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A Study of the Uptake of Silicon and Phosphorus by Wheat Plants, with Radiochemical Methods

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The limit of the classical gravimetric method for the determination of silicon is approximately 100–1000 $\mu\text{g.}$, weighed as silica. With the development of absorptiometric methods, which use either the yellow colour of the silicomolybdate complex or the blue colour obtained on reduction, the lower limit is approximately 10 $\mu\text{g.}$ A recent paper by Miller & Chalmers (1953) describes a sensitive microgravimetric method which is based on the precipitation of the quinoline salt of the heteromolybdate and gives a sensitivity of a few micrograms. The application of these methods in biological and medical investigations is somewhat restricted owing to the frequently encountered presence of phosphates, which form similar complexes with molybdate, and to the presence of other organic and inorganic substances which interfere with the methods. Though attempts have been made to overcome these difficulties, e.g. by bleaching the phosphate complex with citrate, oxalate or tartrate (Sesa & Rogers, 1954a), adding excess of phosphate (Krummholz, 1933), or by extracting with organic solvents (Wadelin & Mellon, 1953; Sesa & Rogers, 1954b), the methods are not applicable to the exact determination of very small quantities of silicon in the form of silica or silicate.

Holt & Yates (1953) have used radioactive silicon, ^{31}Si , to determine the silicon content of organs in

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mice and rats by injecting the animals with active silicate solution and following the distribution in the body for a period of about 6 hr.

Previous work on the uptake of silicon by plants (Hull & Morison, 1906; Jennings, 1919; Nemec, 1927; Whittenberger, 1945) deals with the absorption from soils by mature plants and the published figures only give a measure of the total silicon content in the later stages of development. It was hoped to show that silicon is readily absorbed in the very early stage of growth and to determine the rate of uptake over short intervals of time. It was also desirable to study the influence of added phosphate on the silica uptake.

There appears to be no published work in which radioactive silicon has been used to study the rate of silicon uptake by plants. Experiments are reported in this paper which show that it is possible to follow the uptake of silicon by wheat plants for at least 15 hr. by the use of radioactive silicon, even though the half-life of ^{31}Si is very short (157 min.) and the rate of uptake is comparatively small. The accuracy of the method is of the order of 0.5 $\mu\text{g.}$ of silica 6 hr. after taking from the pile.

To facilitate comparison, experiments are described in which radioactive phosphorus is used to determine the rate of absorption of phosphate by similar plants, and to show the influence of added silicate on the phosphate uptake.

METHODS

Preparation of ^{31}Si -labelled silicate solution. Rock crystal containing at least 99.95% of silica was powdered and irradiated in the Harwell pile for 3 days to produce ^{31}Si . Best results were obtained by irradiating the sample in an aluminium container rather than in a fused-silica tube because, when the silica tube was opened, fine particles from it sometimes contaminated the irradiated specimen and introduced traces of longer-lived isotopes of sodium and potassium. The purity of the specimens irradiated in aluminium containers was shown by determining the specific activity over several hours, from which the half-life was found to lie between 149 and 157 min. On account of the short half-life of ^{31}Si it was necessary to perform all operations with the minimum delay.

The specific activity of each supply of irradiated silica depended upon the pile factor used for each irradiation and upon the time interval which elapsed while the material was being transported from Harwell and prepared ready for use in the laboratory. It was therefore necessary to prepare a calibration curve of each consignment.

Irradiated silica (12.5 mg.) was fused with Na_2CO_3 , the melt dissolved in water and the volume made up to 1 l. Portions of this solution were withdrawn and made up to a standard volume of 10 ml. in the liquid counter designed by Veall (1948) and their activities determined with an EHT unit, scaler and dekatron-timing unit. In each case the actual time of counting was recorded so that allowance could be made for the loss of activity due to radioactive decay. Allowance was also made for lost counts and background counts in the usual manner.

Table 1 shows the counting rate and background-counting rate obtained with four consignments of irradiated silica.

The figures indicate that about 0.5 μg . of silica may be estimated 6 hr. after removal from the pile. The sensitivity will decrease with time and will be about 0.25 μg . 3.5 hr. and about 1 μg . 8.5 hr. after removing the silica from the pile. The loss in activity due to decay is partially compensated by the increase in the uptake of silica by absorption by the plant.

