SOME EFFECTS OF SILICA ON ASSIMILATION EFFICIENCY AND DIET DISCRIMINATION IN PRAIRIE VOLES (MICROTUS OCHROGASTER)

by

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INTRODUCTION

A considerable amount of literature exists on the role and occurence of silicon dioxide (silica) in plants (Van Soest 1970). The silica bodies or phytoliths from grasses occur in plants, soils paleosols, and sediments dating back at least to the Pleistocene (Twiss et al. 1969).

Soil phytoliths are distinct enough in morphology to distinguish their origin to the level of tribe in many grasses (Metcalfe 1960, Gould 1968). Explanation of their ecological importance could be useful in examining the evolution of the grasses.

The presence of silica in plants has been known since 1804, yet only recently has its function been investigated (Jones and Handreck 1967). Certain plants, in particular the grasses, diatoms, sedges, arthrophytes (Equisetum) and palms are known as "cumulator" species (de Pomar 1975). It appears that they either absorb the SiO_2 preferentially or do not prevent its uptake, unlike other plants.

The grasses have been the group most studied because of their abundance and their economical importance. This study will deal solely with the grass family, but conclusions may be applicable to other groups.

An important distinction is made between the solid and soluble silica in the grasses. Opaline silica (solid) is a hydrated mineral deposited in the epidermis of the plant in "silica cells" which appear to be specialized deposition sites. Monosilicic acid is the soluble form of silica which is brought into and transported within the plant. This acid, $Si(OH)_4$, polymerizes to opaline silica in the silica cells and occasionally in other epidermal cells (including prickle hairs), and intercellular spaces (Blackman and Parry 1968). The silica bodies can be observed in the lumen of the cells or as incrustations of the cell walls. Organic linkages may also occur with structural carbohydrates in the cell wall (Van Soest et al. 1970).

The concentration of silica in various plant parts and total plant silica vary considerably for different species. In oats; 28% of the total plant silica was in the blade, 17% in the sheath, 10.9% in the culm and 40.8% in the inflorescence (Handreck and Jones 1967). In rice, Okamoto found the leaves to contain more silica than the inflorescence of British grasses, and suggest that it protects the grain.

Upper leaves contain more silica than lower leaves. The apical region of a single leaf is more silicified than the basal region (Parry and Smithson 1964).

So much variability exists between species and varieties of species Lanning and Linko 1961) that Van Soest (1970) suggests varieties could be selected and bred for higher silica content. (See Table 1 for a list of silica contents of different species.)

Still unresolved is the issue of active versus passive uptake and deposition. Does a plant use metabolic energy to either transport the substance within the tissues of the plant or to bring it in at the root level? Okuda and Takahashi (1964) and Mitsui and Tokatoh (1963) found that silica is absorbed from the soil faster than can be explained by isotonic uptake in the transpiration stream in rice. This has also been found in barley (Barber and Shone 1965). Others have found no hypertonic uptake in other dry soil grasses (Jones and Handreck 1967). Something, as yet unknown, occurs in the roots of grasses that results in a greater uptake of silica than is found in most other groups.

Although uptake may be an energetic process, deposition does not seem to be. The silicic acid simply moves with the transpiration stream and is deposited both intracellularly and extracellularly (Parry and Smithson 1970). Polymerization occurs and the result is an opal phytolith (Sangster and Parry 1971).

One of the criticisms of passive deposition in cells is the pattern of silica deposits. Prat (1948) suggests that senescence may take place in silica cells which would reduce the pH. This reduction in pH would allow the silicic acid to polymerize. Silica is susceptible to gel formation at pH 5.5 (Iler 1955.) and if the cell could change to a pH at that level silica deposition would be likely.

Jones and Handreck (1965) found that solid silica can be almost completely recovered in the feces and urine of sheep fed on pasture forage with only a very small fraction of total silica found in the urine. In sheep it has been calculated by Saur, as reviewed by Jones and Handreck (1967), that only 0.016 percent of the ingested silica would remain in the tissues.

Soluble silica (silicic acid) as well as the insoluble form has effects on herbivores. These effects will be discussed later. The experiments in this study pertain only to the solid or opaline form. I undertook the experiments to examine some of the effects of solid silica on assimilation of foods by small herbivores and to determine the effect silica has on palatability of feeds when all other variables are held constant. It should be pointed out that I am only considering the effect of free silica bodies in the diet. Other studies (Brazle et al. in press) have shown that the phytoliths may be firmly attached to the cell walls and provide structural inhibition to microbial degradation in the rumen of cattle.

Microtus ochrogaster, whose diet consists of up to 80% grass, was used as a test organism. This animal, although not a ruminant, has a lengthy alimentary canal and large cecum (Golley 1960) to support an extensive microflora to utilize as much of the plant tissue as possible. In addition, voles show preferences amoung different grass species as well as parts of the same plant (Hall 1955). Therefore, the results of the experiments should be applicable to field observations of food preferences.

MATERIALS AND METHODS

Voles (Microtus ochrogaster) used in the two experiments were sexually mature males and females obtained from a breeding colony at the University of Kansas (K. U.). Exact ages of the animals were not known but were estimated at three months for the first experiment (February) and therefore six months for the second experiment (May). Litter mates were kept in the same cages to avoid breeding at an early age and also to avoid aggressive behavior common among unfamiliar individuals. The animals were maintained in small plastic cages (12 in. X 6 in. X 5 in.) with wood shavings as bedding. At. K. U., prairie hay was kept in the cages and therefore prior exposure to a siliceous diet could not be avoided. After acquiring them from K.U. no further hay was provided. To avoid possible sexual differences in the assimilation studies, only males were used. Both males and females were used in experiment #2 as no sexual differences were anticipated in the choice experiment.

Experimental cages were larger (18 in. X 10 in. X 5 in.) and made of plastic with a top made of ½-inch mesh screen. They were kept on shelves in a room with 14 hour photoperiod from fluorescent lights. Water was provided by bottles with tubes extending down from above the screen. Changes from the above will be covered in each section.

Pellets referred to in this study were "simulated grass" pellets of the formula Z-33A, a diet that has been used at Kansas State University to raise and breed Microtus. The pellets simulate grasses only in the fact that silica is added to the basic formula. The basic formula contains alfalfa, soybean meal and ground yellow corn, with other trace nutrients listed in Table 2.

Silica was added in concentrations of 2, 4, 6, 8, and 10% of the weight of the pellets. The source of SiO_2 was diatomaceous earth. This substance is similar in shape and identical in chemical structure to the silica bodies found in grasses. The requirement for large amounts of pellets made the extraction of silica from grass impractical.

Part I Assimilation Efficiency

Experiments for determining the effect of silica on assimilation efficiency consisted of feeding diets of known silica concentration to voles and subsequently collecting their urine and feces for calorimetric and gravimetric analyses. Thirty males were weighed and placed in numbered cages. They were blocked by weight into five groups of six animals; the lightest six in one group, etc. Within a block each animal was randomly assigned a treatment. The grouping allowed monitoring of any bias due to weight of the animals.

Each cage had a bottom made of $\frac{1}{2}$-inch mesh screen one to two inches above the plastic floor of the cage. Urine, feces and crumbs could fall through this screen, but pellets were held above the bottom of the cage. Paper towels ("kimwipes") were opened out and spread over the cage floor to absorb urine and minimize its mixing with feces and pellet crumbs. As soon as urine hit the towel, it would spread and dry. No record was made of the amount of water consumed during the experiment.

