer	Total silicic acids
S-1	20	43.4± 7.1	11.1± 1.8	4.0± 0.7	4.0±0.7
	50	36.2± 6.4	8.3± 1.6	3.0± 0.6	3.0±0.6
	100	25.0± 2.0	6.4± 0.5	2.3± 0.2	2.3±0.2
	250	11.1± 0.9	2.8± 0.2	1.0± 0.1	1.0±0.1
	500	4.7± 0.5	1.2± 0.1	0.4± 0.1	0.4±0.1
S-2	20	29.0±10.3	15.1± 5.4	2.3± 0.8	2.3±0.8
	50	30.9± 5.9	16.0± 3.1	2.4± 0.5	2.4±0.5
	100	23.0± 2.2	11.9± 1.1	1.8± 0.2	1.8±0.2
	250	10.1± 1.7	5.2± 0.9	0.8± 0.1	0.8±0.1
	500	3.7± 0.5	1.9± 0.3	0.3± 0.1	0.3±0.1
S-3	20	85.7±92.9	54.5±59.1	24.9±26.5	0.6±0.7
	50	86.1±50.0	56.4±32.7	25.2±14.6	0.6±0.4
	100	76.1±25.4	49.5±16.5	22.0± 7.3	0.5±0.2
	250	73.0±12.4	47.6± 8.1	21.1± 3.6	0.5±0.1
	500	34.1± 7.9	22.2± 5.1	9.8± 2.3	0.2±0.1

Each value represents the mean±S.E. of at least eight rats.
a) Absorption (%) was calculated using equation (I).

Discussion

The present experiments show that the gastrointestinal absorption of silicic acids in rats depends on the degree of polymerization. Namely, the lower the degree of polymerization, the higher the absorption rate. Silicic acid monomer was absorbed at a very high rate. Although, the absorption appeared to decrease at a large dose of silicic acids, it was proportional to the dose up to 100 mg/rat kg of S-1 or S-2. The maximum urinary concentration of silicic acids observed was 0.102 mg/ml (S-1, 250 mg/rat kg), and this concentration is nearly the saturated concentration of soluble silicic acids in water, as determined by colorimetry. As the silicic acids determined by the silicomolybdate method are restricted to low molecular weight compounds,[16] some urine samples studied both by gravimetry using hydrofluoric acid and by colorimetry with sodium carbonate to determine colloidal silicic acids. However, we failed to detect polysilicic acids due to the presence of phosphate. However, polysilicic acids may be formed in urine containing silicic acids in excess of the saturated concentration.[17]

Various silicic acids are formed in the gastric juice by reaction (II) from silicates contained in antacid preparations.

$$\text{Silicate} + HCl \longrightarrow H_4SiO_4 + H_6Si_2O_7 + H_8Si_3O_{10} + H_8Si_4O_{12} + \cdots\cdots\cdots\cdots \quad (II)$$

The molecular forms and the distribution are dependent on the kind of silicate. The silicate composition varies widely depending on the preparation procedure; *e.g.*, sodium silicate used, concentration, and pH and composition of the reaction mixture.[1,11,12] The urinary silicic acid excretion reaches a maximum at a dose of around 250 mg/rat kg. It is thought that the amount of silicic acid absorbed depends on the quantity of antacid decomposed in the

16) G.B. Alexander, *J. Am. Chem. Soc.*, **75**, 5655 (1953); T.L. O'Connor, *J. Phys. Chem.*, **65**, 1 (1961).
17) C.B. Bailey, *Can. J. Biochem.*, **50**, 305 (1972).