Germination of the wheat seeds. Wheat seeds (*Triticum*) were germinated in closed Petri dishes on wet filter paper for 3–4 days. The roots of the young plants were then inserted through holes in paper covering a beaker which contained nutrient solution (see Fig. 2). Care was taken to immerse only the roots. The nutrient solution consisted of 1.21 g. of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.275 g. of KCl, 0.275 g. of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.011 g. of NaCl, in 1 l., and was renewed three or four times.

Light and air were provided. After 3 weeks, plants which showed similar development were selected for the experiments.

Assay of the uptake of silica. After taking the plants from the culture beakers they were either (a) transferred to a beaker containing 400 ml. of the same nutrient solution equivalent to 12.5 mg. of active silica/l. or (b) washed free from nutrient solution and then placed in 400 ml. of an alternative solution containing 0.5 g. of KNO_3 , 0.275 g. of KCl, 0.011 g. of NaCl, in 1 l., to which was added the same amount of silica. Both solutions were adjusted to pH 5.5. The alternative solution was used to show that the activity found in the roots was not caused by precipitation of calcium and magnesium silicates, no detectable difference was found in the uptake rate from each solution. Throughout the experiments the illumination was kept constant at 500 lux.

After varying intervals, two or three plants were withdrawn from the solution and carefully washed with water to remove any adsorbed active material. They were then dried in a current of warm air, weighed (air dry), ashed at as low a temperature as possible, and then fused with 500 mg. of Na_2CO_3 in a platinum crucible. The resulting mass was dissolved in dilute HCl, transferred to the liquid counter and diluted to the standard volume. The proportion of silica taken up by the plants was determined by comparing the counting rates with those of standard solutions. Allowance was made for background, and lost counts were taken into consideration in the usual way.

Assay of the uptake of phosphate. A series of experiments was carried out to determine the rate of uptake of phosphate, by a similar method to that utilized in the silicate experiments. A sufficient amount of carrier-free ^{32}P as phosphate (obtained from the Radiochemical Centre, Amersham) was added to the nutrient or the alternative solutions containing 30 mg. of $\text{KH}_2\text{PO}_4/\text{l.}$, to give approximately the same initial specific activity as the silica solutions.

The proportion of phosphate taken up by the plants after varying intervals was determined by the same procedure, but no detectable difference was found in the uptake rate from each solution. The counting rates of the solutions were compared with standard solutions and allowance was again made for background and lost counts.

RESULTS

Uptake of ^{31}Si silicate without added phosphate. The absorption by wheat plants of ^{31}Si silicate from a solution free from phosphate ions was determined by the radiochemical method described

Table 1. Counting rate and background-counting rate of four consignments of irradiated silica

The solution (10 ml.) in the liquid counter contained 12.5 mg. of irradiated $\text{SiO}_2/\text{l.}$ The pile factor of each irradiation is shown, together with the time of determining the activity. In each case, the sample was withdrawn from the pile at approx. 7.30 a.m.

Date	Pile factor	Time (p.m.)	Background count (counts/min.)	Corrected count (counts/min.)	Calc. counts/min./ μg . of SiO_2	Ratio of counts/min./ μg . of SiO_2 to background
7 July	14	1.26	18	6407	51.3	2.85:1
14 July	12	2.17	20	7018	56.1	2.81:1
21 July	12	12.25	26	7310	58.5	2.25:1
11 August	12	12.23	23	6243	50.0	2.17:1

Table 2. Typical results of uptake of silica by wheat plants from solutions free from phosphates with ³¹Si

Plants were withdrawn from the solution and their combined leaf-root systems worked up separately as indicated.

