SILICON METABOLISM IN DIATOMS

III. Respiration and Silicon Uptake in Navicula pelliculosa*

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INTRODUCTION

The mechanism of ion accumulation by plant cells has been shown to be dependent on aerobic metabolism (Machlis, 1944; Robertson, Wilkins, and Weeks, 1951; Kelly, 1953). Although there is no evidence that diatom cells accumulate silicate ions, they nevertheless possess a very effective mechanism for removing silicate from solution and depositing silicon dioxide in the external walls or frustules. The present paper deals with the evidence that aerobic respiration is necessary for this process.

Materials and Methods

The cells used in the experiments to be described were of two types, morphologically and physiologically distinguishable. (*a*) *Si-deficient* diatoms were cultured as described previously in media containing 1 to 3 mg. Si/liter (Lewin, 1954). After 10 days of growth, the cells had removed all silicon from the medium, and had ceased to divide as a result of the limitation of this element. Such non-dividing cells continued to photosynthesize, and were characterized by the presence of a polyuronide capsule accumulated around each cell. When starved cells were required, the cultures were aerated in darkness for 4 days at the end of the growth period. (*b*) *Non-Si-deficient* cells were obtained by adding a further 10 to 30 mg. Si/liter to the medium when growth became apparent. After 3 days, the cells were still in an active state of cell division, had not depleted the silicon from the culture medium, and had not formed any capsular material. A 2 day starvation period was found to be sufficient to deplete the endogenous reserves of these cells.

The cells were harvested by allowing them to settle in the culture flask. The bulk of the medium was drawn off and the cells were concentrated to the volume required for the experiment. They were not washed, since washed cells are inhibited in their ability to take up silicon (Lewin, 1954). For Warburg respirometry studies, cell concentrations of 1 to 2 $\times$ 10^8 cells per vessel, and silicon concentrations of 20 to 30 mg. Si/liter, were employed. For those few experiments in which silicon uptake was

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measured independently, final cell concentrations of 6 to 10 $\times$ 10^6 cells per ml. and silicon concentrations of 5 to 10 mg. Si/liter were established. A Braun circular Warburg apparatus was employed. Each vessel held 2 ml. of a cell suspension, 0.2 ml. of 10 per cent KOH in the center well, and 0.5 ml. of inhibitor, substrate, or other reagent in the side arm. All experiments were carried out in darkness at 25°C.

Soluble silicate (expressed as milligrams of Si/liter) was determined according to methods previously described (Lewin, 1954). Silicon was supplied to the cells in the form of potassium silicate adjusted to pH 5.0 in determinations of respiratory quotient and to 7.0 in all other cases.

In order to compare the effects of various respiratory inhibitors on silicon uptake and on respiration, oxygen uptake was followed for a 4 hour period after the addition of inhibitor *plus* silicate. Silicon measurements were made on supernatants of cell suspensions removed from Warburg vessels at the end of this period. In the use of KCN, a modified procedure based on the method of Krebs (1935) was followed. In some cases, depending on the stability of the inhibitor and the reproducibility of the results, uptake of silicon was determined in separate experiments, 5 hours after the addition of the inhibitor.

