# THE METABOLISM OF SILICON IN THE RAT AND ITS RELATION TO THE FORMATION OF ARTIFICIAL SILICEOUS CALCULI*

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Interest in silicon metabolism stems from the rather universal incorporation of silicon into cells, its intracellular transformations, its possible functions, and its role in siliceous urinary calculi in the bovine and ovine species.

The metabolism of silicon in biological systems has been an object of intermittent investigation for some time. The possibility that silicon might even be a required element has been suggested by various workers. However, very little good evidence exists for an absolute silicon requirement in any but a few unique organisms. Lewin (1) has shown an obligate silicon requirement for the fresh water diatom, *Navicula pelliculosa*. Evidence in other cases is less conclusive. It was thought for a time that silicon might be required in certain enzyme systems. Crane and Glenn (2) showed that a silicomolybdate complex was very active in the reactivation of aldehyde oxidase from which the molybdenum had been removed by dialysis. However, the complex apparently was not present in the system *in vivo* (3). Other reports (4) implicated silicate as an agent increasing the activity of molybdate dependent enzymes. Keeler *et al.* (5) have, however, demonstrated no correlation between the metabolism of silicon$^{31}$ and molybdenum$^{99}$ in *Azotobacter vinelandii*, an organism with an obligate molybdate requirement for a number of its enzyme systems. Holzapfel (6, 7), in a series of papers on silicon metabolism, has contended that there must be some metabolic function of silicon due to its universal presence in biological materials. However, here again the evidence is not direct. Thus the question of a general biological role for silicon is still open.

The chemistry of silicon is very interesting. A solution of inorganic silicate usually undergoes polymerization rather quickly. Furthermore, inorganic silicate will complex with a number of compounds under the most mild conditions. Complexes of silicon with protein (8), as well as with fatty acids, cholesterol and its homologues, and galactose (6), have been reported.

With further reference to the metabolism and biological function of silicon, the following considerations are of interest. Silicate in polymeric form might be expected to behave differently from the inorganic monomer in its transport across biological membranes and hence in its effect on both the level and rate of incorporation of silicon.

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Silicon is found in various forms in biological systems. In addition to the inorganic form (monomeric or polymeric) various organic forms have been reported particularly by Holzapfel (7) and Holt (8). The latter has shown that this formation of organic silicon compounds is probably non-enzymatic. Other reports support enzyme involvement in silicon metabolism. Fontana (10) has reported that the siliceous spicules present on the grass *Panicum maximum* are apparently enzymatically formed, and Lewin (11) has shown that the uptake of silicon, and perhaps its incorporation into the frustule in the diatom cell are linked with aerobic respiration, implicating a coupling enzyme system.

In the western United States a serious pathological condition involving silicon metabolism exists in the bovine and ovine species. The condition is urinary calculosis resulting from the formation of a silicon-mucoprotein urinary stone which blocks urine flow when lodged in the urethral passage. Thus, aside from any functional considerations of silicon, one phase of its metabolism, urinary excretion, may be quite important in the etiology of this disease.

In an effort to develop a method of producing siliceous calculi in laboratory animals, we have been led to an examination of the phase of silicon metabolism involving its urinary excretion as a function of the form and level of its administration. This paper reports the results of such excretion and tolerance studies in rats, along with results obtained in producing siliceous calculi artificially on zinc implants placed in the urinary bladders of rats. In addition, a rapid analytical method suitable for comparative studies of silicon excretion in rat urines is reported.

## *Methods*

*A. Silicon Analysis.—*

1. Buffer solution: 98.4 gm. of anhydrous sodium acetate plus 1200 ml. of water plus 230 ml. of glacial acetic acid.

2. Molybdate solution: 1 gm. of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ added to 100 ml. of the buffer solution. This reagent must be prepared immediately prior to use.

3. Fusion solution: 10 gm. of anhydrous sodium carbonate and 10 gm. of sodium nitrate dissolved in distilled water and diluted to 100 ml.