Specimen no.	Time in soln.	No. of plants	Position of cut above seed (cm.)	System	Weight (mg.)	Counts/ min. (corrected)	Counts/ min./g. of plant	SiO ₂ /g. of plant (μg.)
C4	4 hr.	2	2.5	Leaves	792.5	1278	1610	31.4
				Roots	465.6	1540	3310	65.0
				Whole plant	1258.1	2818	2240	43.4
C9	11 hr. 50 min.	2	10.0	Leaves	435.4	1480	3400	66.0
				Roots	861.5	7900	11900	229.0
				Whole plant	1297.0	9380	7210	139.8

Fig. 1. Silica uptake by young wheat plants from the nutrient solution containing 12.5 mg. of silica/l. Uptake referred to 1 g. of plant, 18 days old: temp., 23°. ●, Solution free from phosphate; ○, solution containing 30 mg. of KH₂PO₄/l.; △, solution containing 75 mg. of suspended quartz/l.

above. The amount of absorbed silica/g. of plant was calculated after varying intervals (Fig. 1). The scattering of points may be caused by small deviations in the sizes of the roots and the leaves of the plants. The average uptake of silica by the wheat plants under the described conditions is seen to be 12.4 μg./g. of plant/hr.

To confirm that true absorption of the silica had taken place, a number of the plants were cut into two sections and the activities of both the root system and the leaves determined separately. Fig. 2 indicates the mounting and the cutting of the plants. It was found that the average content of

Fig. 2. Illustration of the setting up and cutting position of plant. The roots only are immersed in the radioactive solution.

silica/unit weight of the leaves was approximately half that of the roots. Some typical results are shown in specimen C4, Table 2.

Specimen C9 (Table 2) illustrates an experiment in which the cut was made 10 cm. above the paper (Fig. 2). It is clear that the amount of silica present in the upper portion is smaller, but nevertheless significant.

Uptake of [³¹Si]silicate in the presence of phosphate. The absorption of [³¹Si]silicate in the presence of phosphate by wheat plants taken from the same batch, at the same time and under the same

conditions as those used in the previous experiments, is shown in Fig. 1. The average uptake of silica can be seen to be $11.2 \mu\text{g./g. of plant/hr.}$ This represents a decrease of 10 % due to the presence of an equimolar amount of P_2O_5 .

Uptake of silicon from solutions containing suspended quartz. Irradiated powdered quartz (75 mg.) was dispersed in 1 l. of nutrient solution and the plants were introduced as described above, the solution being occasionally stirred. The average size of the quartz particles was 6μ . The uptake of silica from these solutions is shown in Fig. 1 and, on an enlarged scale, in Fig. 3. It will be seen that the rate of uptake is very small compared with that obtained with the $[\text{Si}]$ silicate and is of the order of $0.3 \mu\text{g./g. of plant/hr.}$

Activity was again detected in the leaves and roots, and after several hours the slope of the uptake curve became steeper. Preliminary experiments to be described fully elsewhere indicate that this may be due to an increase in the rate of dissolution of the silica. Alternatively, Holzapfel & Engel (1956) suggest that microcrystalline quartz particles are absorbed by the roots of the seedlings and ultimately migrate through the plant and enhance the original silica content due to silicate.

Uptake of phosphate without added silicate. The absorption of phosphate by wheat plants from solutions free from added silicate was determined by the radiochemical method outlined above and the uptake of phosphate calculated as P_2O_5 , after various intervals, is summarized in Fig. 4. An average uptake of $16.5 \mu\text{g. of P}_2\text{O}_5/\text{g. of plant/hr.}$ can be calculated. Similar values were obtained from both nutrient and alternative solutions to which had been added similar amounts of phosphate. The activity was again distributed throughout the leaves and the root systems.

Uptake of phosphate in the presence of silicate. The absorption of phosphate in the presence of

Fig. 3. Silica uptake by young wheat plants from the nutrient solution containing 75 mg. of suspended quartz/l. Uptake referred to 1 g. of plant, 18 days old; temp., 23° . ●, Solution free from phosphate; ○, solution containing 30 mg. of $\text{KH}_2\text{PO}_4/\text{l.}$

$[\text{Si}]$ silicate was followed with plants of the same lot and under the same conditions as those used in the experiments reported in the previous section.

The average uptake calculated from this curve (Fig. 4) is $22.2 \mu\text{g. of P}_2\text{O}_5/\text{g. of plant/hr.}$, an increase of 25 % over the solutions without silicate.