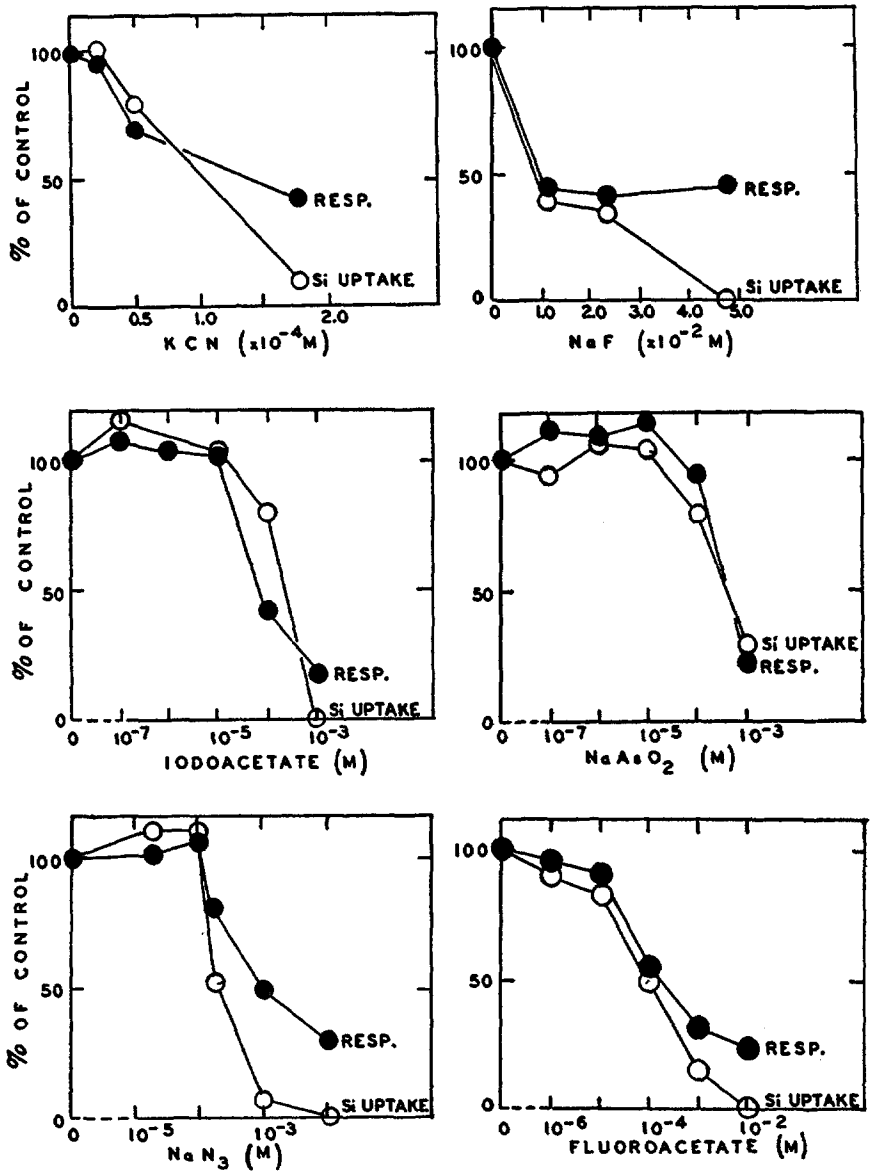
In order to compare the effect of organic substrates on the uptake of oxygen and silicon, the substrate alone and substrate *plus* silicate were added to respiring cells in Warburg vessels. Oxygen uptake was followed for a period of 5 hours after the addition of solutions from the side arm. Respiration during the 4th and 5th hours was averaged and expressed as the ratio of the rate in supplemented flasks compared with that in controls to which no substrate had been added. Silicon measurements were made on solutions removed from Warburg vessels at the end of 5 hours. The effect of substrate was similarly expressed as a ratio of silicon uptake in flasks supplemented with silicate *plus* substrate compared with controls receiving silicate alone.

In experiments to determine respiratory quotients, the pH of the cell suspension was adjusted with HCl to pH 5.0 before the cells were placed in the vessels. Determinations of pH at the end of Warburg runs showed that the pH always remained at this level, and therefore retention of CO_2 by the medium could be ignored. It was not practical to employ phosphate buffer because of the interference of high phosphate concentration in some of the reactions being studied. The quotients were calculated from the gas changes over a 4 hour period, after the gas exchange had become steady, according to the "direct method" (Umbreit, Burris, and Stauffer, 1949). The pH of cell suspensions was also adjusted to pH 5.0 in experiments in which organic acids were tested as substrates. In experiments in which the reaction of the cell suspension was not adjusted, the pH was within the range 6.0–6.5.

RESULTS

1. Necessity of Aerobic Respiration.—Si-deficient cells of *Navicula pelliculosa* were unable to remove silicon from solution under anaerobic conditions. Methylene blue, liver concentrate, and ATP were without effect.

