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THE ABSORPTION OF INORGANIC IONS BY CHLORELLA PYRENOIDOSA [1,2]

H. J. KNAUSS AND J. W. PORTER
THE BIOLOGY SECTION, RADIOLOGICAL SCIENCES DEPARTMENT,
GENERAL ELECTRIC COMPANY, RICHLAND, WASHINGTON

In the course of experiments on the effect of radioactive elements on the growth of *Chlorella pyrenoidosa*, Porter and Knauss (9), determinations were made of the content of a radioactive element in algal cells when the ratio of that element to its stable isotope was kept constant, but the total concentration of the two isotopes in the solution was varied. Calculations were then made, assuming no isotope effect, of the total contents of the two isotopes in the algal cells. These determinations were made for the elements P, S, Ca, Fe, Mn, Zn, Cu, and Sr.

A limited amount of information is available on the effect of the nutrient concentration of an element on the uptake of that element by algae. Scott (11) has reported that the ratio of uptake of calcium and magnesium by Chlorella depends upon the ratio of these two elements in the nutrient solution. Scott (12) has also studied the behavior of phosphate-deficient cells during a restoration period when adequate phosphate was supplied to the nutrient medium. He found the phosphate uptake to be proportional to the nutrient concentration only at levels that were limiting for growth.

More information on the effect of nutrient concentration of an element on its uptake has been obtained through studies with higher plants and diatoms. Beckenbach et al (1), in making a statistical analysis of the relationship between the ionic content of the culture solution and the element content in corn plant tissues, found the most important single factor affecting the absorption and accumulation of an element in plant tissues to be its absolute concentration in the nutrient solution. Similar results were reported by Tidmore (14) on corn, sorghum, and tomatoes, and by Beeson et al (2) on tomato plants. Recently, Rediske and Selders (10) reported the uptake of strontium by plants to be proportional to the concentration of this element in the nutrient solution. Goldberg et al (5), working with diatoms, found the phosphate uptake to be proportional to the concentration in the medium over the range 0.5 to 3.5 μg/l. Similar findings were reported by Ketchum (7).

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[2] This paper is based on work performed under Contract No. W-31-109-Eng-52 for the Atomic Energy Commission.

EXPERIMENTAL METHODS

The strain of Chlorella used was American Type Culture No. 7516. Inocula to use in experiments were obtained by the same procedure reported previously (9).

Seven and one half ml of nutrient solution (KNO_3, 3.0 gm/l; $MgSO_4 \cdot 7\,H_2O$, 4.8 gm/l; $Ca(NO_3)_2$, 1.0 gm/l; KH_2PO_4, 3.0 gm/l; $MnSO_4 \cdot H_2O$, 2.0 mg/l; H_3BO_3, 2.0 mg/l; $CuSO_4 \cdot 5\,H_2O$, 0.2 mg/l, and $ZnSO_4 \cdot 7\,H_2O$, 0.2 mg/l), less the element being investigated, were added to 20×150 mm culture tubes. Varying amounts of the element being studied and the appropriate amounts of water were added to each tube. After sterilization each tube received 0.2 ml of a sterile iron solution (1 gm $FeSO_4 \cdot 7\,H_2O$/500 ml), 1.0 ml of inoculum (an optical density of 0.60 at 660 mμ/18 mm tube, approximately 17×10^6 cells/ml) and amounts of a solution containing a radioactive isotope of the element under study to yield a final specific activity of one mc/gm of that element. In experiments on the absorption of copper the specific activity was 3 to 8 mc/gm of element. Radioisotopes used were P^{32}, S^{35}, Ca^{45}, Fe^{59}, Mn^{54}, Cu^{64}, Zn^{65}, and Sr^{90}. The final volume of solution was 18.2 ml, and the final pH was 4.2 to 4.6. Duplicate tubes were set up for each concentration of the element under study. Control tubes, lacking in radioactivity, were also inoculated in each experiment.

The cotton plugs of the tubes were replaced with sterile 0.5 mm capillary tubing wrapped in cotton. The cells were then aerated through these tubes at a rate of approximately 100 ml per minute with a mixture of 8 to 9 % CO_2 in air. The gas mixture was saturated with moisture by passing through water. Light intensity (white fluorescent) incident to the tubes during the growth period was 300 to 500 fc.