4. Reducing solution: Seven gm. of anhydrous sodium sulfite is dissolved in 100 ml. of water and 1.5 gm. of 1,2,4-aminonaphtholsulfonic acid is added. 90 gm. of sodium bisulfite is now dissolved separately in 800 ml. of water. The two solutions are combined and brought to 1 liter with distilled water.

5. Method: 1 ml. of the unknown solution or suspension containing less than 100 $\mu$g. of silicon is pipetted into a 20 ml. platinum crucible. 1 ml. of the fusion solution is added and the mixture is dried under a heat lamp. The dried material is carefully fused. The cooled crucible is placed in a glass funnel supported in a 30 ml. spectrophotometer tube. 20 ml. of the molybdate solution is added to the crucible to dissolve the silicon and to form the silicomolybdate complex. After 15 minutes the fusion mixture will have dissolved and the silicomolybdate will have formed. The crucible is now tipped into the funnel and the contents allowed to drain into the spectrophotometer tube. The crucible and funnel are washed with 5 ml. distilled water from the jet of a fine hypodermic needle and the washings also allowed to drain into the tube. Now with the funnel and crucible removed, 1 ml. of reducing solution

is pipetted into the tube and immediately mixed with an up and down motion with the flattened end of a stirring rod. This operation, for all tubes used simultaneously, should require less than 1 minute total time. Exactly 120 seconds from the time the last tube was mixed, the tubes are immersed in a cold water bath (below 14°C.) and the absorption read at 700 mμ. This should be accomplished for all tubes within 3 minutes after being placed in the water bath. The tubes are read against a reagent blank. Optical density is linear with silicon concentration up to 100 μg. of silicon per tube. A standard containing 50 μg. of silicon is also run for use in calculation. Experiments concerned with the suitability of this method are found in the results section.

*B. Rat Housing and Feeding.*—

Rats were housed according to treatment and sex in groups of 2 or 3 rats in metabolism cages. Provision was made for collecting feces-free urine. Further, each cage was fitted with a 15 cm. long side tunnel at the end of which the water and food were kept to insure that the urine would not be contaminated with feed or drinking water. Rats were fed a ground laboratory rat chow with additions as noted in the text. The silicon additions were administered *via* stomach tube or by mixing in the feed as noted in the individual experiments. Water was given *ad lib* except when noted otherwise.

*C. Implanting of Zinc Pellets.*—

In experiments when zinc was implanted in the rat bladders (12, 13), the following procedure was used. Each rat was anesthetized with ether, restrained in dorsal recumbency, the ventral abdominal wall clipped, and the skin cleansed with 70 per cent alcohol. The suture, instruments, and drapes to be used were kept immersed in a solution of quaternary ammonium disinfectant.

A suprapubic incision 2 cm. long was made to expose the urinary bladder. The bladder was incised near the fundus and the zinc pellet placed within. The bladder incision was closed with one or two stitches of 6-0 surgical silk. No attempt was made to effect a tight closure. The peritoneum and muscles were sutured as one layer, and the skin incision was closed as a separate layer, 6-0 silk being used in both instances. The operation required 15 to 20 minutes per rat. Aftercare consisted of withholding water overnight. Mortality following surgery was approximately 10 per cent.

The zinc pellets used were weighed and placed in 70 per cent ethanol prior to implantation. In the first experiment the pellets were cut from pieces of mossy zinc. The average weight of the irregularly shaped pellets was 0.05 gm. For the second experiment, pellets were cut from sheet zinc having a thickness of 2 mm., and they averaged 0.12 gm. in weight.

All rats including controls with no zinc implantation were sacrificed, and the bladder contents removed 5 weeks after implantation in the first experiment and 9 weeks following implantation in the second.