stomach.[18] In the present report, the proposed mechanism of absorption of silicic acid is based on the increment of silicic acid excreted in the urine at doses in the range of 20 to 100 mg/rat kg. The absorption capacity in this range is proportional to the concentration of low molecular weight (M.W.) silicic acids. The silicic acids are hydrophilic, and thus are probably absorbed from the digestive tract through the lipoid membrane pore route. The absorption of silicic acid by this mechanism may be expected to decrease with increasing degree of polymerization of the silicic acids. The order of decreasing absorption rate is estimated to be as follows:

$$H_4SiO_4 > H_6Si_2O_7 > H_8Si_3O_{10} > \cdots\cdots$$

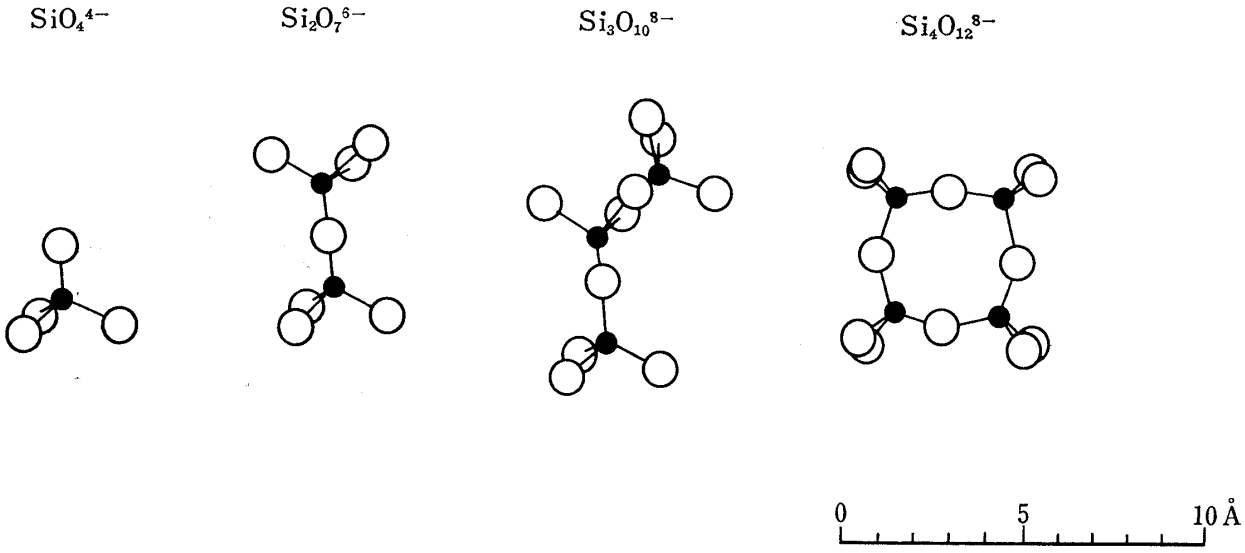

Fig. 3. Various Silicic Acid Ions
●: Si, ○: O.

Percentages of absorption were calculated separately on the basis of both total silicic acids and silicic acid monomer contained in a preparation and are shown in relation to dose in Fig. 4 and 5, respectively. The absorption rate of total silicic acids varied with the preparation,

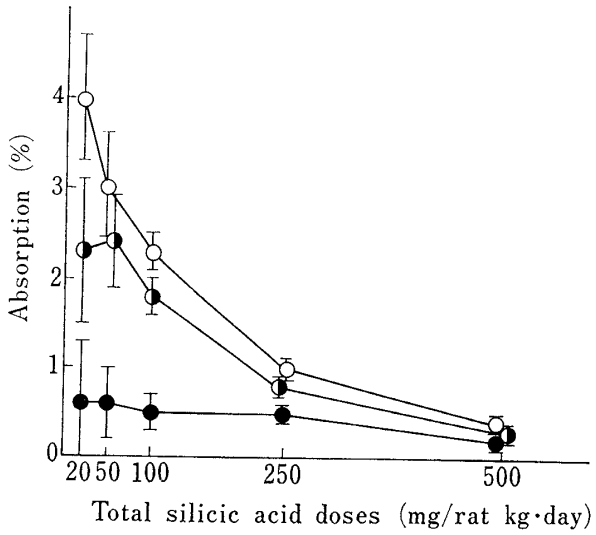

Fig. 4. Relation between Total Silicic Acid Doses and Absorption
○: S-1. ◑: S-2. ●: S-3.