After the growth period, usually 72 hours, the suspensions of cells were made to a common volume such that light absorption readings could be made. These were then made on the cell suspensions in 18 mm tubes at 660 mμ with a Beckman Model DU Spectrophotometer. From these readings the dry weight for each sample was determined by reference to a previously determined relationship between dry weight and optical density (fig 1).

The procedure of a typical experiment in which

the relationship between dry weight and optical density was determined was as follows. A liter Erlenmeyer flask containing 500 ml of nutrient medium was inoculated with 32 ml of a washed suspension of Chlorella that had an optical density of 0.61 in an 18 mm tube at 660 mμ, a cell number of 16.6×10^6/ml and a dry weight of 0.15 mg/ml. The solution was aerated with a mixture of 8 to 9 % CO_2 in air at such a rate as to prevent sedimentation of the cells.

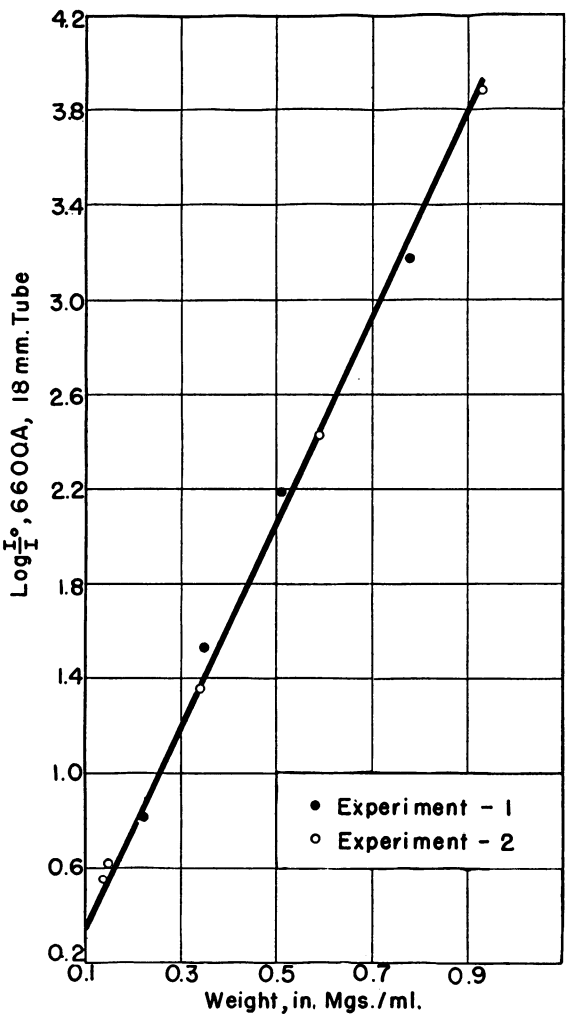

FIG. 1. The relationship between optical density and dry weight of Chlorella.

Light intensity at the surface of the solution was approximately 200 fc. Temperature was approximately 25°C. Daily, after inoculation, aliquots were taken for dry weight and light absorption determinations. When the optical density of the solution exceeded 1.00, the suspension was diluted to an optical density of approximately 0.60. Weight of the samples was determined as follows. Twenty-five ml of the original suspension was centrifuged at $10,000 \times g$, then 3 times resuspended with distilled water and

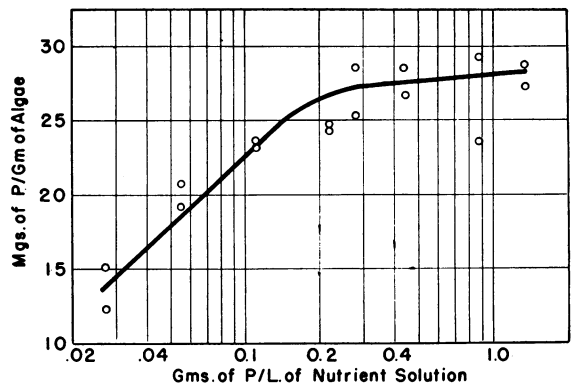

FIG. 2. The phosphorus content of Chlorella grown at different nutrient concentrations of phosphorus.

centrifuged. The residue was transferred with a small amount of water to a weighed boat. The sample was dried on a microheater with a stream of nitrogen, then in a vacuum oven at 62°C for 2.5 hours. Weighings were made on an analytical balance. The data of figure 1 are those obtained in 2 experiments.