2. Effect of Respiratory Inhibitors on Silicon Uptake.—A series of respiratory inhibitors were tested at various concentrations for their effect on respiration



FIGS. 1 to 6. The effect of inhibitors (KCN, NaF, iodoacetate, $NaAsO_2$, NaN_3, fluoroacetate) on respiration and silicon uptake by Si-deficient cells of *Navicula pelliculosa*.

and on silicon uptake by Si-deficient cells. The results are plotted graphically in Figs. 1 to 6. It can be seen that those concentrations of cyanide, fluoride, iodoacetate, arsenite, azide, and fluoroacetate which are inhibitory to respira-

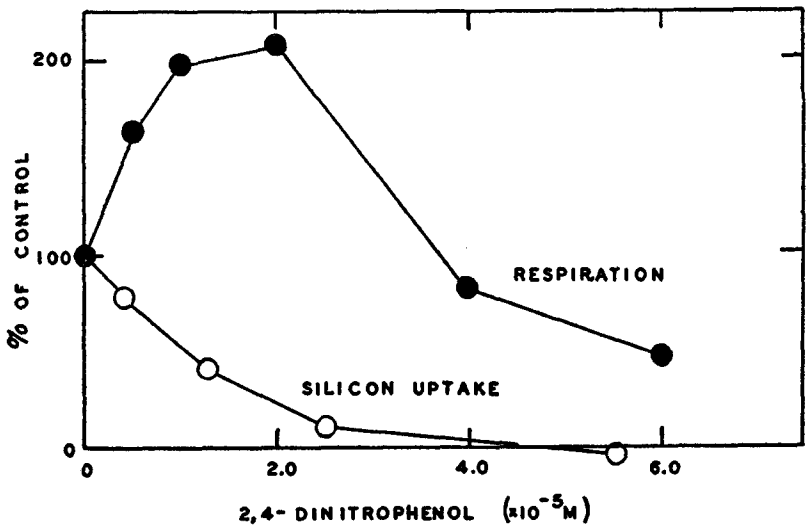


FIG. 7. The effect of 2,4-dinitrophenol on respiration and silicon uptake by Si-deficient cells of *Navicula pelliculosa*.

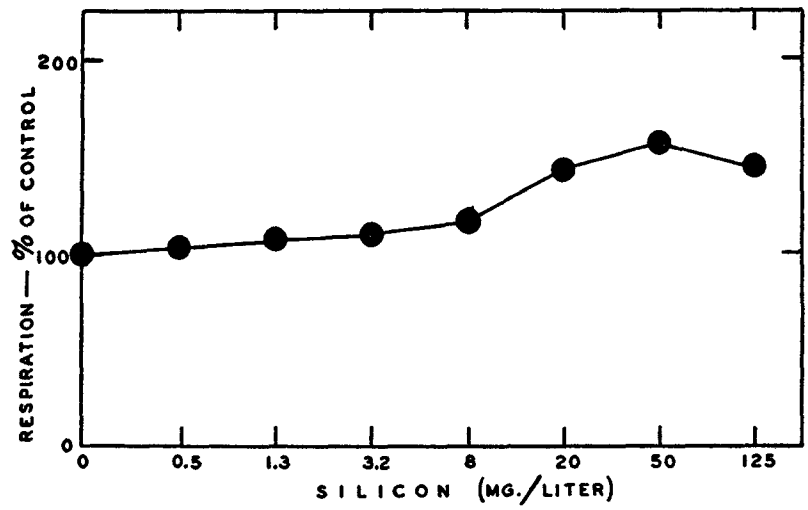


FIG. 8. The effect of silicon on respiration of Si-deficient cells of *Navicula pelliculosa*.

tion are also inhibitory to uptake of silicon. At the highest concentration of certain agents some respiration remained resistant to inhibitor, but silicon uptake was completely inhibited.

3. Effect of 2,4-Dinitrophenol.—Dinitrophenol (DNP) is a reagent which uncouples oxidation from phosphorylation. Concentrations of 1.0 to 2.0 ×

10^{-5} M DNP, which cause a 100 per cent increase in respiration, are strongly inhibitory to silicon uptake (see Fig. 7).

4. Respiratory Quotients of Non-Si-Deficient Cells.—Respiratory quotients of actively dividing cells were found to be 0.92, 0.93, 0.96 (replicates). At the end of a 2 day starvation period the R.Q. had dropped to 0.57, 0.62: when glucose (1 per cent) was added it rose to 0.99. In cells starved for 4 days the R.Q. had dropped to 0.72, 0.78: when glucose was added, it rose to 1.05, 1.06.