A preweighed pellet of the assigned SiO_2 concentration was introduced daily and the old one removed and stored in an aluminum pan. Unused pellets were kept in plastic bags. The experiment lasted eight days, but only feces, urine and crumbs from the last six days were saved for analysis, since the first two days may have contained feces and urine derived from previous food sources. The feces, crumbs and urine were collected and stored in preweighed aluminum pans and placed in drying ovens at $60^{\circ}C$. The cages were cleaned each day with the material added to that of the previous days. The ovens prevented decay of organic materials and also dehydrated the urine.

Each day the animal was removed and the screen taken out. The paper towels were shaken to remove crumbs and feces and rinsed with distilled water into the pan to remove urine, and discarded. Feces were picked out with forceps and crumbs were brushed out with a small paint brush. New towels were then laid down and screen and animal returned. Feces, urine and crumbs were dried to a "constant weight" and stored in the same oven until calorimetry could be done. Pellets were dried to determine moisture content.

A randomized sample of material was used to determine the energy content of the above materials after it was ground up with mortar and pestle. The techniques of Paine (1964) were used on a Parr Semimicro Oxygen Bomb Calorimeter, Model 1411, to determine the caloric value of the materials.

The percent ash was determined for the feces, urine and pellets by placing two samples of each material into a muffle furnace for 12 hours at $450^{o}C$.

Silica content was obtained by treatment of the ash with HCl to remove impurities and then filtering out the acid on ashless filter paper. The paper was then burned in the muffle furnace in a platimum crucible for one hour. After complete combustion, the crucible was weighed and treated with HF and returned to the furnace. After HF and SiF_4 were driven off, the crucible was removed and weighed. The difference in weight is the amount of SiO_2 which is lost as a gas with the fluorine.

Values in calories per gram dry weight are multiplied by the dry weight of each material to obtain caloric intake and output. Assimilated energy is the calories of food ingested minus the calories of feces produced. Metabolizable energy is assimilated energy minus calories of urine produced. Assimilation efficiency is the assimilated energy diveded by the total calories consumed.

Feces and urine were treated a bit differently. Energy values were determined for each cage on feces but because of the loss of accuracy resulting from contamination of the urine by crumbs, the energy values were obtained by lumping the urine samples for those voles on the same diet.

Part II Palatability and Selectivity

In the experiments for determining the effect of SiO_2 on food choice, voles were allowed to choose between pellets of differing SiO_2 concentration to determine their ability to discriminate. Fifteen combinations of two concentrations of pellets were used with four animals on each combination. Animals were weighed before the experiment and blocked into four groups of fifteen animals based on the ranks of the weights. Within each group of fifteen, animals were assigned at random to a particular combination.

Cages were prepared as in Part I except bedding was placed under the screen to absorb urine. Crumbs and feces were not collected.

The experiment ran for five consecutive days with new pellets introduced daily. Each day pellets were dropped into the cage together and allowed to roll around on the screen at random. The voles subsequently moved them around the cage at will. Leftover pellets were returned to the plastic bag to maintain moisture content. Pellets were distinguished from each other in the cage by a thin black mark placed lengthwise on one of the two with a marking pen. So as to avoid this influencing the choice, the mark was placed on the lower concentration one day and the higher the next.

Voles were weighed at the end of the five days and removed from the cages.

The leftover pellets were weighed and this value subtracted from the original weights to obtain the amount actually chewed off. A "crumbing factor" was used to estimate the amount of crumbs lost through the screen. This number was derived from the assimilation experiment by the ratio of crumbs collected to the amount eaten of a particular pellet type. No significant differences between the crumbing factors were found. Variances between animals were considerably greater than between pellets. For this reason the crumbing factor was not applied.

Part III Analysis of Prairie Grasses

To demonstrate that the pellets did in fact closely simulate the SiO_2 content of grasses actually available to Microtus in the field, grasses were collected on the Konza Prairie Research Natural Area just south of Manhattan, Kansas. Several species were collected over different soil types, which were noted. The list of species collected can be seen in Table 3. All plants were brought to the lab in plastic bags to avoid moisture loss and weighed. This was followed by drying in an oven at $60^{\circ}C$. until a "constant weight" was attained. Subsequently, the plants were ground into a homogenized coarse powder and ashed as described earlier. Silica content was determined also by techniques mentioned previously.

RESULTS

Part I Experiment for Determining the Effects of SiO_2 on Assimilation Efficiency

An analysis of feces and pellets yielded assimilation efficiency values for animals on the various concentrations of silica. Means, and standard errors of these efficiencies appear in Table 4. Since the variance of the 6% mean seemed very large, a Bartletts test (Snedecor and Cochran 1967) for homogeneity of variances was done. This yielded a significant chi-square value ($p < .01$). Therefore an AOV was not appropriate since it requires equal variances for the means. A test for equality of means with unequal variances (Sokal and Rohlf 1969) revealed no significant differences between the means of the assimilation efficiencies ($F'_{(5, 10)}=1.75$, $p \approx .22$). The grand mean of the efficiencies was 77.6%.

Figure 1 represents the plot of assimilation efficiencies at different silica levels. The line is the regression through those points. The slope of the line is not significantly different from 0.

Since silica was added to the diet as a percentage of the wet weight of the diet, corrections for amount of water in the pellet caused the percent silica to increase when measured as the percentage of dry weight. Values for silica are therefore higher than reported but if only the amount of added silica is considered, the tables listing 0, 2, 4, 6, 8, and 10% are accurate. The average amount of water in the pellets was 5.8%. Weight values of consumed food were corrected accordingly.

Table 5 shows the amount of food consumed per gram body weight per day. The values show an increase from 0 to 2 percent but a decline from 2 percent on. Figure 2 illustrates the trend.

Calories of urine, subtracted from calories assimilated gives metabolizable energy (Table 6). Although the number of calories per gram of urine decreased with increasing silica in the diet, little confidence can be placed on the relationship because of contamination of urine by crumbs. Contamination by crumbs was not uniform (see below). Urine production of the voles is also given.

Table 7 shows the amount of ash and silica in feces with silica in food. The values increase with increasing silica in diet. A concentration factor, the ratio of silica in food to silica in feces, is given. The concentration factors decline with increasing SiO_2 concentration, excluding the 0% value for its low original concentration, and the 6% value. The greatest variance can also be seen in the 6% value. This coincides with the large variance of the 6% assimilation efficiency values and could be significant. I will discuss these outliers later.

Except for those voles fed on unaltered pellets, all animals lost weight during the six day period. The amount lost was increased significantly over increasing silica diets (Table 8, Figure 3). Values are given in grams/vole.

Animal #7 died on day five, #2 and #20 on day seven. Vole #28 died the day after the experiment was concluded, but death obviously was due to experimental treatment. Voles #2 and #7 were on an 8% silica diet. Voles #20 and #28 were on a 10% silica diet. Specific cause of death was not immediately apparent.

Crumbing factors (CF) were determined and found to be more dependant on individual animal differences than on different feeds (ie. large σ^2 between animals).