Radioactive algal cells and nutrient solution were transferred (1-ml aliquots) from each tube on which light absorption readings were made to 1-inch stainless steel plates for counting. Orthodox beta counters employing a Geiger-Müller tube were used for counting radioactive P, S, Ca, Fe, Cu, and Sr. A scintillation counter was used to count radioactive Zn and Mn. Calculations were then made, using the appropriate factors for geometry, self-absorption, etc, of the radioactivity and the specific activity of the solution.

The remaining cells in each tube were centrifuged at aproximately $10,000 \times g$ for 2 minutes, and the supernatant solution was decanted. The cells were resuspended in 10 ml of nutrient solution that contained the element in question at a concentration of 0.1 % (except iron which was held at a concentration of 0.04 %). The cells were again centrifuged, the supernatant solution decanted, and the cells suspended in a volume of 10 ml. A 2.0 ml aliquot from each suspension was transferred to a 1-inch plate for counting. Calculations were then made of the

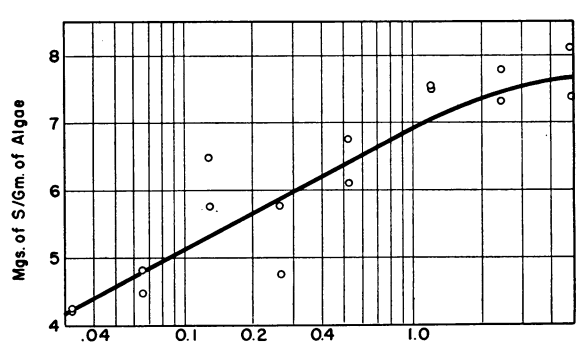

FIG. 3. The sulfur content of Chlorella grown at different nutrient concentrations of sulfur.

TABLE I

A SUMMARY OF THE GROWTH AND ELEMENT ABSORPTION BY CHLORELLA

ELEMENT	MASS OF CELLS AFTER 72 HOURS	k VALUES (y = kx + C)	RANGE IN NUTRIENT CONCENTRATION	RANGE IN CONCENTRATION IN CHLORELLA	NUTRIENT ELEMENT REMOVED BY CELLS
	gm/l		*mg/l*	*mg/gm*	*%*
Fe	0.389 ± 0.016[a]	2.19 ± 0.30	0.104–13.31	0.237 –34.53	85.04 ± 10.82
Mn	0.285 ± 0.021	2.49 ± 0.12	0.165–10.56	0.241 –25.95	70.45 ± 6.82
Ca	0.325 ± 0.025	0.0085 ± 0.0016	4.28 –547.8	0.059 –4.00	0.28 ± 0.06
Sr	0.942[b] ± 0.037	0.0075 ± 0.0010	0.550–70.4	0.0044–0.507	0.70 ± 0.09
Cu	0.434 ± 0.024	0.65 ± 0.17	0.017–0.281	0.0082–0.337	27.65 ± 12.02
Zn	0.702[c] ± 0.099	0.14 ± 0.04	0.021–0.856	0.0038–0.093	9.27 ± 3.44
P	0.576 ± 0.041	16.7[d]	27.5 –200	13.5 –23.4	24.4 — 11.9[e]
P	0.576 ± 0.041	.....	200 –1375	23.4 –27.6	.....[f]
S	0.969[b] ± 0.104	1.77[d]	31.9 –1020	4.23 –7.45	14.8 — 0.75[e]
S	0.969[b] ± 0.104	.....	1020–4078	7.45 –7.67	.....[g]

[a] All ± values are standard errors at 95 % confidence level.
[b] 120 hour growth period.
[c] 96 hour growth period.
[d] y = k log x + C; for (P), y = 16.7 log x – 10.53; for (S), y = 1.77 log x + 1.57.
[e] Range of percent of element removed.
[f] Concentration in cells remained constant at approximately 27.5 mg/gm above a nutrient concentration of 200 mg/l.
[g] Concentration in cells remained constant at approximately 7.5 mg/gm above a nutrient concentration of 1020 mg/l.

radioactivity and the total content of the stable and radioactive isotopes in the cells. Corrections were made for the decay of the radioisotopes within the algal cells during the time elapsing between the determination of specific activity of the nutrient solution and the radioactivity of the cells.