## RESULTS

### *A. Silicon Determination.*—

Initial determinations of urinary silicon excretion in the rat using a standard analytical procedure involving formation of silicomolybdic acid (14), elimination of phosphate by tartaric acid (15), and reduction with aminonaptholsulfonic acid (16) were unsatisfactory. High values and a variable degree of turbidity indicated possible interference by phosphate and iron. There exists a rather extensive literature on silicon methodology much of which reports the elimination of the difficulties caused by phosphate, iron, and other contaminants. This literature was reviewed by King *et al.* (17) who proposed a method of their own de-

signed to eliminate these difficulties. This method entails removal of the phosphate and in addition takes advantage of the lack of reduction of phosphomolybdate in an acid medium, first reported by Dienert and Wandenbulcke (18).

As a routine, however, we use a method of our own design (Methods section) and this has proven satisfactory for comparative studies on rat urines. This method also utilizes the lack of reduction of phosphomolybdate in acid conditions, but requires fewer steps than the method of King *et al.* (17). Using the method of our design, absorption is linear with silicon concentration below 100 μg. of silicon. Phosphorous contamination is not a serious problem in the range ordinarily found in urine samples in studies in which one is interested in the relative silicon levels between rats as a function of dietary silicon additions. However, since the ratio of P to Si in normal rat urine may be as high as 30 to 1 (17), the method is not satisfactory for determining absolute silicon concentration in rat urine unless a correction for phosphorous is made. It was found

TABLE I

*The Development of the Reduced Silico- and Phosphomolybdate Color as a Function of Time after Addition of the Reducing Solution (Reaction temperature 14°C.)*

| Treatment | Relative optical density | | |
|---|---|---|---|
| | 2 min. | 30 min. | 60 min. |
| 100 μg. Si | 0.540 | 0.730 | 0.750 |
| 500 " P | 0.018 | 0.385 | 0.725 |

that an equivalent amount of phosphorous gave a reading of only $\frac{1}{150}$ that of silicon by this method.

Very careful attention must be paid to proper timing. If the optical density is not read within 3 minutes after the tubes are placed in the water bath, differences greater than an acceptable 1 to 2 per cent will be found due to a progressive deepening of the color. Placing the tubes in an ice bath, of course, slows this change, but for routine determinations in which an error of 1 to 2 per cent can be permitted a 14°C. bath is acceptable. This problem cannot be eliminated by allowing the blue color development to proceed to completion. This is because reduction of the phosphomolybdate also occurs with increased time and becomes proportionally greater as the silicomolybdate reduction nears completion (Table I). It has been found desirable to run only about 8 samples at a time to insure exact observance of the time intervals. Further, one of these samples should be a standard containing 50 μg. of silicon from which calculations can be made for the concentrations of the unknowns. This method is used rather than comparison with a standard curve since there will be slight differences in the value of the standard in different runs due to variation in tempera-

ture of the reagents on mixing. This procedure minimizes the effects of variation from one run to the next.

*B. Silicon Metabolism.—*

It was desirable initially to make a comparison of the urinary silicon excretion in rats as a function of the forms of silicon administered. It seemed likely that rapid polymerization of some inorganic forms with possible retarding of absorption would occur at the acid pH of the stomach.

Groups of 3 rats were given either sodium metasilicate, magnesium trisilicate, water glass, or tetraethylorthosilicate (ethyl silicate). The silicon was administered *via* stomach tube at a level of 1 ml./rat of a 10 per cent solution or suspension of the silicon compound in cottonseed oil. The silicon content of the various forms is nearly equal; hence the quantity of silicon administered was approximately the same in each case.

TABLE II

*Urinary Silicon Excretion as a Function of the Form Administered*

| Form of silicon fed | Total Si supplemented per 3 rat group | Extra* Si excreted in 72 hrs. per 3 rat group | Percentage of administered Si excreted in the urine |
|---|---|---|---|
| | *mg.* | *mg.* | *per cent* |
| Water glass | 43.1 | 3.3 | 8.0 |
| Sodium metasilicate | 33.8 | 1.4 | 3.0 |
| Magnesium trisilicate | 35.1 | 2.6 | 7.0 |
| Tetraethylorthosilicate | 43.1 | 7.1 | 17.0 |

* These values were corrected to exclude the average daily silicon excreted by control rats, and represent the average values of 2 runs for 72-hour Si excretion per 3 rat group.