Part II Experiment on Effects of SiO_2 on Food Choice

In the fifteen combinations of pellets given to the voles, no vole chose the higher concentration over the lower with statistical significance (Table 9). Chi-square tests are for deviations from equal use of the pairs of concentrations. Values used in the chi-square test were totals of four animals on the assigned combination. In all combinations except those with 2 percent differences in silica (ΔS), a highly significant ($p < ,01$) preference for the lower concentration is exhibited. At the 2 percent level, discrimination was not uniform. In 3 of the 5 combinations where a ΔS was 2 percent there were no significant preferences for either pellet.

In all cases, some of the pellet with higher silica concentration was chewed indicating a testing behavior involving comparisons that occur when the animal is confronted with new food sources.

Relative Preferance Indexes (R.P.I.), calculated as the fraction of the total consumed which was of the higher silica diet, are given in Table 10. These values reflect the amount of selectivity for the higher silica pellet. A value of 0.5 would mean no preference for either; a low value would mean preference for the lower concentration. An AOV test showed a highly significant ($F_{(14,\ 45)} = 3.69$, $p < .01$) difference amoung the means.

Plotting the means against the difference in the silica concentrations between pellets (ΔS) yields the graph in Figure 4. If a linear regression is done on these points, an equation of a line R.P.I. = $-.032\Delta S + .3474$ is obtained ($r^2 = .273$), S being the silica concentration. This is significant with 13 degrees of freedom ($p < .05$). Since the Y intercept is considerably lower than the theoretical Y intercept at 0.5, (for no preference), a non-linear model was also attempted (Figure 5). The relationship R.P.I. = $0.5e^{-0.23\Delta S}$ was found to have an r^2 of .33. Since more of the variance is explained by the curvilinear relationship and it makes more biological sense (see discussion), the non-linear model was chosen. It should be noted that a number of models could fit the data with such variance and small sample size. Since some of the variances in the preference values can be explained by the fact that there were different total silica values in the choices, the means of like differences may fit the curve better and indeed show an r^2 of 0.78.

Weight changes of the animals on the different combinations are shown in Table 11. These weight changes are not much different from each other, but strikingly different from experiment I (Table 8).

DISCUSSION

Assimilation efficiency as defined by Odum (1957) is the ratio of energy absorbed from the food to the energy of the total food ingested. The values for assimilation efficiency in my experiment were high (77%) for a vole. Golley (1960 b) found assimilation efficiency in Microtus pennsylvanicus feeding on old field vegetation to be 70%. The higher assimilation efficiency for my animals could be due to (1) higher caloric content of the food (2) less cellulose to digest for gut microflora (3) higher lipid content of pellets (4) lack of any organic linkage of silica (5) lack of incructation in organic matter. Cattle, sheep and other ruminating organisms achieve a 50% assimilation efficiency or less (Brody 1945).

The pellets are quite different structurally than the grasses they simulate. The silica is mixed evenly into the food, grasses have silica in rows of epidermal cells. Normally the food is made up of water in addition to cell parts, the pellets contain only traces of water. The shapes of the silica particles in diatomaceous earth are different from those in grasses, although not drastically. These differences are quite real and any conclusions need to take them into consideration.

The benefits of using pellets are: (1) a uniformity of silica in the diet. (2) Caloric value of the pellet is uniform. (3) Pellets of different silica concentrations are consistant in every other variable, allowing conclusions to be based only on the silica variability.

The highly abrasive silica did not alter the ability of the animals to assimilate energy from their food. The quantity eaten was greatly reduced yet the efficiency with which the food was digested was not altered.

Although the efficiencies did not change, the weight of the animals and number of calories consumed decreased with higher silica diets. That can only be interpreted as reflecting the palatability of the different diets since the assimilation was no different for each diet. Interestingly, the 2% animals showed an increase in the amount of food consumed. This could mean that the diet was not terribly unpalatable and yet the animal needed to ingest more to make up for the caloric deficit of the 2% diet forced the animals to reduce their intake. By reducing caloric input and with the added effect of a lower quality diet (in calories per gram), the voles lost weight.

Prior to the experiment it was thought that silica would interfere with digestion, perhaps by blocking the microvillae where food absorption takes place or by damaging the lining of the gut. The fact that assimilation efficiency was not altered leads to the conclusion that the silica had either no effect on the ability to absorb food or that its effect is only seen on a long term basis.

Baker et al., (1961) found that the stratum corneum and stratum granulosum were better developed in lambs fed on alfalfa rather than grass. They suggest it could be due to particulate mineral differences in the two feeds. Adult sheep whose rumen contents were examined showed the particulate minerals to be opaline silica from plants, with small amounts of soil quartz silica. The irregular shape and hardness could affect the rumen lining through abrasive action. Poorly developed areas of stratum corneum in the mucosal surface can exist in the rumen wall which they suggest may be due to this abrasion.

Although <u>Microtus</u> has no rumen, it does have a very large cecum that contains an extensive microflora (Golley 1960). Possibly the muscular contractions of a rumen (Magee 1932) account for the strong abrasion in the sheep. Perhaps this may also occur in the cecum of Microtus. My experiment lasted only one week. A longer period of time on the abrasive diet might show these dramatic effects and eventually cause decreased assimilation.

Digestibility of pasture plants has been under study by Van Soest and Jones (1968). They found silica to be an important factor in reducing digestibility of cell-wall constituents. By using an artificial rumen, they were able to measure digested material after treatment with neutral detergent to remove silica. The more silica removed, the more cell-wall digested. They suggested that there is a silica-cellulose linkage that retards the ability of gut microorganisms to break down structural carbohydrates. That may be true for a fraction of the silica in plant tissues, but since solid silica bodies which occur in the lumen of the cell make up most of the weight of the total plant silica, it may only be a relatively small amount that is actually linked to the cell wall carbohydrates. However, the amount of total silica may be highly correlated with the organically linked form. Still, Van Soest reports an average decline of three units of digestibility for each unit of silica. He points out that lignin is also a factor in deducing digestibility and the effect of silica and lignin are additive.

In other studies Van Soest (1970) and Van Soest and Lovelace (1969) found that acid pretreatment of the silica in the plant tissues before extraction yielded higher digestibility than without. He concludes that the sensitivity of forage silica to acid may be indicative of organic complexes.

It seems from these studies that the organo-silica linkages may be important. These linkages had not been considered before 1967 (Jones and Handreck 1967). However, other studies (Brazle et al. in press) indicate silica bodies may be important structural inhibitors to degradation by gut microorganisms. Electron microscopic examination of grass tissue taken from the rumen of cattle revealed less degradation in silica rich areas. They interpret this observation as mechanical resistance by reducing the surface area exposed to the gut microorganisms. My study only considers loose phytoliths and tends to support the idea that association with the cell wall is important for reduction in digestibility.

The second part of my study was directed at the abilities of Microtus to detect subtle differences in silica concentrations. From the results, it is not totally clear if a 2 percent difference can be detected. Alternately the results show conclusively that differences of 4 percent or greater are immediately detected. These conclusions may be somewhat misleading. Grasses contain a large quantity of water in the cellular spaces. The percent of the live grass that is silica by weight can be reduced dramatically by the water content. Microtus will eat both dried and living a critical criteria for selection, yet moist vegetation may not require as much mastication or time in the digestive system.