In experiments with Sr^{90}, which forms a radioactive decay equilibrium mixture with Y^{90}, decay curves were obtained for the radioactivity in cells and in cells plus nutrient solution. Counts obtained at equilibrium, 30 days after harvest of the cells, were used in calculating the radioactivity and the amount of Sr present in the cells.

In experiments with Fe^{59}, carrier iron was supplied as either Fe^{++} or Fe^{+++}. Absorption of this element by the algae was the same in either case.

RESULTS AND DISCUSSION

The results of the various experiments are presented in figures 2 to 9 and in table I. These figures, and the table, show that the amount of an element absorbed by the algae is a function of the element and its concentration in the nutrient solution. The

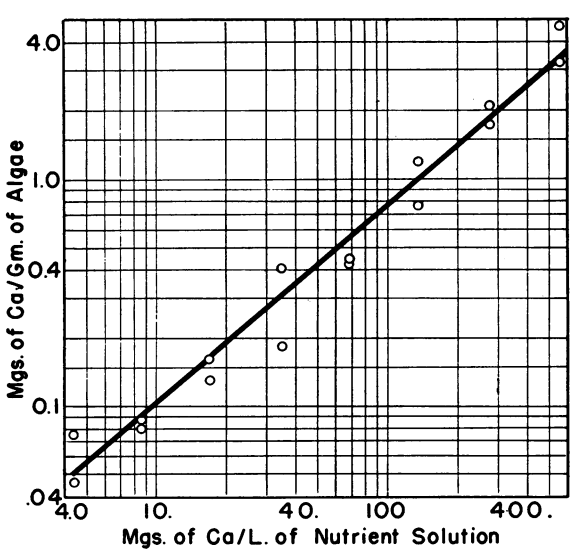

FIG. 4. The calcium content of Chlorella grown at different nutrient concentrations of calcium.

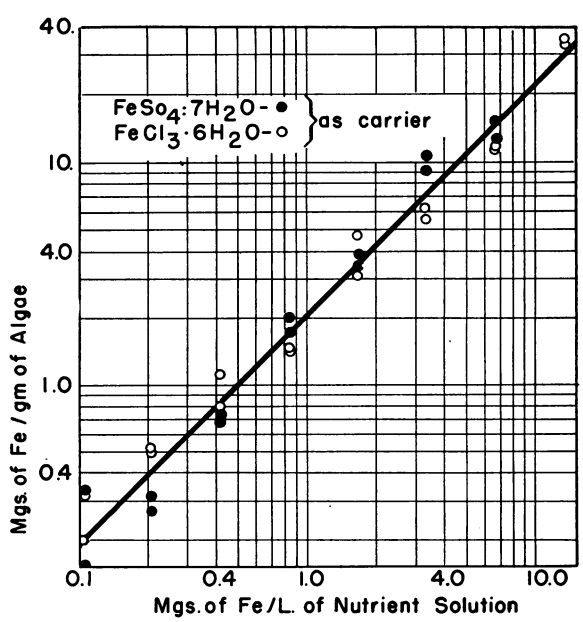

FIG. 5. The iron content of Chlorella grown at different nutrient concentrations of iron.

