8. J. Warner, *Bull. observatoire Puy de Dome* 2, 33 (1957).
9. E. Wahl, *Bull. Am. Meteorol. Soc.* 33, 380 (1952).
10. G. W. Brier, *ibid.* 35, 378 (1954).
11. The study reported here is a preliminary phase of an investigation of ice nuclei being undertaken jointly by the National Science Foundation and the U.S. Weather Bureau.

13 May 1959

Effect of Strontium Replacement for Calcium on Production of Motile Cells in Protosiphon

Abstract. Protosiphon botryoides Klebs grows without evident zoosporogenesis in inorganic media in which strontium is substituted for calcium. Growth is 50 to 90 percent of that obtained with calcium; when sodium is substituted for calcium, there is no appreciable growth. Motility was observed at or above $CaCl_2 \cdot 2H_2O$ levels of 5 mg/lit., with or without strontium.

Earlier reports of partial or complete substitution of strontium for calcium as a required nutrient in certain plant species (*1*) led, in connection with a study of the mineral nutrient requirements of *Protosiphon botryoides* Klebs, to an attempt to substitute $SrCl_2 \cdot 6H_2O$ in the growth medium for the $CaCl_2 \cdot 2H_2O$ ordinarily supplied in Bold's inorganic salt medium for the culture of algae (*2*). Three treatments were set up, one with calcium chloride, a second with a molar equivalent of reagent-grade strontium chloride, and a third lacking both added calcium and strontium. A uniform inoculum, obtained from medium containing calcium was washed and added in equal small quantities to each of the three preparations. The preparations were placed under illumination of approximately 300 ft-ca from cool white fluorescent bulbs at 22°C, under a light-dark cycle of 12 hours of light and 12 hours of darkness (*3*). By 12 days after inoculation a phototactic ring of *Protosiphon* cells, indicating zoospore formation, became evident in the preparation containing calcium chloride but did not appear in either of the other two preparations, although growth was evident in the medium containing strontium chloride. Microscopic examination of the cultures revealed an abundance of zoospores in the calcium-containing medium; however none were seen in the medium containing strontium. Turbidimetric growth measurements, reported as total cell volume per liter, were made 37 days after inoculation, with results shown as replicate 1 of Table 1. Subsequent replicates, which showed the same responses in relation to zoospore production, are also shown in the table.

That the growth of *Protosiphon* in the presence of the strontium chloride was not due directly to the addition of calcium as a contaminant in the strontium salt is clear from a study of the growth response to different levels of calcium chloride (Fig. 1) and from spectroscopic analysis of the reagent-grade strontium chloride used (*4*). The rate of growth in the strontium chloride was equivalent to what was obtained at a calcium level of about 0.4*M* equivalent, whereas the spectroscopic analysis revealed a calcium contamination of the reagent-grade strontium chloride of only 10 to 20 parts per million. Replicates with Johnson Matthey, and Co.'s Specpure strontium salt containing 2 parts of calcium per million gave similar results. It appears that strontium replaces the major part, if not all, of the calicum required in this medium for optimum growth of *Protosiphon,* since the controls, containing all the other salts of the medium but the calcium or strontium salts, failed to sustain appreciable growth.

Table 1. Cell volume of *Protosiphon* in nutrient medium at end of culture period (ml/lit.).

Replicate	Medium		
	With Ca	With Sr	Without Ca or Sr
1	5.8	2.9	0
2	12.9	9.4	0
3	8.0	4.0	0
4*	7.7	6.9	0.7
5*	10.6	6.6	0

* Replicates with Specpure strontium salt.

The two curves of Fig. 1 show the relative growth rate of *Protosiphon* at varying levels of calcium chloride. To maintain the chloride content uniform in these cultures, the calcium chloride was replaced by sodium chloride in one instance and by strontium chloride in the other. Other tests have indicated that results would have been similar if the sodium chloride replacement had been omitted from the medium. Careful microscopic examination of the cultures revealed that zoospore production, whether or not strontium was present, began at a calcium level of approximately 5 mg/lit., 0.2 the concentration of the calcium supplied in the basal medium.

The results of these studies on *Protosiphon* point to a critical role for calcium, which cannot be assumed by strontium, in the production of motile cells.

JOSEPH C. O'KELLEY
WALTER R. HERNDON
Biology Department,
University of Alabama, University

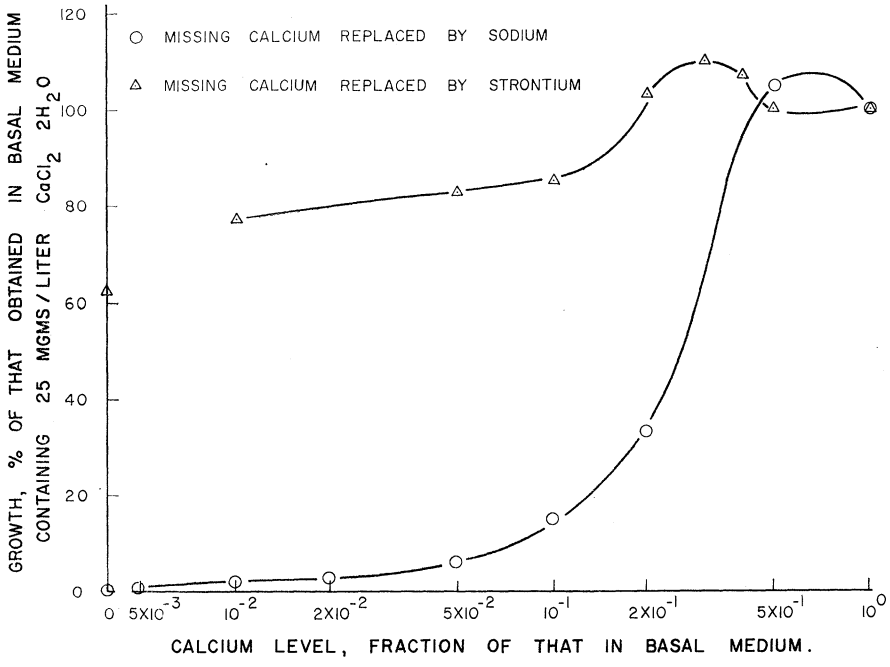

Fig. 1. Effect of strontium and sodium replacement of calcium on the growth of *Protosiphon botryoides* Klebs, measured as percentage of total cell volume obtained with 25 mg of $CaCl_2 \cdot 2H_2O$ per liter.

References and Notes

1. A. Rippel and U. Stoess, *Arch. Mikrobiol.* 3, 492 (1932); G. Lindeberg, *Symbolae Botan. Upsaliensis* 8, 1 (19→ R. A. Steinberg, *Science* 107, 423 (1948); J. B. Walker, *Arch. Biochem. Biophys.* 46, 1 (19→ J. L. Ingraham and R. Emerson, *Am. J. Botany* 41, 146 (1954).
→ H. C. Bold, *Bull. Torrey Botan. Club* 76, 101 (1949).
3. We gratefully acknowledge the assistance of the research committee of the University of Alabama in providing the culture facilities used in this study.
4. We gratefully acknowledge the help of E. L. Grove of the School of Chemistry, University of Alabama, who supplied ion-free water for use in preparing the media and who provided the spectroscopic analysis of the reagent-grade strontium chloride.

28 April 1959