The relationship of selectivity to difference in silica between choices suggests that an increase in silica concentration of one plant over another can dramatically alter the selection of one of the plants by a herbivore, although my data suggest that more than a 2% increase is probably needed. They also suggest that an increase of 2% can have a greater effect when both of the plants have low initial silica concentrations. Moreover, this difference could also be the cause of selection of one plant part over another. Individual Microtus apparently pick blades of grass and chew the basal region leaving the apical region untouched (Hall 1955). As stated earlier, the basal region contains less silica than the apical region (Parry and Smithson 1964).

The means for weight loss between combinations show no pattern. Whereas the assimilation experiment showed considerable weight loss. The only differences between the two experiments were (1) a choice was given in one and not the other (2) the animals were 3 months older in the choice experiment, and (3) wood shavings were added as bedding below the screen to absorb urine in the choice experiments. The animals were not able to build nests with material but it may have provided some insulation effect. The temperature in the room was always between 65^{o} to $75^{o}F$. So, stress due to temperature would not be expected.

The choice experiment also points out that testing must occur by Microtus when a new food choice is presented. Each day the animal had to choose between pellets of differing quality. No visual cues other than a subtle color difference were given. Therefore it appears likely that a "tasting" of the pellets led to the eventual choice. This may indeed be the case in nature (Krueger et al. 1974). Although more visual cues occur in the wild, at some time testing and perhaps learning should occur to lay down patterns of selectivity.

The data also suggest a predictive model for estimating effects of silica on choice (Figure 5). The model, although based on a small sample size could explain food selection of species that do not differ drastically in other variables such as alkaloids and other toxic substances (Freeland 1974).

The low r^2 for the model (0.33) can be partially explained by the inherent variances in the preference means, especially for the 2% differences. Several of the combinations differed by only 2% but the total amount of silica in the diets were higher. It suggests that at high silica levels discrimination may not be as effective (see Table 9). This trend does not hold for the larger ΔS's likely due to small sample size. The 6% - 8% combination appears to be an outlier in the above mentioned trend of 2% differences. This outlier can be partially accounted for. In both the assimilation efficiency values and amount of silica in the feces, much larger variances were observed for the 6% diet than the other diets. Perhaps this diet was not adequately mixed in the initial preparation. Some pellets could possibly have a much higher silica content and others have less.

No other explanation can be suggested but at least the data are consistant. This could account for the low preference value of the 6% to 8% combination, particularly if the 6 percent diet was too low in silica. It could also account for the high preference value on the 4% to 6% choice.

Considering my results, silica likely has some benefit to the plant with respect to palatability, but the non-linked, non-attached amorphous silica probably doesn't alter the plant's digestibility.

Other effects of opaline silica have been observed. Many animals show morphological adaptations to abrasive diets. Simpson (1951) points out the changes that occured in the molar teeth of horses. The changes appear to correspond nicely with the advance of grasses into the North American temperate zone in the Miocene. Baker et al. (1959) examined tooth wear in sheep raised on pasture grasses. They noticed the fractured condition of many of the phytoliths in the feces and speculated that considerable wear may occur in the teeth due to this highly abrasive diet. They conducted hardness tests and found silica to be harder than the tissues of the sheep's teeth. They calculated that if three pounds of dry matter containing 2 percent silica were consumed daily by a sheep, a yearly intake of twenty-two pounds of silica would result. Since a two percent concentrarion is common, the estimate seems realistic. Van Soest (personal communications) criticized the Baker study by saying that the amount of soil contamination in the diet would be more than the silica.

The occurence of continuously growing hypsodont molars in microtine rodents (Miles 1967) could also be an adaptation to abrasive diets. Tooth wear may not be as limiting to longevity if the molars can continue to grow. However, increased tooth growth must require an increase in mineral consumption and energy for tooth growth.

Grasshoppers also show morphological adaptations to grasses (Isely 1944, Mulkern 1967). Mandibles in grass-eaters greatly resemble molars in grass eating mammals. Although a new set of mandibles is acquired at each molt, advantages in dealing with a very tough diet seem obvious.

Soluble silica in plant tissues has also been shown to affect animals. Reduced reproductive success (among other things) was found in white rats raised on a water source containing silicic acid (Smith et al. 1973).

In another study, female lambs given silicic acid in the water showed a loss of weight of 6%, yet males actually gained weight significantly greater than controls (Smith et al. 1972).

Dissolved silica can polymerize in the urinary system of many ruminants, especially sheep, to cause uroliths or urinary calculi (reviewed in Jones and Handreck 1967).

In conclusion, it seems that the evidence for detrimental effects of silica on herbivores is overwhelmingly in favor of considering opaline silica as a defensive substance in grasses. Whether it requires metabolic costs, or not, it appears the benefits justify at least some cost to the plant. If this substance can be absorbed from the soil and deposited passively in plant tissues, then it seems grasses have a very inexpensive defense system.

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Table 1: Silica composition of various grasses selected from the literature to illustrate the range of silica concentrations that commonly occur in grasses.

Species	Parts of Plant	Amount of SiO_2 (% dry-weight)[2]
Cynodon dactylon	All	0.7
Spartina pectinata	"	3.0
Calamovilfa longifolia	"	3.1
Panicum antidotale	"	3.5
Bromus sp.	"	5.2
Setaria sp.	"	6.6
Buchloe dactyloides	"	6.9
Phalaris arundinacea	"	8.8
Festuca sp.	"	8.9
(from Van Soest et al. 1970)		
Andropogon gerardi	Leaves	5.07
Phalaris arundinacea	"	5.46
Setaria faberi	"	9.88
Sorghastrum nutans	"	10.31
Spartina pectinata	"	4.54
(from Jones in de Pomar 1975)		

Table 2: Ingredients of pellets (Z-33A) used in the feeding experiments. Values are given as percent of total weight at time of mixing. This basic formula was then supplemented with silica.

Ingredients	% Total Weight
Soybean Meal	20.0
Alfalfa Meal	21.0
Ground Yellow Corn	29.0
Wheat Midds	20.0
Corn Oil	1.8
Wheat Germ Oil	0.2
Brewers Yeast	2.0
Dicalcium Phosphate	2.0
Salt	1.0
Stapel	2.0
Trace Minerals	0.05
Vitamin D_3	trace
Vitamin B_{12}	trace
Aurofac-10	0.3
Ground Corn	0.6

Table 3: Percentage of silica, ash and water in five grass species collected on Konza Prairie Research Natural Area July 27, 1977 with soil types on which they were collected.

Species	% Ash (dry-wt.)	% Silica (dry-wt.)	% H_2O	% SiO_2 (wet-wt.)	Soil Type
Bouteloua certipendula	8.71	6.63	43.52	11.58	*T
Bromus inermis	8.92	4.46	53.17	9.52	T
Panicum virgatum (1)	6.88	3.51	61.99	9.24	T
Panicum virgatum (2)	3.69	1.43	57.91	3.38	**B-A
Andropogon gerardi	5.04	2.23	64.22	6.25	T
Spartina pectinata	2.88	0.77	46.51	1.87	B-A

* T = Tully

** B-A = Breaks-Alluvium

Table 4: Mean assimilation efficiencies with standard errors of prairie voles fed diets of differing silica concentration for six days.