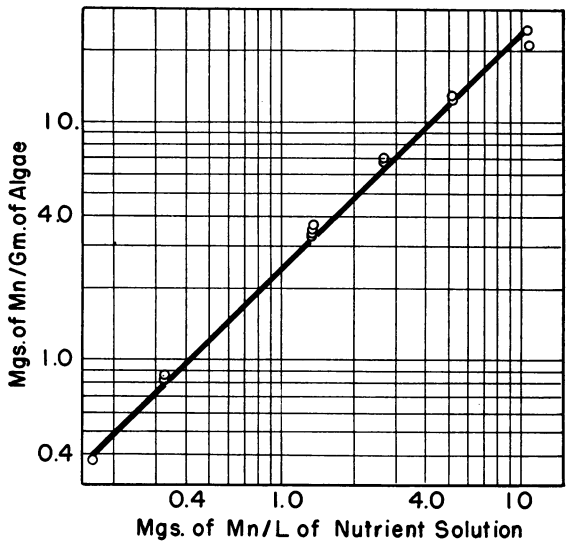

FIG. 6. The manganese content of Chlorella grown at different nutrient concentrations of manganese.

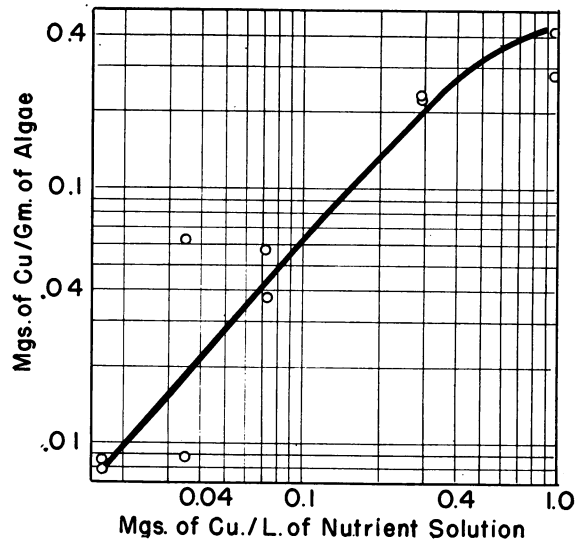

FIG. 8. The copper content of Chlorella grown at different nutrient concentrations of copper.

absorption of iron and manganese from the nutrient solution by Chlorella was much greater per equal quantity of element than it was for the other elements used. In fact, since the absorption of these elements was so great, we are inclined to believe that most of the iron and manganese was adsorbed on the cell surfaces, in complexes which did not exchange with nutrient element during the washing procedure, rather than absorbed into the cell. Also noteworthy are the large quantities of phosphorus that were absorbed into algal cells, and the small proportions of nutrient sulfur, calcium and strontium that were absorbed by the cells.

The effect of concentration of a nutrient element on the absorption of that element by Chlorella is also interesting. Phosphorus and sulfur contents of algal cells remained constant at the higher levels of nutrient concentration of these elements. At lower levels of nutrient concentration, absorption into the cells was equal to a factor (k) times the log of the nutrient concentration plus a constant, (C). Absorption of other elements into the algal cells was equal to a constant (k) times the nutrient concentration plus a constant, (C).

Growth of the cells (table I) in experiments with different elements varied, but the growth within an experiment did not indicate limiting or toxic element concentrations. Whatever caused variation in growth between experiments was common to each tube in an experiment. Concentrations of elements within cells at growth-limiting levels of nutrient concentration were not determined.

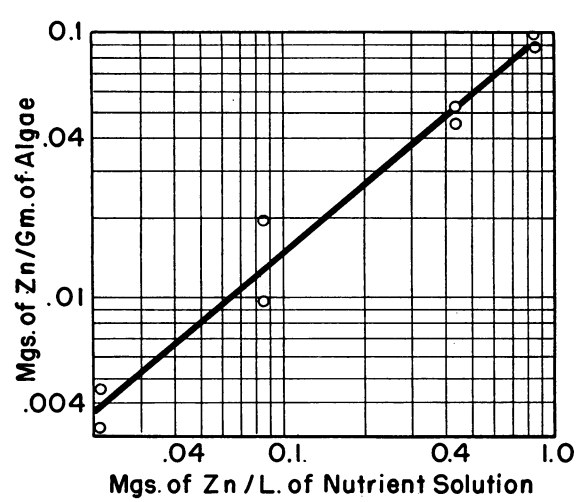

FIG. 7. The zinc content of Chlorella grown at different nutrient concentrations of zinc.

