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Strontium and Calcium Uptake by the Green Alga, Oocystis eremosphaeria

Abstract. *The uptake of calcium and strontium by a green unicellular alga cultured in media containing these elements in concentrations found in nature is directly proportional to the concentration in the media. Variation in the concentration of either element has a slight inverse influence on the uptake of the other. The increase of strontium uptake when the calcium concentration is very low indicates that strontium is substituted for calcium when calcium is limited and suggests that the alga requires either element.*

The fact that ^{90}Sr is a potentially dangerous contaminant in an environment has resulted in many investigations of both its biogeochemistry and its metabolism by plants and animals (*1*, *2*). Many of these studies have centered on the relation and similarity between Sr and Ca metabolism. As a result, ^{90}Sr-Ca ratios have been used widely to predict or interpret the behavior of ^{90}Sr in the environment. However, in many of the otherwise controlled experiments the Ca and Sr compounds used in media or diet preparations probably contained significant amounts of the complementary element as an impurity (*3*). Thus study in the lower range of Sr concentrations in the environment has been difficult because Sr-free calcium compounds are not readily available.

The object of this investigation was to study, under rigorously controlled chemical conditions, the uptake of Sr and Ca by an alga, in order to obtain information regarding the fate of Sr, especially ^{90}Sr, in an aquatic environment, and to attempt to interpret Sr uptake with regard to environmental Sr concentrations rather than Sr-Ca ratios. A green, unicellular alga, *Oocystis eremosphaeria*, was used. Concentrations of Ca and Sr in the media were selected to cover the usual range found in freshwater environments. Stock algae were cultured in modified Hoagland's solution, and cells were transferred, after centrifugation, to a series of 250-ml erlenmeyer flasks. The conditions in the flasks—fluorescent light (4400 lu/m^2, 12-hour photoperiod), temperature (29°C), solution volume (150 ml), and nutrients other than Sr and Ca—were kept constant. The solutions were aerated and agitated by a filtered stream of air bubbles. The series of flasks was established according to a factorial design (3 Sr × 3 Ca) with two replications, thus giving nine combinations of Sr and Ca in the media. Strontium concentrations were 1.5×10^{13}, 15×10^{13}, and 150×10^{13} atoms per milliliter, and Ca concentrations were 1.5×10^{16}, 15×10^{16}, and 150×10^{16} atoms per milliliter. All chemicals used for media preparation were cross-checked for Ca and Sr impurities by the Analytical Chemistry Division of Oak Ridge National Laboratory, and certain Ca compounds were specially purified to eliminate Sr impurities; the Sr impurity of the $CaCO_3$ used in the study was reduced to 1.8 μg of Sr per gram of $CaCO_3$.

Immediately after the flasks had been inoculated with algae, 0.116 μc of carrier-free ^{85}Sr and 0.124 μc of ^{45}Ca, with negligible carrier (1.5×10^{13} atoms of stable Ca), were added to each flask as tracers. Samples of algae were taken at 1, 4, 24, 48, 72, 144, and 215 hours during the first run of the study and at 1, 4, 24, 48, 96, 168, 240, 360, 552, and 840 hours during the second run. Samples were collected by pipette, and the algae were removed with membrane filters (filter area, 2.54 cm^2; pore diameter, 0.45 μ) and then rinsed on the filters with tracer-free medium. The filters bearing the algae were oven-dried at 35°C and weighed; the tare weight of the filters was subtracted to obtain the dry weight of the algae. The filters were glued to counting planchets and counted for ^{45}Ca and ^{85}Sr, respectively, in gas-flow and gamma scintillation counters. The volume of the algal samples was maintained at a dry weight below 1.3 mg (0.5 mg/cm^2) to keep self-absorption of beta particles at negligible levels. After correcting the radioisotope counts for background and counter efficiencies, and on the assumption of no discrimination between radioisotope and stable isotope by the algae, the uptake of stable Ca and Sr was calculated from specific activities; for example,

$$\text{Stable Sr}_{\text{al}} = \frac{(\text{Sr}^{85}{}_{\text{al}})\ (\text{stable Sr}_{\text{med}})}{\text{Sr}^{85}{}_{\text{med}}}$$

where the subscripts *al* and *med* represent algae and media.

