

itself among the particles according to  $x^{k_i}$  where  $2 \leq k_i \leq 3$  is given (5) by:

$$\frac{f_i}{f_{80}} = \left( \frac{x}{x_m} \exp \frac{-(k_i + 2) \sigma^2}{2} \right)^{(k_i - 2)}$$

so that

$$\frac{f_i}{f_{80}} = \left( \frac{f_{95}}{f_{80}} \right)^{(k_i - 2)} \left( \exp \frac{\sigma^2}{2} \right)^{(3 - k_i)(k_i - 2)} \quad (2)$$

By taking logarithms, letting  $(k_i - 2) = b_i$  and

$$a_i = \frac{b_i (1 - b_i) \sigma^2}{4.606}$$

this expression becomes identical with Eq. 1.

As with all models, misimpressions may be obtained by too literal interpretation of either Stewart's approach or my modification even though lognormal distributions have been in fact observed (6). During particle formation periods, the particle population of the cooling fireball is continually changing due to the arrival of new particles and departure of old ones, with the result that fallout particles of a given size are extremely heterogeneous. A more realistic expression of the derived relation would be to say that the effects of the various particle-formation processes have the cumulative result of producing radionuclide frequency functions which are lognormal with respect to particle size, and that for  $Zr^{95}$  and  $Sr^{90}$  respectively, 3 and 2 are approximate values for the power to which the particle diameter must be raised before it is weighted by the lognormal frequency function of the particles.

The model should be more valid for air bursts, where the particles are in contact with the condensing radionuclides for longer periods of time. Airburst debris also correlates logarithmically, is extremely heterogeneous, and can be fitted with lognormal distribution functions (7).

One important consequence of this development is that, if Eq. 2 holds for actual debris, no single particle size would have a completely unfractionated composition. Thus, if the ratio  $f_{95}/f_{80}$  were representative (equal to unity), the ratio  $f_i/f_{80}$  would be unrepresentative. The maximum departure from unity would be attained if  $k_i$  were  $5/2$ , in which case

$$f_i/f_{80} = \exp(\sigma^2/8),$$

a significant departure from unity. Another important result is that the rela-

tionships presented offer a means of estimating the partition between local and world-wide fallout of biologically important radionuclides, like  $Sr^{90}$ , from their observed values of  $b_i$ . Additional details are in press (5; 8).

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#### References and Notes

1. E. C. Freiling, *Science* **133**, 1991 (1961).
  2. K. Stewart, *Trans. Faraday Soc.* **52**, 161 (1956).
  3. A variate  $x$  is said to be lognormally distributed with mean  $\mu$  and variance  $\sigma^2$  if  $\ln x$  is normally distributed with mean  $\mu$  and variance  $\sigma^2$ . If  $\Lambda(x' | \mu, \sigma^2)$  is the probability that  $x \leq x'$ , the normalized frequency function is described by
- $$\frac{d\Lambda}{d \ln x} = \frac{1}{\sigma(2\pi)^{1/2}} \exp \left[ -\frac{1}{2} \left( \frac{\ln x - \mu}{\sigma} \right)^2 \right]$$
4. J. Aitchison and J. A. C. Brown, *The Log-normal Distribution* (Cambridge Univ. Press, 1957).
  5. E. C. Freiling, U.S. Naval Radiol. Defense Lab. Tech. Rept., in press.
  6. A. D. Anderson, *J. Meteorol.* **18**, 431 (1961).
  7. E. C. Freiling, in *Radioactive Fallout from Nuclear Weapons Tests*, (U.S. Atomic Energy Commission, 1962) book 1, p. 47.
  8. Supported by the Division of Biology and Medicine, U.S. Atomic Energy Commission. The applicability of Stewart's conclusions to this subject was suggested by Mr. Sanford Baum in a personal communication.
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#### Algae: Nitrogen Fixation by Antarctic Species

**Abstract.** *Algae from terrestrial and fresh-water habitats in Antarctica were examined for ability to fix atmospheric nitrogen. Nostoc commune was the only species capable of growing in nitrogen-free medium; nitrogen fixation by this species was verified by assimilation of  $N^{15}$ . The importance of nitrogen-fixing algae to terrestrial life in the Antarctic is discussed.*