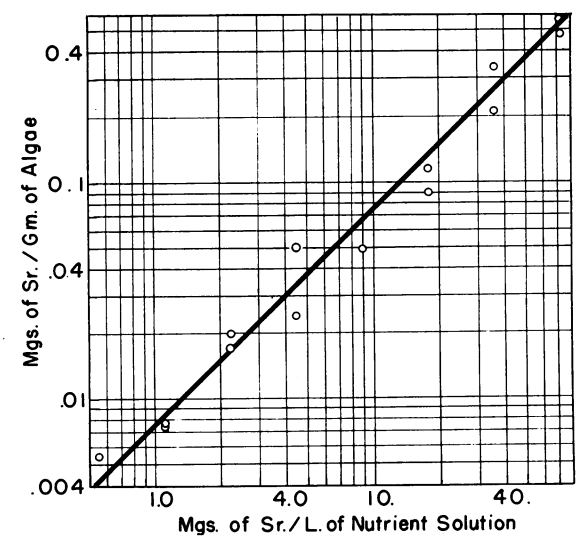

FIG. 9. The strontium content of Chlorella grown at different nutrient concentrations of strontium.

The tremendous differences in absorption of elements by Chlorella suggests that absorption (and adsorption) pathways of elements entering the cell may be quite different. If so, then a considerable number of enzymes would be involved in elemental absorption, and a considerable variation in elemental absorption could be effected by varying factors which affect the rates of reaction of these enzymes. A factor of paramount importance in determining the amount of an element absorbed by cells is the concentration of this element in the nutrient solution; and more important is the fact that the uptake of elements by Chlorella has been related mathematically to nutrient element concentration, other factors kept constant. An example of the remarkable precision with which the forces within a cell work, and the effect of changing nutrient concentration on this system, is seen in the uptake of calcium and strontium by Chlorella cells. The k values for these elements, which are quite similar chemically, are the same even though nutrient concentrations of these elements were quite different.

Several sources of error exist in tracer experiments which, like those reported in this paper, are designed to determine the actual content of an element in cells. These include the possibility of (1) differences in valence state or compound between the tracer and the carrier, (2) radiochemical impurities, (3) an isotope effect, (4) selective adsorption of radioisotope on the walls of the container, (5) exchange between the radioisotope of the cells and the carrier of the solution during the washing process, and (6) adsorption of the radioisotope on the surface of the cells. By far the most difficult sources of error to cope with are (4), (5), and (6). In our experiments (4) was found to be unimportant. Activity counts made on the culture solution, when corrected for decay, checked quite closely with the activity added. Considerable disagreement exists in the literature as to the importance of (5) and (6). This difference seems to be attributable to (1) the method (tracer or non-tracer) used and (2) the quantity of the element in the nutrient solution. Scott (13), using non-tracer methods, found that mineral constituents were not removed by suspending Chlorella cells in distilled water. He also found that potassium is not removed by centrifuging and resuspending the Chlorella 6 times in potassium-free Detmer's medium. Goldberg (5), using tracer methods, found that at a minimal concentration in the nutrient solution (approximately 0.5 μg P/l), the phosphorus present in diatom cells is strongly bound and is not removed by permitting the cells to stand three weeks in suspension. He also found, however, that when the cells were grown at higher concentrations of phosphorus, washing removed a considerable portion of the element. The latter results are generally in agreement with those of Gest and Kamen (4) who found, by radiochemical methods, that Chlorella cells grown in a solution containing the amount of phosphate recommended for optimum growth, contain excess inorganic or highly labile phosphate. This phosphate can be washed from the cells, but the phosphate present in cells grown in a medium of low phosphate content cannot be so removed.

As a check on the effect of washing cells with a stable isotope on the removal of the radioisotope, algae were grown in the presence of several levels of phosphorus and then washed 0, 1, 2, and 3 times with a solution containing 0.1 % of stable phosphorus. The results presented in table II indicate that 1 wash did not remove appreciable quantities of P^{32} from cells grown in the presence of low and medium concentrations of phosphorus. Some P^{32} was removed from those cells grown at the higher levels of nutrient phosphorus. Additional washes did not materially change the phosphorus content of the cells, except at 3 lower levels of nutrient phosphorus content. The procedure of a single wash of the cells with a solution of the nutrient under study was then arbitrarily adopted as one which would give most accurate values for elemental content within the cell structure.