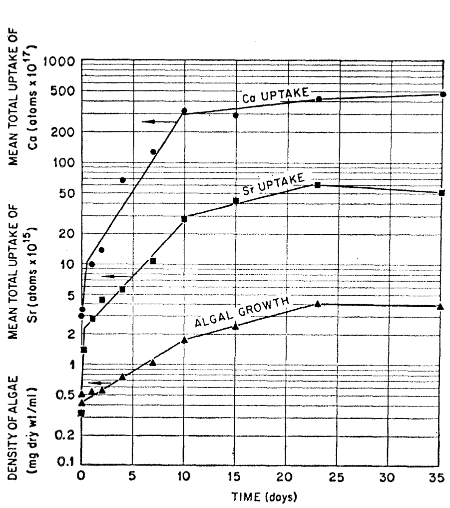

Fig. 1. Mean accumulated uptake of Sr and Ca by algae, and mean population growth of algal cultures (arrows indicate proper legend on ordinate for each curve).

Figure 1 shows the uptake of the two elements and the growth of algal populations during the second run of the study. Results for the first run were similar but less complete. Each point in Fig. 1 is the average of results from samples from all 18 flasks. Since the uptake of either element was dependent on its concentration in the medium, the points are not true averages, but are only intended to demonstrate the relation of the uptake to the growth. The algae demonstrated, at all concentrations of media, a rapid uptake of both elements during the first 24 hours, indicating a large initial uptake, probably exchange of both elements between cells and media. Uptake of both elements continued at a rate slightly greater than the population growth rate during the period from 24 to 240 hours. After 240 hours, the uptake paralleled the growth rate of the algae, indicating equilibrium between cellular and media concentrations. Three-dimensional graphs of the uptake of Sr and Ca by *O. eremosphaeria* appear in Figs. 2 and 3. The plotted points are the averages of samples from each pair of flasks from near equilibrium until termination of both runs of the study. Thus each point is the average of duplicate samples taken at 144 and 216 hours during the first run, and at 168, 240, 360, 552, and 840 hours during the second run. The response plane for Sr (Fig. 2) reveals that uptake is directly proportional to Sr in the medium, with enhanced uptake at very low media concentrations of Ca. At each concentration of Ca, the Sr uptake increased about nine times with each tenfold increase in the concentration of Sr in the medium. The response plane for Ca (Fig. 3) was primarily a function (natural logarithm) of the Ca concentration in the medium, with a slight inverse influence by Sr in the medium. Regression formulas satisfying the response planes are:

$$\ln \mathrm{Sr}_{\mathrm{uptake}} = 0.693 + (0.990 \pm 0.017) \ln \mathrm{Sr}_{\mathrm{input}} - (0.098 \pm 0.017) \ln \mathrm{Ca}_{\mathrm{input}}$$

$$\ln \mathrm{Ca}_{\mathrm{uptake}} = 0.432 + (0.932 \pm 0.058) \ln \mathrm{Ca}_{\mathrm{input}} - (0.013 \pm 0.058) \ln \mathrm{Sr}_{\mathrm{input}}$$

where Sr uptake is in units of 10^{12} atoms per milligram of algae (dry wt.), Ca uptake in units of 10^{15} atoms per milligram of algae (dry wt.), Sr input in units of 10^{13} atoms per milliliter, and Ca input is in units of 10^{16} atoms per milliliter, the standard error being included. Other mathematical expressions for Sr uptake were tested from which the Ca input was omitted, but they failed to predict Sr uptake as well as the above formula, which includes the inverse influence of Ca.

The increased uptake of Sr at very low concentrations of Ca suggests that Sr was used by the algae in place of Ca when Ca was in short supply. Strontium substitution for Ca has been reported for algae by Walker (*4*). Accordingly, a significant reduction of Sr uptake by adding Ca to the medium