5. Effect of Silicate on Endogenous Respiration of Silicon-Deficient Cells.—When potassium silicate was added to Si-deficient cells, the rate of respiration was increased within 1 hour. As little as 1.3 p.p.m. Si produced a detectable stimulation of respiration. On the addition of silicon to a final concentration of 20 p.p.m., the oxygen uptake in several experiments was stimulated by about 40 per cent (see Fig. 8). It could be shown that the effect was due to

TABLE I

Respiratory Quotients of Silicon-Deficient Cells After Starvation in Darkness

	Respiratory quotient			
	Control	After addition of silicate (27 p.p.m. Si)	After addition of glucose (1 per cent)	After addition of glucose and silicate
Experiment I	0.30	0.47	0.81	0.87
Experiment II	0.34	0.53	0.74	0.71
Experiment III	0.29	0.51	0.80	0.75
Experiment IV	0.29	0.37*	0.73	0.86

* 24 hours after addition of 32 p.p.m. Si.

silicon and not to potassium, since K_2SO_4 did not stimulate respiration. The effect was not caused by a change of pH, since silicate solutions adjusted to pH 9.5, 7.4, and 3.5 before tipping all stimulated respiration to a comparable extent. (The pH after the addition of silicate to the cell suspension in the vessels was 6.4–6.8.) Respiratory quotients (R.Q. = $\Delta CO_2/-\Delta O_2$) during endogenous respiration of Si-deficient cells were found to be 0.85, 0.87, 0.87 (different experiments). The values for comparable cells after the addition of silicate were 0.89, 0.90, 0.90. Since the respiratory quotients were virtually unchanged after the addition of silicate, both oxygen uptake and carbon dioxide evolution appear to be stimulated to a comparable extent.

When cells were starved by aeration in darkness for 4 days, the effect of silicate was not as marked as in cells taken directly from the light. With concentrations of 27 p.p.m. Si the respiration in several experiments was increased by 20 to 27 per cent. Respiratory quotients for silicon-deficient cells which had been starved in the dark were very low, around 0.3, and on such cells the addition of silicate had a pronounced effect. Although oxygen uptake was stimulated

by only about 25 per cent, carbon dioxide evolution was stimulated by 90 per cent, with the result that the R.Q. was raised to about 0.5 (see Table I).

TABLE II

Effect of Substrates on Respiration and Silicon Uptake

		Respiration	Silicon uptake
		Ratio of rates $\left(\frac{+\text{ Supplement}}{\text{Control}}\right)$	Ratio of Si taken up $\left(\frac{+\text{ Substrate}}{\text{Control}}\right)$
Controls		1.00	
Experiment I.	Silicate (27 p.p.m. Si)	1.25	1.00
	Glucose (1 per cent)	4.00	—
	Glucose + silicate	4.00	1.56
	Na lactate (0.1 per cent)	3.03	—
	Na lactate + silicate	3.03	1.66
Experiment II.	Silicate (27 p.p.m. Si)	1.21	1.00
	Glucose (1 per cent)	4.42	—
	Glucose + silicate	4.72	1.58
	Galactose (1 per cent)	2.25	—
	Galactose + silicate	2.64	1.49
Experiment III.	Silicate 27 p.p.m. Si)	1.27	1.00
	Succinate (0.1 per cent)	1.34	—
	Succinate + silicate	1.44	1.33
	Citrate (0.1 per cent)	1.30	—
	Citrate + silicate	1.46	2.00
Experiment IV.	Silicate (27 p.p.m. Si)	1.24	1.00
	Glucose (1 per cent)	3.50	—
	Glucose + silicate	3.80	1.77
	Fructose (1 per cent)	1.46	—
	Fructose + silicate	1.37	1.77
	Glycerol (1 per cent)	1.00	—
	Glycerol + silicate	1.36	1.69