% Silica in Food (added)	Mean ± s.e. (n = 5)
0	76.68 ± 2.45
2	77.38 ± 0.48
4	78.54 ± 0.92
6	69.36 ± 4.47
8	79.57 ± 1.86
10	78.30 ± 2.29

Table 5: Grams of food consumed by prairie voles on diets of differing silica concentration for six days.

% Silica (added)	Grams consumed/weight-day X 10^{-2} (n = 5)
0	10.94 ± 0.96
2	14.22 ± 1.19
4	10.46 ± 0.69
6	7.48 ± 1.01
8	8.39 ± 1.47
10	8.87 ± 0.69

Table 6: Weight and caloric content of urine from prairie voles fed diets of differing silica concentrations for six days. Percentage of ingested energy that was metabolized is also given.

% SiO_2	Mean Urine Production (gms. dry-weight per vole-day)	Mean Caloric Content (kcal. per gm. dry-weight)	Metabolizable Efficiency (%)
0	0.255 ± 0.028	2.702	74.04 ± 2.6
2	0.273 ± 0.023	2.187	75.38 ± 0.5
4	0.220 ± 0.048	2.169	76.66 ± 1.1
6	0.210 ± 0.006	2.015	66.32 ± 4.6
8	0.184 ± 0.016	1.981	76.56 ± 2.2
10	0.210 ± 0.063	1.891	75.80 ± 2.5

Table 7: Silica and ash values for feces, and silica content of diets fed to the prairie voles. A concentration factor for silica from food to feces is given. This value is the ratio of the percentage silica in feces to percentage silica in food.

% SiO_2 in Food (added)	% SiO_2 in Food (corrected)*	% Ash in feces	% SiO_2 in feces	Conc. Factor
0	0.08	00.88 ± .01	00.49 ± 0.00	6.12
2	2.25	09.31 ± .12	07.01 ± 0.70	3.11
4	4.42	16.99 ± .01	12.88 ± 0.31	2.94
6	6.60	19.79 ±1.88	15.65 ± 1.43	2.37
8	8.78	27.59 ± .12	22.04 ± 0.15	2.51
10	10.95	31.25 ± .10	25.23 ± 0.40	2.31

* Corrected for amount of moisture in the pellets and amount of silica in the pellets unsupplemented with silica.

Table 8: Weight changes of voles kept on the different silica diets. Means are from five animals during six days.

Percent SiO_2 in Diet	Mean Weight Change (percent of total)	Mean Weight Change (grams/vole)
0	.4	.18 ± 2.13
2	5.4	-2.00 ± 0.94
4	9.8	-3.74 ± 2.236
6	14.25	-5.24 ± 1.27
8	30.45##	-11.56 ± 2.67
10	19.05#	-6.76 ± 1.62

# indicates number of animals that died during the experiment

Table 9: Total amount of food consumed and chi-square values for deviations from equal use of silica diets by prairie voles fed diets of different silica concentrations for five days. Each value is a total for four animals.

Combinations	Percent SiO_2						
	0	2	4	6	8	10	2
1	101.3	18.3					57.6 **
2	190.0		31.0				114.3 **
3	150.6			12.4			117.1 **
4	170.0				13.5		133.4 **
5	150.8					24.9	90.2 **
6		60.5	46.5				1.8 NS
7		108.9		41.0			30.7 **
8		106.8			27.5		48.0 **
9		181.2				11.5	149.4 **
10			104.4	95.7			0.3 NS
11			133.8		13.4		49.2 **
12			74.9			43.2	8.5 **
13				137.4	7.8		115.6 **
14				129.5		25.8	34.6 **
15					56.2	89.0	0.9 NS

Table 10: Preference indexes for voles given choices of food with different silica content. Means are taken from four voles over a five day period.

Combination #		Mean Preference Index (amount consumed of higher concentration/total)
1	(0%-2%)	.1678 ± .0976
2	(0%-4%)	.1206 ± .0531
3	(0%-6%)	.0649 ± .0421
4	(0%-8%)	.0619 ± .0512
5	(0%-10%)	.1422 ± .0477
6	(2%-4%)	.4425 ± .1050
7	(2%-6%)	.2647 ± .0358
8	(2%-8%)	.0607 ± .0251
9	(2%-10%)	.0553 ± .0194
10	(4%-6%)	.4487 ± .0662
11	(4%-8%)	.0816 ± .0363
12	(4%-10%)	.3055 ± .0500
13	(6%-8%)	.0492 ± .0140
14	(6%-10%)	.1805 ± .0551
15	(8%-10%)	.5050 ± .1819

Table 11: Weight changes of voles fed combinations of diets differing in silica content for five days.

Combination	Mean Weight Change (percent of total)	n = 4
1 (0%-2%)	-1.71	
2 (0%-4%)	+4.2	
3 (0%-6%)	+0.8	
4 (0%-8%)	+0.8	
5 (0%-10%)	+5.8	
6 (2%-4%)	-2.0	
7 (2%-6%)	+2.2	
8 (2%-8%)	-5.0	
9 (2%-10%)	+6.5	
10 (4%-6%)	-1.1	
11 (4%-8%)	-2.9	
12 (4%-10%)	-3.1	
13 (6%-8%)	-2.8	
14 (6%-10%)	-3.1	
15 (8%-10%)	-2.8	

Figure 1: Relationship between the amount of silica in a manufactured diet and the assimilation efficiency of prairie voles during a six day period.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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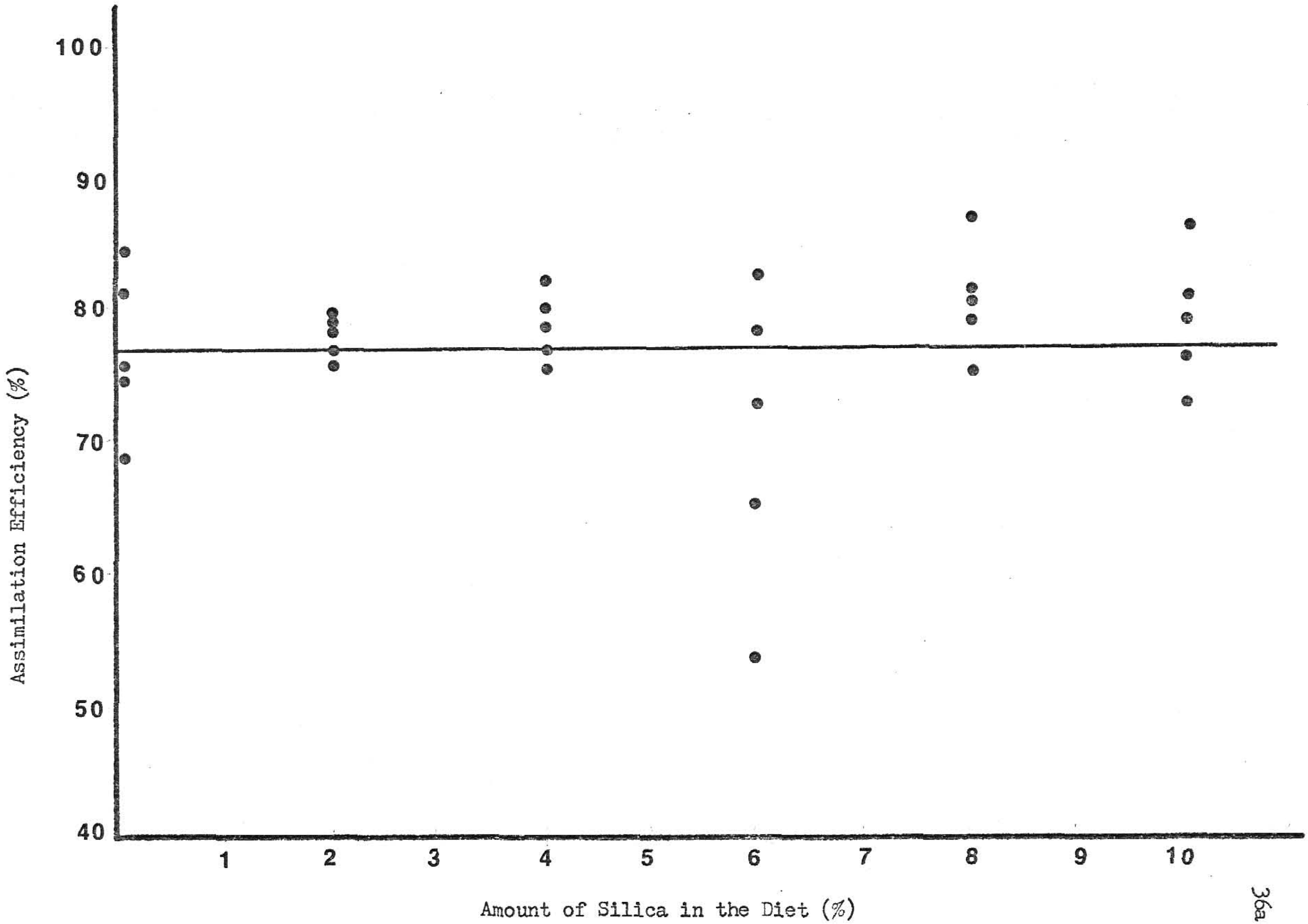