Table II shows the average results of two runs in which the total silicon administered per 3-rat group is compared with the amount excreted in the urine in 72 hours. Experiments showed that urinary silicon excretion was back to the base level 72 hours after discontinuing silicon administration. It is evident that the percentage of administered silicon which is excreted in the urine is much higher with ethyl silicate than with any of the other forms.

Fig. 1 illustrates the results obtained in an experiment designed to determine the tolerance of rats to ethyl silicate administered in single doses, and to measure urinary silicon excretion as a function of the level of ethyl silicate administered. In addition to the three levels shown in Fig. 1, other groups were also given 2 and 4 times the highest level shown. None of these rats survived. Thus it was concluded that a single dose of 40 to 60 mg. of silicon per rat was the maximum tolerance when given by stomach tube. The results in Fig. 1 indicate that an increase in silicon administered resulted in an approximately proportional increase in urinary silicon.

The results of another experiment to examine urinary silicon excretion as a function of the amount administered are shown in Fig. 2. In this experiment groups of 2 rats were given ethyl silicate in cottonseed oil *via* stomach tube at the levels shown. In this instance, however, rats were given silicon each day on a continuous basis. It was felt that since all the silicon given is not excreted

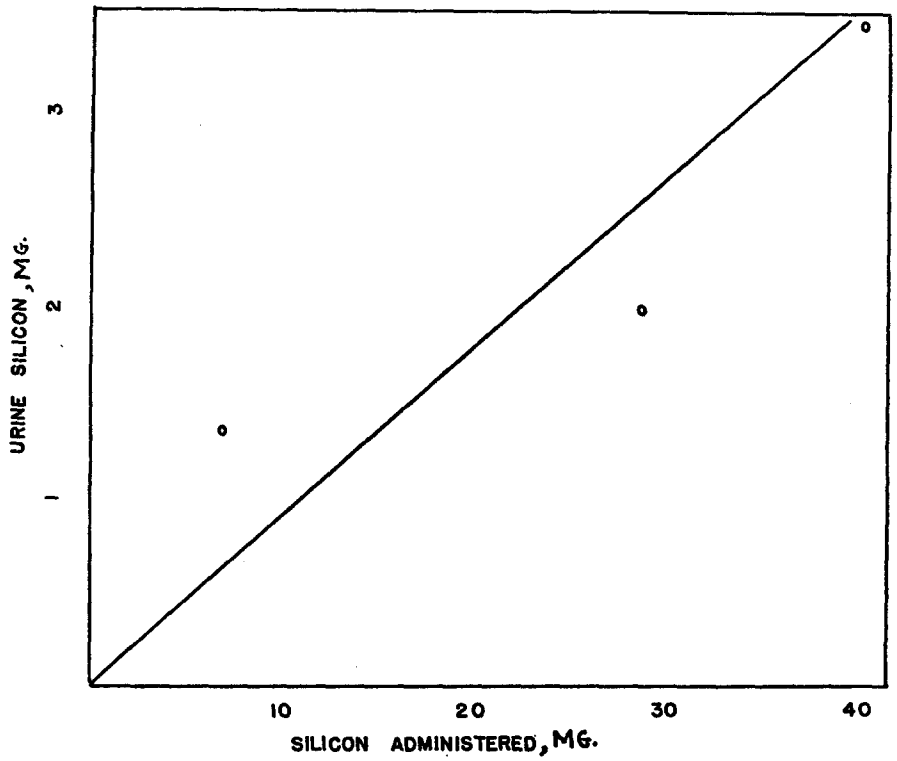


FIG. 1. Urine silicon excretion per rat in a 48 hour collection as a function of the level administered in a single dose *via* stomach tube. The data were corrected to exclude normal background silicon excretion, and were from averages of 2 groups of 3 rats expressed on a single rat basis.