The Antarctic continent is an extensive land mass of over 5 million square miles, only about 5 percent of which is not covered with ice or snow. Although much of this exposed land surface consists of rugged mountains and isolated nunataks, there are areas which are conducive to extensive habitation by algae, lichens, bryophytes, and a mixture of protozoans and simple metazoans such as rotifers, flatworms, nematodes, tardigrades, and two genera of flightless insects. Among the more hospitable areas for plant growth are the Palmer Peninsula and the eastern

part of South Victoria Land. In the latter area are located Marble Point and the Taylor, Wright, and Victoria dry valleys, all of which are characterized by extensive areas that are free of snow and ice. Temperatures in these localities are generally higher than in surrounding areas and the precipitation is very slight. There is evidence that the glaciers feeding into these valleys are receding and exposing new land as the ice disappears. Thus there is opportunity to study biological and physical sequences which are important in the formation of soil.

In the antarctic summer of 1959-60 collections of algal material were made from various places on Ross Island, Marble Point, and all three of the dry valleys. This material was collected aseptically and sent back to Wisconsin. Examination of the samples revealed a variety of filamentous and unicellular Cyanophyta, many representatives from the Chlorococcales of the Chlorophyta, and an assortment of pennate and centric diatoms. A list of algae identified from antarctic collections has recently been compiled (1). The samples were all tested for nitrogen-fixing algae by inoculation into nutrient media free from any source of fixed nitrogen. A variety of nutrient solutions were used in an attempt to include media conducive to the growth of algae belonging to different phyla. Of the 130 samples tested, approximately half of them showed growth in liquid media; samples of these cultures were then plated out on agar medium in petri dishes. The localities from which these samples had been collected included Cape Crozier, Cape Royds, Marble Point, and the top of Hogback Hill; the habitats were shallow ponds of fresh water, glacial streams, moist sand or rocks, and dry scrapings from rocks. By repeated transfer of small colonies on agar medium, 20 unialgal cultures were obtained. These cultures have all been identified as *Nostoc commune* Vauch.

Although the isolates were unialgal, they were not bacteria-free, so it is possible that nitrogen was being fixed by a contaminant and not by the alga itself. Such a contaminant would have had to be present in the antarctic flora, as aseptic techniques were used in collection of the samples and in all subsequent manipulations. The possibility of a nitrogen-fixing contaminant in the isolated cultures of *Nostoc* is unlikely, for other algal species would have been

Table 1. Ability of *Nostoc commune* from the Antarctic to fix atmospheric nitrogen.

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> standard	Air	<i>Nostoc</i> <i>commune</i> *	<i>Chlorella</i> <i>pyrenoidosa</i>
Atom percent N <sup>15</sup>			
0.3570	0.3626	0.4679	0.3617
Atom percent excess N <sup>15</sup>			
		0.1081	0.0019

\* Isolated from glacial melt pond at Marble Point.

expected to grow also at the expense of fixed nitrogen furnished by a microbial contaminant. The *Nostoc* cultures were examined microscopically, but no *Azotobacter* or *Clostridium* cells were seen.

To verify that the cultures of *Nostoc commune* were fixing atmospheric nitrogen, one of the isolates was tested for ability to assimilate N<sup>15</sup>. The methods used for the incubation with N<sup>15</sup> and ensuing determination of amount of nuclide assimilated have been described by Neess *et al.* (2). The results are shown in Table 1. In addition to the culture of *Nostoc commune* from the Antarctic, a culture of *Chlorella pyrenoidosa* was also tested at the same time to serve as a control, since it is known that this organism does not fix atmospheric nitrogen. By the use of 0.3598 as the standard atom percent N<sup>15</sup> (the average value for the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> standard and air), the atom percent excess of N<sup>15</sup> in the *Nostoc* culture was 0.1081. The corresponding value for the *Chlorella* sample was 0.0019. As the mass spectrometer was accurate to about 0.003 percent excess N<sup>15</sup>, it is clear that the culture of *Nostoc* did assimilate atmospheric nitrogen.