100
90
80
70
60
50
40
Assimilation Efficiency (%)
1
2
3
4
5
6
7
8
9
10
Amount of Silica in the Diet (%)

Figure 2: The amount of food consumed per gram body weight per day by prairie voles fed manufactured diets of different silica concentrations. The points are means of five voles on each silica concentration.

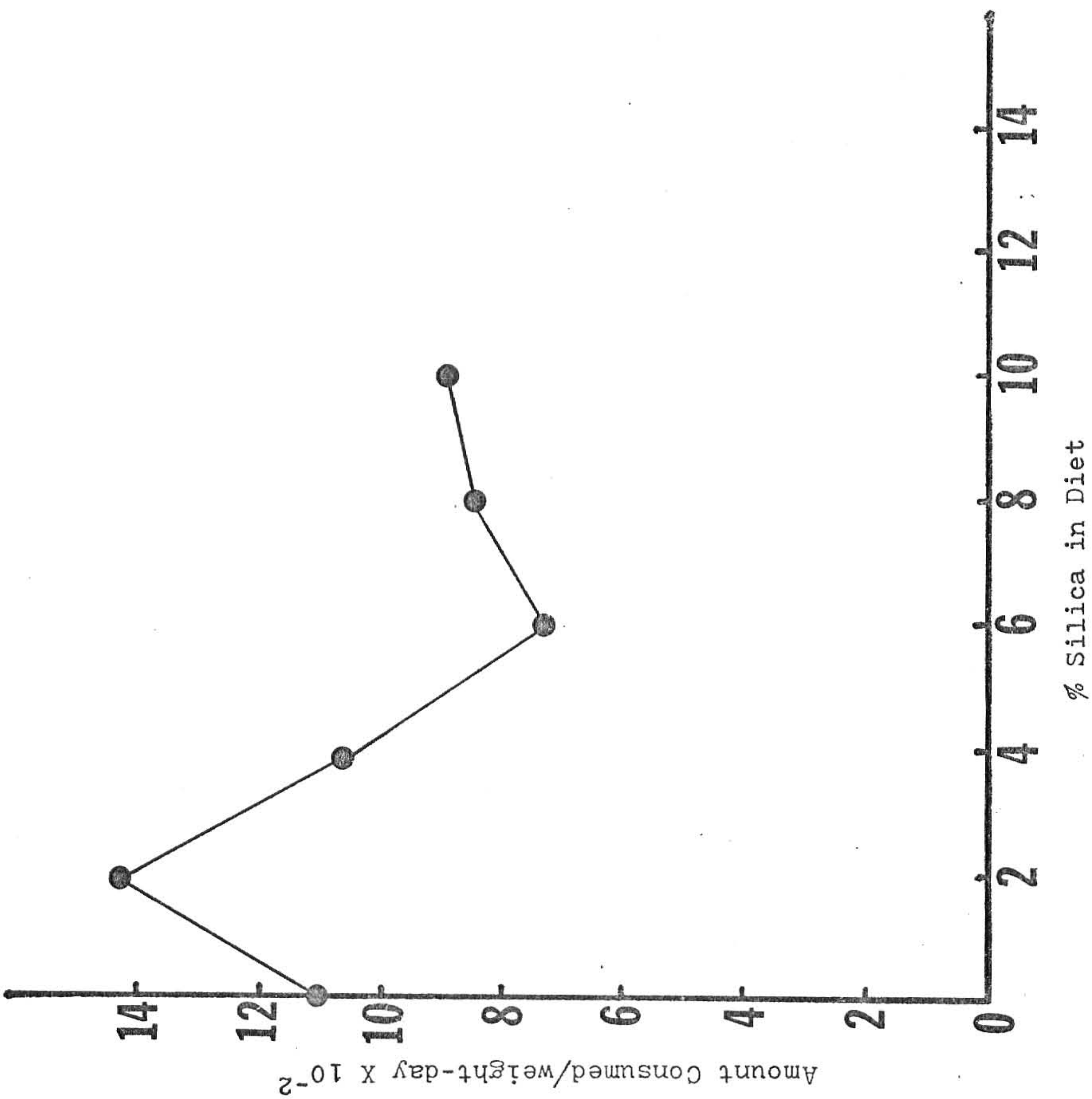

Amount Consumed/weight-day X 10-2
% Silica in Diet
0
2
4
6
8
10
12
14

Figure 3: Weight losses of prairie voles on diets of different silica concentrations. Each point represents the mean of five animals.