6. Effect of Silicate on Exogenous Respiration of Si-Deficient Cells.—Since silicate stimulated endogenous respiration, it was of interest to determine what effect it would have on respiration of carbon compounds supplied externally. In order to obtain cells in a state in which they will respond to exogenous substrates, it is first necessary to deplete their endogenous reserves by subjecting them to a period of starvation in darkness (Lewin, 1952). Cells grown for 10 days in light and starved for 4 days in darkness responded to the addition

of various substrates by an increase in oxygen consumption. Respiratory quotients of Si-deficient, starved cells were measured in the presence and absence of silicate and glucose. Upon addition of 1 per cent glucose the quotients returned to values comparable with those of unstarved cells (Table I). Although the presence of silicate tended to raise the quotient during endogenous respiration it had evidently no effect on the R.Q. during the oxidation of glucose.

7. Effects of Exogenous Respiration on Silicon Uptake by Si-Deficient Cells.— The uptake of silicon has been shown to be intimately connected with endogenous respiration. In order to determine whether silicon uptake was stimulated during exogenous respiration, the effect of various substrates was tested on the respiration and silicon uptake of cells starved in the dark (see Table II). In general, it can be stated that when respiration was stimulated during oxidation of an exogenously supplied substrate, the uptake of silicon was also stimulated.

DISCUSSION

The uptake of silicon by diatom cells is an aerobic process and can be inhibited by respiratory inhibitors. Cyanide, fluoride, iodoacetate, arsenite, azide, and fluoroacetate, at concentrations which are inhibitory to respiration, are also inhibitory to uptake of silicon. Thus diatom cells behave like other plant tissues in which ion uptake and salt accumulation have been shown to be dependent on aerobic respiration. The process of silicon uptake tends to be completely inhibited by the highest concentration of inhibitors tested, whereas some respiration is resistant to inhibitor. Kelly (1953) observed a similar phenomenon in iodine uptake by *Ascophyllum*, and suggested that only a fraction of respiration was involved in iodine uptake. Likewise, in barley roots, Machlis (1944) observed that concentrations of cyanide or azide which completely inhibited the accumulation of bromide ions nevertheless permitted respiration to continue at one third of the control rate.

Organic substrates (glucose, fructose, glycerol, galactose, lactate, succinate, citrate) which stimulate respiration of cells starved in the dark also stimulate the uptake of silicon by Si-deficient, starved cells. Kelly (1953) found that organic substrates stimulated iodine uptake by segments of *Ascophyllum.*

2,4-Dinitrophenol is an inhibitor which impairs the energy-requiring functions of the cell by uncoupling oxidative phosphorylation from respiration. Robertson, Wilkins, and Weeks (1951) observed that in the presence of certain concentrations of DNP the accumulation of ions by carrot cells was inhibited although respiration was increased. Yemm and Folkes (1954) reported that DNP inhibited the assimilation of nitrogen by N-deficient yeast cells whereas oxygen uptake was increased in its presence. Since DNP inhibits uptake of silicon by *N. pelliculosa* at concentrations which stimulate respiration by 100 per cent, it appears that energy from respiration, perhaps in the form of

high energy phosphate, is required for the accumulation of silicon by diatom cells.

The respiration of Si-deficient diatoms was found to be stimulated when silicate was added. A similar response by cells deficient in some particular element has been frequently demonstrated. Yemm and Folkes (1954) observed that oxygen uptake by N-deficient *Torulopsis utilis* increased two- to threefold when $(NH_4)_2HPO_4$ was added. Syrett (1953) reported that assimilation of ammonia by nitrogen-starved *Chlorella* was accompanied by a sixfold increase in respiration rate. Silicate (20 p.p.m. Si) gave a 40 per cent increase in oxygen uptake by Si-deficient *N. pelliculosa.* However, after endogenous reserves had been depleted by starvation in the dark for 4 days the observed increase was only 20 to 25 per cent, which suggests that some intermediate formed in light may be necessary for the silicon to exert its effect on respiration.