within 24 hours after administration, the cumulative effect might make it necessary to revise the toleration level downward. One additional group not shown in Fig. 2 was given a level of silicon five times that of the highest shown. These rats, however, died by the 3rd day of silicon administration and hence do not appear in Fig. 2. The rats receiving a level of 15 mg. of silicon per day per rat (highest level shown in Fig. 2) survived and appeared healthy in all respects. The group in which both rats died received 72 mg. Fig. 2 clearly indicates that urinary silicon excretion beyond normal daily background levels is a straight

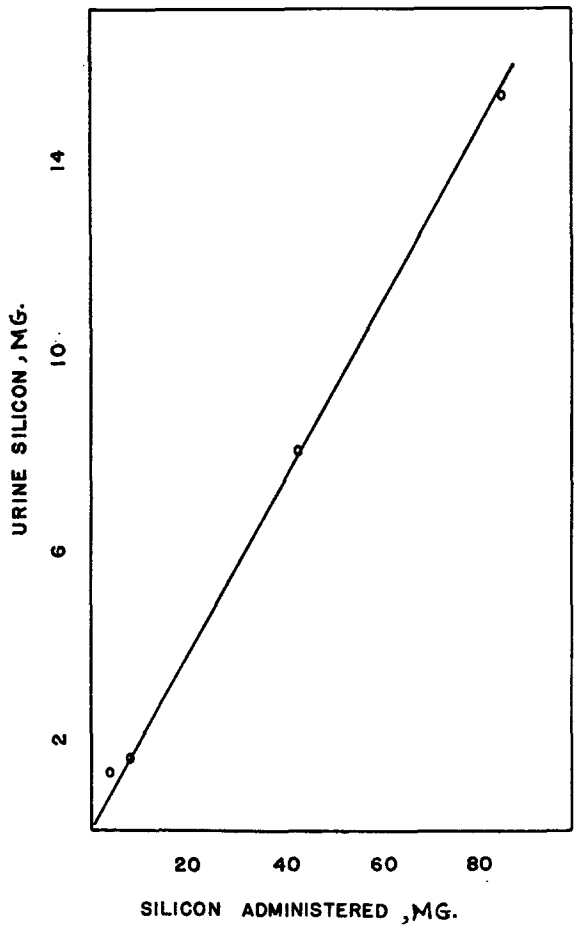


FIG. 2. Urine silicon excretion from 2 rats during a 72 hour period as a function of the total amount of silicon administered during the 72 hour period. The silicon was administered in daily doses *via* stomach tube. The data were corrected to exclude normal background silicon excretion.

line function of the silicon administered. Approximately 18 per cent of the administered silicon appeared in the urine under a sustained silicon load at the levels tested.

The foregoing data indicate that one could best enhance urinary silicon excretion with ethyl silicate. Further, one could perhaps administer individual daily doses of 30 mg. per rat *via* stomach tube on a sustained basis without adverse toxic effects. These data, of course, do not necessarily indicate that the silicon was the toxic factor. The ethyl groups of the compound may have been the factor necessitating tolerance limits.

*C. Artificial Siliceous Calculi.*—

An attempt was next made to produce siliceous urinary calculi artificially in the bladders of rats on implanted zinc pellets.

Both female and male rats were used separately in groups of 3 rats per treatment. The following treatments were used: A. two groups, one of each sex, on 29 mg. of silicon as ethyl silicate per rat per day and with no bladder implants; B. two groups, one of each sex, with no silicon additions and no bladder implants; C. two groups, one of each sex with no silicon additions but with bladder implants; D. two groups, one of each sex, on 29 mg. of silicon as ethyl silicate per rat per day and with bladder implants; and E. two groups, one of each sex, on 24 mg. of silicon as magnesium trisilicate and with bladder implants.