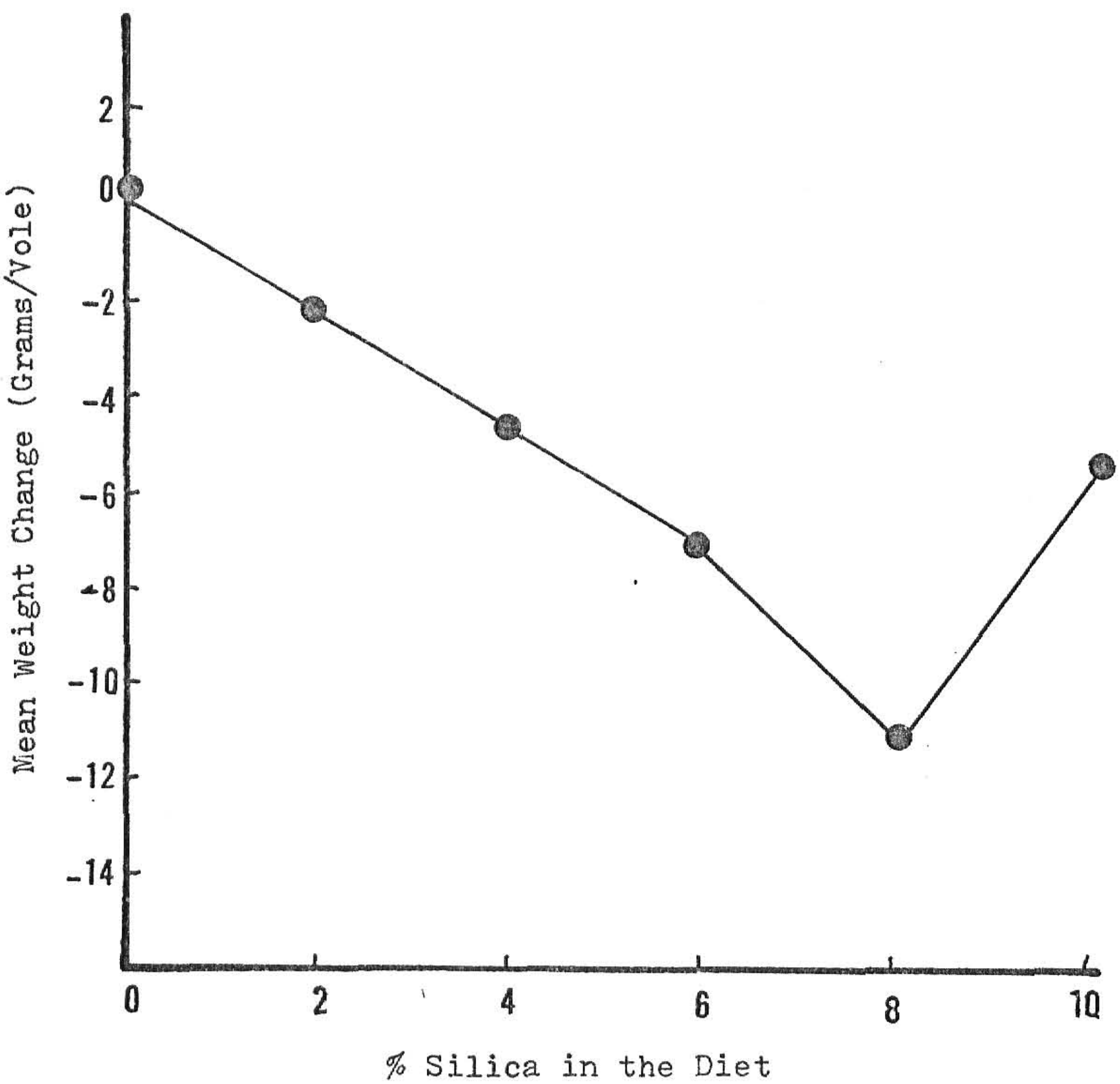

Mean Weight Change (Grams/Vole)
2
0
-2
-4
-6
-8
-10
-12
-14
0
2
4
6
8
10
% Silica in the Diet

Figure 4: The linear regression of the relative preference index (R.P.I.) between diets differing in silica concentration on the extent of the difference between the two concentrations. The index reflects the preference of a prairie vole when given a choice between two diets. Values are means of four animals. The line is the linear regression of the R.P.I. on the silica difference between the two diets.