The respiratory quotients of a few other species of diatoms have been measured. Barker (1935) obtained a value of 0.93 with fresh cells of *Nitzschia closterium*; even after 4 days' starvation in darkness the R.Q. remained around 1.00, indicating that carbohydrate was still available for respiration. For *Nitzschia palea* he obtained a quotient of 0.79, and concluded that fat dissimilation might be taking place. Wassink and Kersten (1945) measured quotients of 0.55, 0.35, 0.61, and 0.43 for *Nitzschia dissipata.* They concluded that these low values could be attributed to oxidation of a reduced substance, probably fat. On the basis of these "abnormal" respiratory quotients measured by Wassink and Kersten, Franck (1951) inferred that intermediate products of respiration in diatoms are quickly reduced photosynthetically in the light before they have a chance to evolve carbon dioxide. However, since these respiratory quotients were measured in darkness, this could hardly be a likely explanation for the low values obtained.

From the data reported in this paper it can be seen that the metabolic state of the cells can have a profound effect on the respiratory quotient. Cells of *N. pelliculosa* in an active state of cell division exhibited a quotient of 0.93. The quotients were lower (0.8 to 0.9) for cells that had ceased to divide and were accumulating a polyuronide capsule. Starved, non-capsulated cells gave a quotient of 1.05 during glucose oxidation, indicating that at least some of the substrate was being completely dissimilated to CO_2 and water. However, starved capsulated cells oxidized glucose with quotients of 0.7 to 0.8. The oxidation of some of the glucose to glucuronic acid, instead of completely to CO_2 and H_2O, could account for these lower quotients of capsulated cells.

Cells which had been actively dividing (*i.e.* which lacked capsules) showed, after a period of starvation in darkness, an endogenous R.Q. of 0.75, indicating that fat reserves were being oxidized. On the other hand, capsulated cells, which had ceased to divide prior to their being starved in darkness, had an endogenous R.Q. of 0.3. Such a low R.Q. might be attributed to an oxidation of

fat and, simultaneously, to uronic acid production from some of the intermediates so formed. Both of these processes would require an excess of oxygen uptake over carbon dioxide evolution. The fact that silicate raises the endogenous respiratory quotient of starved Si-deficient cells from 0.3 to 0.5 is difficult to explain.

Finally, certain observations on the effects of silicon on growth and respiration in *Chilomonas paramecium* (Mast and Pace, 1937) are of interest, in view of the fact that there is no evidence that the Cryptophyceae require or utilize silicon in their cell structure. It was found that 8×10^{-6} M Na_2SiO_3 stimulated growth rate and respiration by 24 per cent, although in the absence of sulfur silicon had only a slight effect. Mast and Pace concluded that silicon exerted its effect by catalyzing the synthesis of complex organic compounds.

SUMMARY

1. Evidence is presented that silicon uptake in the diatom *Navicula pelliculosa* is linked with aerobic respiration.

2. Cyanide, fluoride, iodoacetate, arsenite, azide, and fluoroacetate, at concentrations inhibitory to respiration, were also inhibitory to silicon uptake.

3. 2,4-Dinitrophenol (1 to 2×10^{-5} M) stimulated respiration by 100 per cent, but almost completely inhibited silicon uptake.

4. The respiratory quotient of non–Si-deficient cells decreased from 0.93 to 0.75 after 4 days of starvation in darkness. Glucose (1 per cent) raised the respiratory quotient of such starved cells to 1.05.

5. Silicate (20 mg. Si/liter) stimulated respiration of unstarved Si-deficient cells by about 40 per cent. The effect of silicate on the respiration of Si-deficient cells which had been starved in darkness for 4 days was less marked.

6. The respiratory quotient of Si-deficient cells decreased from 0.8–0.9 to 0.3 after 4 days of starvation in darkness. The addition of silicate to starved cells raised the quotient to 0.5. This represented a 25 per cent stimulation of oxygen uptake concomitant with a 90 per cent stimulation of carbon dioxide evolution.

7. Glucose (1 per cent) caused an increase of respiratory quotient in starved cells from 0.3 to 0.7–0.8. The addition of silicate had no effect on the R.Q. during the oxidation of exogenous glucose.

8. Substrates (glucose, fructose, galactose, lactate, succinate, citrate, glycerol), which caused a stimulation of respiration in starved cells, also stimulated silicon uptake by those cells. However, the stimulation of silicon uptake (50 to 100 per cent) was not proportional to the respiratory stimulation by these substrates (30 to 300 per cent).

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