TABLE III

*Weight and Silicon Content of Calculus Deposits on Zinc Implants (1st Experiment)*

| Stone No. | Treatment | Weight of deposit | Weight of daughter stones when present | Silicon content |
|---|---|---|---|---|
| | | *gm.* | *gm.* | *per cent* |
| 1 | No Si (male) | 0.1046 | 0.0233 | 1.4 |
| 4 | " " " | 0.0000 | | |
| 5 | " " " | 0.0157 | | 0.8 |
| 8 | " " " | 0.0938 | 0.0223 | 0.7 |
| 16 | No Si (female) | 0.0592 | 0.0187 | 0.9 |
| 17 | " " " | 0.0592 | | 3.0 |
| 18 | " " " | 0.0057 | | |
| 19 | " " " | 0.0016 | | |
| 2 | Mg silicate (male) | 0.0090 | | |
| 9 | " " " | 0.0106 | | |
| 10 | " " " | 0.0007 | | |
| 12 | Mg silicate (female) | 0.0795 | | 1.8 |
| 14 | " " " | 0.0028 | | |
| 15 | " " " | 0.0112 | | 1.2 |
| 3 | Ethyl silicate (male) | 0.0148 | | |
| 6 | " " " | 0.0112 | | 43.0 |
| 7 | " " " | 0.0164 | | 12.7 |
| 11 | Ethyl silicate (female) | 0.0550 | 0.0095 | 3.1 |
| 13 | " " " | 0.0104 | | 16.1 |
| 20 | " " " | 0.0685 | 0.0043 | 4.7 |

It is evident from Table III that as much or more total stone material formed on the implants in rats receiving no silicon as in rats with silicon supplementation. The rats which had no implants formed no stones whether receiving silicon

or not. Table III further reports the silicon analysis of the stone material formed on the zinc implants. It is evident from the data that in all instances in which the rats were receiving ethyl silicate the stones contained more silicon than the control stones or the stones in rats on magnesium trisilicate, although the silicon level in the stones of the ethyl silicate groups was not consistently high. Deter-

TABLE IV

*Weight and Silicon Content of Calculus Deposits on Zinc Implants (2nd Experiment)*

| Stone No. | Treatment* | Weight of deposit | Silicon content |
|---|---|---|---|
| | | *gm.* | *per cent* |
| 2 | High water/high silicon | 0.0037 | — |
| 5 | " " " " | 0.0025 | — |
| 19 | " " " " | 0.0035 | — |
| 13 | " " " " | 0.0125 | 22.4 |
| 20 | " " " " | 0.0286 | 13.1 |
| 22 | " " " " | 0.0080 | 30.8 |
| 4 | Low water/high silicon | 0.0102 | 14.7 |
| 6 | " " " " | 0.0151 | 19.9 |
| 21 | " " " " | 0.0187 | 19.3 |
| 9 | " " " " | 0.0170 | 24.2 |
| 11 | " " " " | 0.0110 | 21.5 |
| 18 | " " " " | 0.0296 | 16.0 |
| 8 | Low water/no silicon added | 0.0002 | — |
| 16 | " " " " " | 0.0002 | — |
| 23 | " " " " " | 0.0229 | 3.3 |
| 1 | " " " " " | 0.0270 | 1.9 |
| 3 | " " " " " | 0.0087 | 4.6 |
| 14 | " " " " " | 0.0015 | — |

* Explanation of treatments: High water indicates consumption of three times normal level, low water indicates consumption of ½ normal level, and high silicon indicates the addition of 86 mg. of silicon to the feed per rat per day (further explanation in text). Female rats were used exclusively.

mination of the level of silicon being excreted in the urine showed that the control groups, B and C, excreted silicon at a level of about 75 μg. silicon/ml., the groups receiving magnesium trisilicate at a level of about 120 μg. Si/ml., and the groups on ethyl silicate at a level of about 300 μg. Si/ml.