Statistical analysis of results. The regressions of SiO_2 uptake on time have been fitted by the method of least squares with the following results (the lines are shown in Fig. 1):

	Without phosphate	With phosphate
Equation of regression	$y = 12.4x + 0.8$	$y = 11.2x - 1.5$
Slope of regression line	12.39	11.22
Standard error of slope	0.75	0.58

The t test for the difference between the slopes gives the following value:

$$t = 12.39 - 11.22 / \sqrt{(0.75^2 + 0.58^2)} = 1.3$$

with 14 degrees of freedom. This corresponds to a probability of about 0.2 (20 %) that the difference is only caused by statistical scattering. Thus although the difference between the slopes of the two lines in Fig. 1 is not statistically secured, there is a typical trend to form a difference.

Fig. 4. Uptake of phosphate calculated as P_2O_5 by young wheat plants from the nutrient solution containing 30 mg. of $\text{KH}_2\text{PO}_4/\text{l.}$ Uptake referred to 1 g. of plant, 19 days old; temp., 23° . ●, Solution free from silicate; ○, solution containing 12.5 mg. of $\text{SiO}_2/\text{l.}$

Similarly, the regressions of P_2O_5 uptake on time have been fitted and the lines are shown in Fig. 4:

	Without silicate	With silicate
Equation of regression	$y = 16.5x + 2.4$	$y = 22.2x - 7.2$
Slope of regression line	16.50	22.24
Standard error of slope	1.52	2.16

The t test gives a value of 2.2 with 9 degrees of freedom, corresponding to a probability of about 0.05 (5%). These figures tend to show that the difference in the slopes of the two lines is statistically significant.

DISCUSSION

The results show that the uptake of silicon by young wheat plants and calculated as silica is of the same order as that of phosphorus calculated as P_2O_5 . However, when it is taken into consideration that P_2O_5 contains two atoms of phosphorus, the uptake ratio, i.e. the ratio of the number of silicon atoms to phosphorus atoms, is about one to three.

From the figures given in Table 2 it can be concluded that there has been true absorption of [^{31}Si]silicate. The solution contained only 12.5 μg . of silica/ml., yet after approximately 12 hr. the concentration of silica in specimen C9, for example, had increased sixfold in the top leaves, 20-fold in the roots and an average of tenfold in the whole plant.

The average uptake of silicon and phosphorus/g. of plant under the condition of the experiments and calculated from regression forms are: SiO_2 from solutions free from phosphate, 12.4 μg /hr.; SiO_2 from solutions containing phosphate, 11.2 μg /hr.; P_2O_5 from solutions free from silicate, 16.5 μg /hr.; P_2O_5 from solutions containing silicate, 22.2 μg /hr.

The presence of phosphate appears to have a quenching effect on the uptake of [^{31}Si]silicate though this is not yet statistically secured. This may be explained by assuming that the extra amount of silica which is taken up in the absence of phosphate is used instead of phosphate (cf. Holzapfel, 1951, 1954). On the other hand, the uptake of phosphorus is slightly stimulated in the presence of silicate. These results appear at first to be conflicting, but it will be noticed that the total silicate and phosphate uptakes are approximately constant. Consequently, a decrease in the silicate uptake will cause an increase in the phosphate uptake and vice versa, and the close connexion of their metabolism as observed in other systems becomes evident.

The influence of silicate on the uptake of phosphorus in the early stages of growth and under the conditions given above is, however, not as significant as that reported in an early publication (Hull & Morison, 1906) when the effect of the combined addition of phosphate and silicate on the field crop yield of cereals was investigated.

Further experiments are planned to investigate the influence of other compounds on silicon uptake of different model organisms and to evaluate the role of silicon in plant and animal metabolism. It is hoped that later when the longer-lived radioactive isotope of silicon becomes available long-term experiments may be undertaken.

SUMMARY

1. The described technique involving the use of ^{31}Si is able to determine silica in the form of silicate or SiO_2 with an accuracy of 0.5 μg .

2. It has been shown that even young wheat plants have a significant capacity to absorb silica. After only half an hour (the shortest duration of experiment) considerable amounts were detected in various parts of the plants. The distribution of the absorbed silica over the whole plant was followed.

3. Added phosphate slightly depressed the uptake of silicate, and added silicate enhanced the absorption of phosphate, showing the close relation of their metabolism.

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