TABLE II

THE PHOSPHORUS CONTENT OF ALGAL CELLS WASHED 0 TO 3 TIMES WITH A SOLUTION CONTAINING 0.1 % OF PHOSPHORUS

NUTRIENT CONC. OF P	P CONTENT, MG/GM ALGAE			
	NUMBER OF WASHES			
MG/L	0	1	2	3
2.75	7.5	7.0	5.4	5.0
5.50	16.6	15.8	14.5	10.6
11.00	38.1	38.0	34.0	25.7
22.00	46.5	44.4	42.8	28.8
44.00	36.3	33.5	34.0	32.8
88.00	39.1	37.8	33.0	33.2
176.0	39.9	34.5	35.4	33.5
352.0	35.2	21.7	29.2	29.4
704.0	43.6	36.4	37.5	33.4
1408.0	43.4	27.1	26.0	27.8

In comparing the values reported in this paper with those of others it was noted that our values for calcium and iron agree with those found in the literature (8, 11, 13). Our values for phosphorus are, however, 10 to 100 times those reported by Scott (11, 12, 13), but they are within the range of 1 to 4 times those reported for bean plant leaves (3). No explanation, other than a difference in method, is immediately apparent for the difference between our results and those of Scott.

The fact that the absorption of an element by algae is a function of the concentration of that element in the nutrient solution has importance in research designed to improve the control of hazards from radiations and radioactive elements. By controlling the quantity of element in the nutrient solution and the ratio of radioisotope to stable isotope, it should be possible to obtain algal cells which will have the same quantity of radioactivity per mass of algal cells, but a difference in the number of disintegrations in vitally important compounds (particu-

larly enzymes and genes) of the cells. This is essentially the same technique as employed by Hungate and Mannell (6) in studies with Neurospora. It should then be possible through subsequent studies of growth rate, numbers of killed cells, and numbers of mutations, to determine the relative hazard of transmutation of radioelements in algae.

Summary

The isotope method was used to determine the quantities of an element present in Chlorella when the nutrient concentration of that element was varied. Elements used in these studies were P, Ca, S, Fe, Mn, Zn, Cu, and Sr.

The absorption, by the algae, of all elements except P and S was directly proportional to the concentration of that element in the nutrient solution. The quantities of P and S in the algal cells were constant when the cells were grown in the higher nutrient concentrations of these elements.

A brief discussion is presented of: 1) the sources of error existent in the isotope method of determining the elemental content of algae; 2) the effect of the nutrient concentration of an element on its absorption by algae.

The authors wish to acknowledge the technical assistance of Mrs. D. G. Watson. The assistance of members of the Biological Services Unit of this Section in preparing and calibrating solutions of the isotopes and in counting samples is also gratefully acknowledged.

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THE INFLUENCE OF HYDROGEN ION CONCENTRATION ON CATION ABSORPTION BY BARLEY ROOTS [1,2]

HUSSEIN FAWZY,[3] ROY OVERSTREET, AND LOUIS JACOBSON

Department of Soils and Department of Plant Nutrition, College of Agriculture, University of California, Berkeley, California

Hoagland and Broyer (2) working with excised barley roots and Arnon, Fratzke, and Johnson (1) working with intact plants demonstrated a pronounced effect of certain concentrations of hydrogen ion on mineral absorption.

In a former research of this laboratory dealing with the absorption of potassium by barley roots (3), it was postulated that the absorption process involves the following reversible reaction,

$$HR + K^+ \rightleftarrows KR + H^+$$

where HR represents a metabolically produced cationic binding substance and KR, a labile organic complex of potassium. To a large extent, the experiments were designed to study the reverse of this reaction; that is, the effect of hydrogen ion on the absorption of potassium by roots.

In a number of respects the results were con-

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[2] This paper is based on work performed under contract No. AT-(11-1)-34, project 5, with the Atomic Energy Commission.

[3] Present address: Faculty of Science, Fouad I University, Cairo, Egypt.