The lack of high silicon percentages in all stones formed in rats receiving ethyl silicate prompted a second experiment. Rats were given higher levels of ethyl silicate (86 mg. silicon per rat per day, or three times that of the previous experiment), and, in addition, their water intake was varied. Preliminary experiments indicated that the rats would tolerate this level of ethyl silicate provided it was mixed with the feed rather than fed *via* stomach tube. Some groups

received less than ½ their normal daily water consumption and some groups were stimulated to drink about 3 times normal level by the use of 5 per cent glucose in the drinking water (13). Table IV compares the weight and silicon content of the calculus deposits on the zinc implants in this experiment. It is evident that the weight of the deposit was consistently greater in rats on low water and high silicon than in either rats on enhanced water consumption and high silicon, or in rats on low water and no silicon. Much less deposit formed on implants in rats receiving no silicon in this experiment than was evident in the experiment reported in Table III.

Reference to Table IV also indicates that the content of silicon in the stones was consistently high in rats receiving silicon additions whether on low or high water ration. The rats receiving no silicon additions formed stones low in silicon in all instances.

TABLE V

*Average Urine* Volumes and Silicon Excretion in the 2nd Implant Experiment‡*

| Treatment | Total urine volume | Urine silicon concentration | Total Si excreted |
|---|---|---|---|
| | *ml. per rat/day* | *μg Si/ml.* | *μg Si/rat/day* |
| High water/high silicon | 45 | 125 | 5450 |
| Low water/high silicon | 12 | 390 | 4700 |
| Low water/no silicon added | 6 | 151 | 860 |

* Urine collection made during the 5th week.

‡ The values are averages of 2 groups of 3 rats for 3 day periods expressed in terms of single day values for one rat.

The results in Table V show that all groups in the 2nd experiment were excreting silicon in the urine at levels above 125 μg. of silicon/ml.

On necropsy of rats in this experiment it was noted that the kidneys from rats on high silicon/low water and to a lesser degree those on high silicon/high water were enlarged, misshapen, mottled, and pale. The capsule was easily stripped. Some of the rats showed a trace of blood on the hair around the nostrils. No other pathological changes were noted at necropsy, but silicon-fed rats were thinner and smaller than the controls. Control rats on no silicon and low water averaged 186 gm., rats on high silicon and enhanced water consumption averaged 148 gm., while those on high silicon and low water were very small, averaging only 105 gm.

One final urine collection was made in the 2nd experiment just prior to sacrifice (9th week). The proportion of the silicon eaten which was subsequently excreted in the urine was found to have decreased by ½ in the rats on low water and high silicon intake, which may have been a reflection of the kidney damage shown by this group at necropsy.

## DISCUSSION

The results reported here clearly demonstrate that it is possible to produce artificial siliceous urinary stones in rats by using bladder implants and feeding a diet which will result in high urinary silicon excretion. It seems likely that this will serve as a useful method in further studies on the mechanism of formation of siliceous stones. Using this method in both the rat and in the bovine species, studies are now being planned which may lead to information on the factors affecting the initiation and rate of stone formation.

Rats have been used previously as test animals in studies of urinary stone formation on bladder implants (12, 13). These authors demonstrated the usefulness of the implant technique.

One might question the direct relationship of information gained by artificial stone formation in rats with the natural occurrence of stones in other species. Until more is known about both, one could hardly resolve the problem. There are probably many factors in common in the etiology of urinary calculi from one species to another. Furthermore it would seem likely that extreme differences do not occur in the formation of artificially induced calculi on bladder implants as compared with naturally occurring calculi, aside, perhaps, from the means by which calculi formation is initiated. Although the stones formed in the bovine may be predominantly silica as contrasted with the phosphate, urate, or oxalate in the human, interesting similarities exist in the organic fraction of the stones. The amino acid and carbohydrate composition reported for human calcigerous urinary calculi (19) is similar in some respects to the composition of bovine siliceous calculi reported by the author (20). Although the composition of artificial siliceous rat calculi has not been thoroughly investigated, it may be similar to the human and bovine stones. If this proves to be true, one might expect some similarities in the mechanism of formation, especially if the organic fraction plays the important role in stone formation that has been suggested by Boyce and coworkers (19, 21). This would further support the use of implantation methods as techniques for the investigation of urinary stone formation in general.