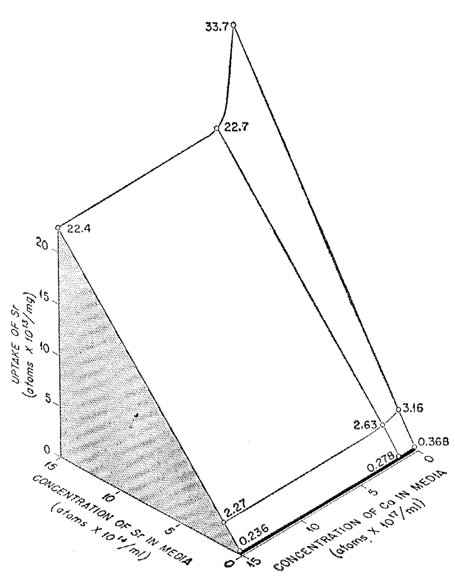

Fig. 2. Mean uptake of Sr per milligram of algae (dry wt) as a function of environmental concentrations of Sr and Ca (0.15×10^{14} atoms of Sr per milliliter = 2.8 parts per billion; 0.15×10^{17} atoms of Ca per milliliter = 1.0 ppm).

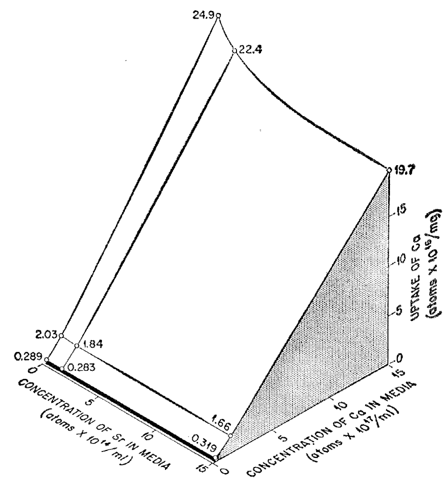

Fig. 3. Mean uptake of Ca per milligram of algae (dry wt.) as a function of environmental concentrations of Sr and Ca (0.15×10^{14} atoms of Sr per milliliter = 2.18 parts per billion; 0.15×10^{17} atoms of Ca per milliliter = 1.0 ppm).

would be possible only if the existing Ca concentration was limiting. Martin *et al.* (*5*) reported similar results: addition of lime up to a certain point to acid soils reduced ^{90}Sr uptake significantly, but further addition had only slight effect.

When the uptake of Sr or Ca is proportional to the concentration of the respective ion in the medium, and when the same amount of tracer is present in each medium, it follows that a constant amount of the tracer will be taken up by the organism regardless of the concentration of the stable element. This is true over the range of concentrations tolerated by the organism, if there is no isotopic discrimination. A constant uptake of ^{90}Sr was noted by Corcoran and Kimball (*6*) for phytoplankton cultured in media of various Sr concentrations. Uptake of ^{85}Sr in this study was not significantly different when tested statistically at the three concentrations of Sr, but the uptake was significantly greater ($P = 0.05$) at the lowest Ca concentration than at the two higher Ca concentrations. This was revealed as an increase in stable Sr uptake at the low Ca concentration and was assumed to be a result of a substitution of Sr ions for Ca ions when the Ca concentration was very low. Likewise then, uptake of ^{90}Sr fallout could be reduced significantly by Ca addition, only if the system involved were one in which Ca was in short supply.

Uptake of Sr or Ca by *O. eremosphaeria* is best explained as a chemical equilibrium reaction. Adsorption might explain the uptake. To test adsorption as a factor in the uptake, algal samples on membrane filters were washed with 0.1*N* HCl or EDTA solutions for periods of 1 to 1.5 minutes. Washing removed less than 10 percent of the Sr and Ca, thus indicating that adsorption was a minor factor in the uptake. Spooner (*7*) used "ion-exchange equilibrium" to explain Sr uptake by marine algae, and his explanation may be consistent with that offered here. The equilibrium ratio of cellular Sr to medium Sr reached the same approximate value at all concentrations of Sr and Ca in the media except at the lowest Ca concentration where the equilibrium ratios were higher but still consistent for the three Sr concentrations at that Ca level. Deviation from a direct proportion, as at the lowest Ca concentration, can be explained by allowing an increase in cellular sites, normally filled by Ca, to be available to Sr. Calcium uptake is explained by the same process, with an increase in uptake occurring at very low Sr concentrations. A slight relative increase in Ca uptake at very low Ca concentrations follows a pattern noted for many nutrients and is usually termed an increase in uptake efficiency at limiting levels of the nutrient. Hence these very low concentrations are inadequate for supplying algal requirements in the equilibrium ratio existing at higher concentrations. Therefore the cells must expend additional energy to shift the equilibrium in favor of uptake.

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