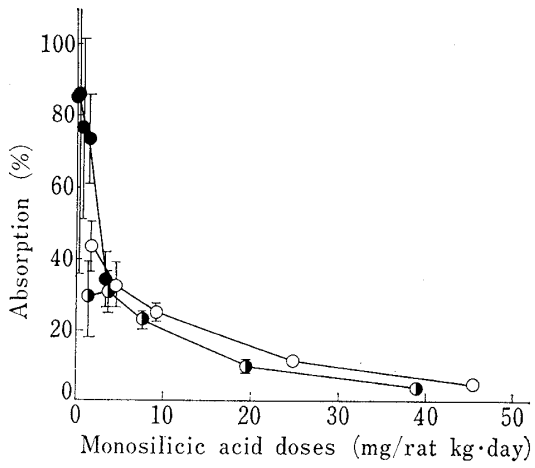

Fig. 5. Relation between Monosilicic Acid Doses and Absorption
○: S-1. ◑: S-2. ●: S-3.

18) M.I. Grossman, *Am. J. Digest. Diseases*, **1**, 453 (1956).

as shown in Fig. 4, suggesting that low molecular weight silicic acids may be absorbed exclusively. The approximate coincidence of the three lines representing the relation between percentage absorption and dose of the monomer given also suggests absorption of the monomer. The effective radius of a lipoid membrance pore is about 4 Å,[19] which would allow a silicic acid monomer to pass. A hydrated tetramer is considered to be unable to pass through the pore, since its molecular diameter is too large. Hogben noted that water-soluble substances with molecular weights of more than 100 are little absorbed.[20] Although the amount of silicic acids absorbed suggests possible absorption of the dimer ($H_6Si_2O_7$; M.W.=174.2) as well as the monomer (H_4SiO_4; M.W.=96.1) in the present experiments, it is considered that the monomer is chiefly absorbed, because the solubility of silicic acid decreases exponentially as the degree of polymerization increases. The absorption rate decreased sharply as the amount of silicate given increased. The decrease is attributed to polymerization of low molecular weight silicic acids in solution over the neutral to acid pH range and the formation of chemically active silicic acid at a high concentration in the gastric juice. Ishikawa[21] has reported urinary silicic acid concentrations both high and within normal range in different patients receiving silicate antacids. Based on the results obtained from the present experiments, the difference in the excretion can be attributed to a difference in the contents of low molecular weight silicic acids in the preparations administered.

With respect to mechanism of formation of renal and urinary calculi, silicic acids absorbed from the digestive tract, largely by physical or diffusion processes, are concentrated in the urine to exceed the saturated concentration, and polymerize. The polymer formed is converted into insoluble precipitates *via* colloidal silicic acids. In the formation of urinary stones, various substances, such as urine proteins and salts, act as promoters.[22] For example, Bailey noted that polysilicic acids were formed when the silicate concentration increased in the urine of a cow, or when the silicic acid solubility decreased due to the presence of urinary proteins.[17] Thus, a decrease in silicic acid solubility in the bladder may cause the formation of silicic acid calculi.

Glomerular polymerization of low molecular weight silicic acid may lead to physiological problems or may damage the renal tissue. Thus, care should be taken in administering silicate antacid preparations containing large amounts of low molecular weight silicic acids to patients with renal insufficiency. Further clinical investigation is required. On the other hand, silicate antacids composed of highly polymerized silicate are desirable in order to avoid calculi.

In the present study, it has been demonstrated that the degree of polymerization of silicic acids in antacid affects the absorption of silicic acid from the digestive tract. The mechanism of formation of siliceous calculi is discussed. These results may also be useful in connection with elucidation of the mechanism of gastrointestinal absorption of polymer particles of different molecular sizes.

Acknowledgement The authors are grateful to Prof. H. Takahashi of the University of Tokyo for his kind encouragement, and to Prof. Y. Koizumi of Toyama Medical and Pharmaceutical University for valuable suggestions. The authors are also indebted to Misses C. Fukuda and Y. Yamada for technical assistance.

19) B. Lindemann and A.K. Solomon, *J. Gen. Physiol.*, **45**, 801 (1962).
20) C.A.M. Hogben, *Ann. Rev. Physiol.*, **22**, 381 (1960).
21) J. Ishikawa, *Seikagaku*, **30**, 413 (1958).
22) H. Fleisch, *Kidney Int.*, **13**, 361 (1978).