An assumption that some forms of ingested silicon would polymerize and thereby reduce intestinal absorption seems justified on the basis of the results in Table II since ethyl silicate, the form showing the greatest urinary excretion (a function of absorption), is the form likely to polymerize least rapidly. This form must be hydrolyzed before polymerization can begin. It would, of course, be of interest to pursue this question further by a study of the rate of and the conditions effecting the hydrolysis of ethyl silicate, and by an *in vitro* study of the transport of silicon across the intestinal membrane as a function of the degree of polymerization of the silicate.

The lack of consistently high silicon analysis for the stones formed in the ethyl silicate group in Table III is very interesting in view of the level of

urinary silicon excretion. The saturation level of silica ($SiO_2 \cdot nH_2O$) in water at 37°C. (rat body temperature) is about 75 to 80 μg. of Si per ml. The control groups, B and C, excreted silicon in the urine at a base level of about saturation, while those receiving ethyl silicate excreted it at a 4-fold increase over saturation. The groups receiving magnesium trisilicate showed only about a 1½ fold increase over saturation. If one can assume that saturation values in distilled water can be carried over to conditions in the urine, then concentrations were certainly very favorable for the polymerization of silicon in the urine, at least in the groups on ethyl silicate, and yet the stones were not consistently high in silicon.

The rather marked decrease in the amount of deposited material on the implants in rats receiving no silicon in the experiment of Table IV as compared with that in Table III may be a function of the nature of the zinc surface. In the Table III experiment very rough textured pieces of mossy zinc were used while in the experiment of Table IV the zinc implants were cut from smooth sheet zinc. It is possible that the rough surface of the mossy zinc may have aided in the initial adhesion of stone material and hence caused more rapid formation. This assumes that the initial step of binding stone material to the zinc is the limiting step in artificial stone production.

Reference to Table V indicates that the low water, high silicon group showed a 5- to 6-fold increase in urinary silicon excretion over saturation. The rats receiving supplemental water and silicon additions excreted silicon at a rather low level per ml. of urine, but did show a very high total amount of silicon excretion per day. Since this group consistently produced silicon stones, these results support a contention that factors in addition to a simple high concentration of urinary silicon are important in stone formation.

It is interesting that in both implantation experiments in which the silicon was mixed in the feed, silicon excretion was much less than 18 per cent of that administered, the level found in sustained silicon feeding by stomach tube. This may be a function of the length of time the ethyl silicate is in contact with the feed, resulting in its hydrolysis and polymerization prior to being eaten. The rats were able to tolerate much higher levels when the silicon was in the feed as compared with stomach tube feeding.

It would be of interest to determine the silicon balance and the nature of silicon complexes formed under such high increases in silicon consumption. It has been suggested (9) that there is no large accumulation of silicon in any organ of mice except the kidney, and excretion of any accumulation in this organ occurs rapidly. However, no information is available on accumulation under such high silicon absorption as occurs when feeding ethyl silicate.

## SUMMARY

The urinary excretion of silicon in the rat was found to be enhanced beyond normal levels by the administration of various chemical forms of silicon. The

excretion was enhanced to a much greater degree by the administration of ethyl silicate than by magnesium trisilicate, sodium metasilicate, or water glass. The tolerance level of rats to sustained daily doses of ethyl silicate fed *via* stomach tube was approximately 15 to 30 mg. of silicon per rat per day. Urinary silicon excretion was found to be a straight line function of the concentration of ethyl silicate administered, *via* stomach tube, with approximately 18 per cent of the administered silicon appearing in the urine at all levels tested. Using sustained dietary additions of ethyl silicate as a means of enhancing urine silicon levels, artificial siliceous urinary calculi were consistently produced on zinc pellets implanted in the bladders of rats.

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