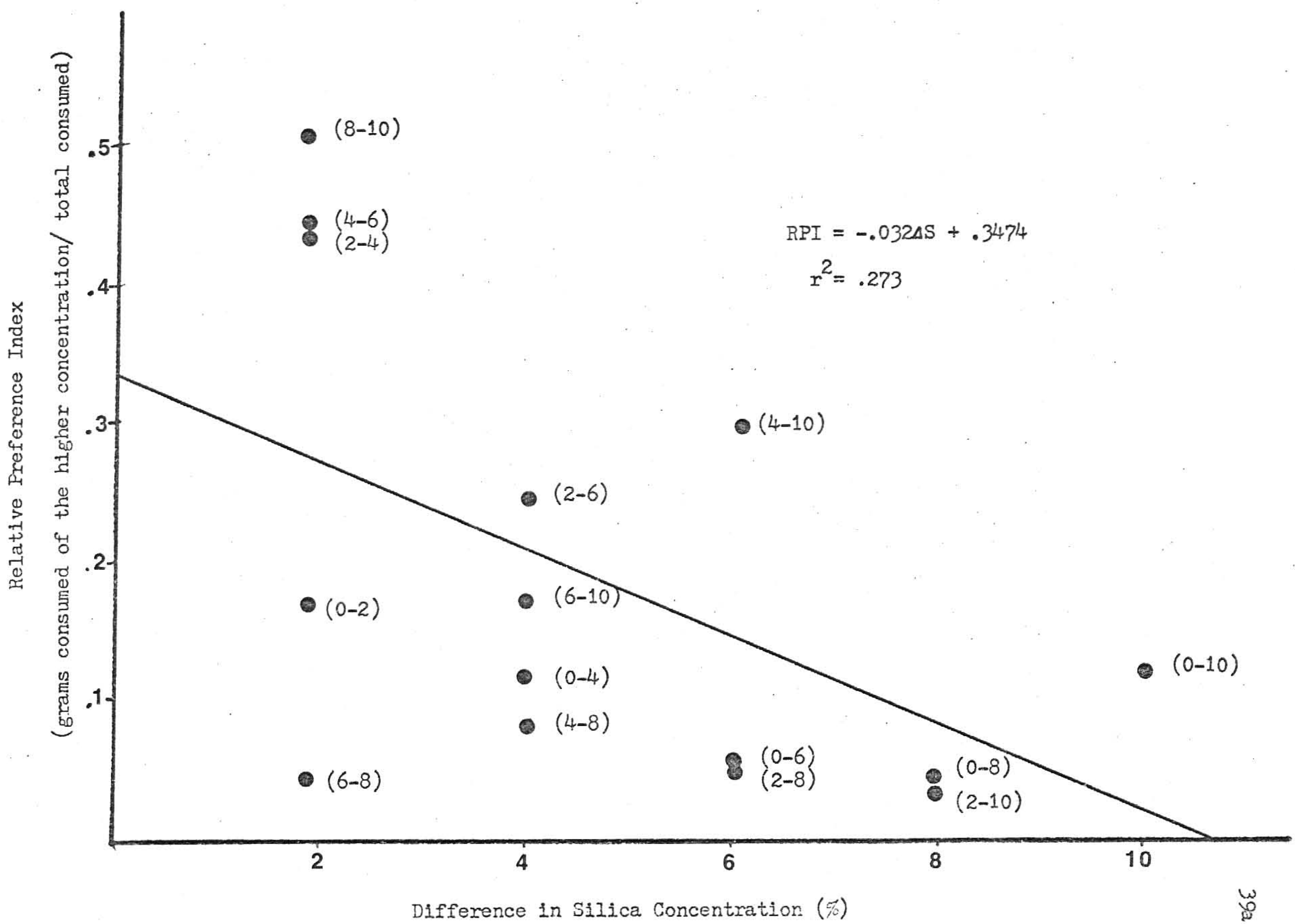
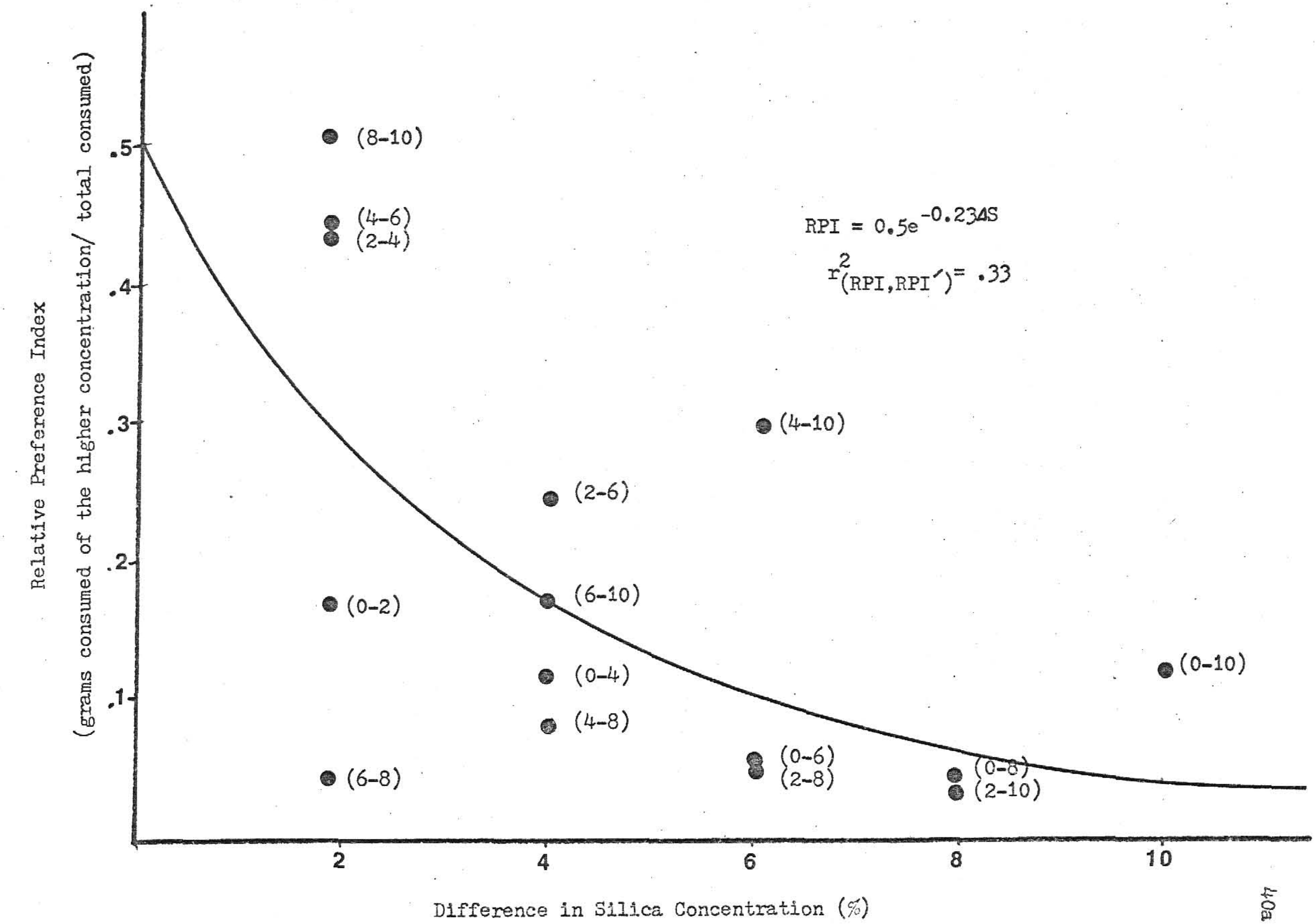

RPI = -.032ΔS + .3474
r2= .273
Relative Preference Index
(grams consumed of the higher concentration/ total consumed)
.5
.4
.3
.2
.1
2
4
6
8
10
Difference in Silica Concentration (%)
(8-10)
(4-6)
(2-4)
(0-2)
(6-8)
(2-6)
(6-10)
(0-4)
(4-8)
(4-10)
(0-6)
(2-8)
(0-8)
(2-10)
(0-10)

Figure 5: A curvilinear relationship of the relative preference indexes between two diets and the difference in silica concentration between the two diets. Values are the same as on figure 4. The curve has been forced through the theoretical value of 0.5 indicating no preference for no difference in silica between diets. The r^2 reflects the fit of the calculated relative preference indexes (R.P.I.) to the predicted relative preference indexes (R.P.I.').


Relative Preference Index
(grams consumed of the higher concentration/ total consumed)
.5
.4
.3
.2
.1
(8-10)
(4-6)
(2-4)
(0-2)
(6-8)
(2-6)
(6-10)
(0-4)
(4-8)
(4-10)
(0-6)
(2-8)
(0-8)
(2-10)
(0-10)
RPI = 0.5e^-0.234S
r^2(RPI,RPI′) = .33
2
4
6
8
10
Difference in Silica Concentration (%)

SOME EFFECTS OF SILICA
ON ASSIMILATION EFFICIENCY
AND DIET DISCRIMINATION IN
PRAIRIE VOLES (*MICROTUS OCHROGASTER*)

by

DALE RICHARD TROTT

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Manhattan, Kansas

1977

ABSTRACT

Grasses differ considerably in their palatability, digestibility and nutritive value. Graminivores have the ability to select those grasses having the optimum combination of the three criteria. One factor that can particularly affect these criteria is the amount of silica in the plant parts. High silica concentration has been shown to reduce palatability, digestibility and possibly nutritive value.

Most of the silica in the plant is in a solid form and can pass directly through the digestive system without being absorbed. Although significant effects of soluble silica in animals have been shown, this study deals only with the solid opaline silica or phytoliths.

Microtus ochrogaster, the prairie voles, utilize grasses to a great extent (up to 80%) as a food. For this reason they should be expected to have evolved adaptive responses to silica concentrations in their food and therefore make good test animals.

Laboratory food pellets were supplemented with varying amounts of silica in the form of diatomaceous earth. Pellets of 0, 2, 4, 6, 8, and 10% SiO_2 by weight were synthesized. These served as different "grass" species for the study. This range was chosen to correspond with dry weight values of SiO_2 for known grasses. Calculation of assimilation efficiencies for *Microtus* fed on each of the different "grass" pellets for 6 days averaged 77% and yielded no significant differences between SiO_2 concentrations. Replicates of fifteen choice combinations of the six "grasses" provide palatability ratings for the higher and lower silica concentrations in each combination. A difference (ΔS) of 2% SiO_2 for a combination was detected by the animals in only two of five of those combinations where it occured, whereas any ΔS greater than 2%

was always detected with the result of strong preference for the lower concentration. A predictive model estimating the percent of the total amount consumed which would be of the higher concentration was obtained: $Y=(0.5)e^{-0.23\Delta S}$.

Weight changes in the voles were also monitored and show a trend of increasing weight loss with increasing silica in the food. Evolutionary patterns and ecological significance of silica in grasses is discussed including other possible effects of silica on herbivores.