The presence of nitrogen-fixing blue-green algae in the Antarctic has significance in relation to the formation of soil in the ice-free areas. Extensive areas consist of sand or gravel of varying coarseness, with little or no organic material. The ability of nitrogen-fixing algae to colonize recently exposed areas is well known: for instance on Krakatoa after the volcanic eruption (3). In some areas of the Antarctic such as Marble Point, the growth of *Nostoc commune* is so luxuriant that areas of dried algal material up to 5 to 6 inches in depth are encountered. This "algal peat," as it is commonly called, also contains a rich assortment of green algae, diatoms, bacteria, protozoa, and some metazoans. It is likely that the productivity of these areas is made possible by the accumulation of nitrogenous organic matter synthesized by

the nitrogen-fixing blue-green algae. The nitrogen-fixing isolates obtained in this study did not include any from the three dry valleys examined. From examination of collections preserved in formaldehyde, it is known that *Nostoc commune* is abundant in the Taylor Dry Valley; it has also been found in a few samples taken from the shoreline of fresh water ponds in the Wright Dry Valley. As yet no *Nostoc* has been detected in collections from the Victoria Dry Valley. As the collection of material from the Victoria Dry Valley was limited in scope, it is certainly possible that *Nostoc* was present in this valley system but was not included in the collected samples.

Thus, nitrogen-fixing species of blue-green algae are present in the antarctic flora. As a supply of fixed nitrogen is one of the prime requisites for growth of other organisms, the occurrence of these algal forms is of importance and interest because of progressive changes wrought in the exposed land surfaces. It will be of great interest in the years ahead to examine carefully the biological development of areas in the Antarctic like the dry valleys, which at the present time do not have the abundance and variety of plant and animal species found elsewhere around Ross Island and South Victoria Land.

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#### References and Notes

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4. Supported in part by research grant RG-7052 from the National Institutes of Health and by grant G-9975 from the National Science Foundation.

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#### "Flickering" in Protoplasts of *Bacillus megaterium*

Abstract. "Flickering," which has been observed in the erythrocytes of many animals, has now been observed in the protoplasts of *Bacillus megaterium*.

"Flickering" is the appearance of a rapid and regular oscillatory movement passing across the surface of erythrocyte cells in a fashion somewhat reminiscent of the movements produced by the wind blowing over a field of wheat.

These fluctuations are different from Brownian movements in that they are more rapid, rhythmic and systematic. Blowers, Clarkson and Maizels (1) have demonstrated a number of similarities between the occurrence of flickering and of active transport. Both phenomena were found to be constrained by changes away from the normal shape of the cell and by agents inhibiting glycolysis, but they were practically unaffected by respiratory inhibitors. Both phenomena occur over the same pH range and seem to be energized by the same sugars. In view of this apparent interrelationship these workers suggested the possibility that the flicker phenomenon is an expression of the activity of carrier molecules engaged in active transport.

Although observed in the erythrocytes of many animals, flickering has not been shown previously to occur in any other type of cell. The present report describes a method whereby this phenomenon may be observed in protoplasts of *Bacillus megaterium* under phase contrast microscopy.

Protoplasts were prepared from cultures of *B. megaterium* strain KM maintained on antibiotic medium 2 (Difco), and harvested during exponential growth in a 2 percent Bacto-peptone (Difco) liquid medium. The cells were washed by and suspended in a medium (2) containing 0.3M sucrose, 0.1M Na<sub>2</sub>HPO<sub>4</sub> and 0.016M MgSO<sub>4</sub> adjusted to pH 6.5 with NaOH. Lysozyme (Nutritional Biochemical Corp.) was added to the medium to a concentration of 0.2 mg/ml, and the resulting protoplasts were washed and resuspended in the same medium.

The slide on which flickering was observed was prepared as follows: A small No. 1 cover slip was placed at each end of an ordinary glass microscope slide; a small drop of melted 20 percent gelatin solution was placed on the slide between them. A piece of plexiglass or similar plastic was placed on top of the gelatin and cover slips; this caused the gelatin to spread out in a layer of the same thickness as the cover slips. After the gelatin became firm, the plastic was removed carefully so that the gelatin film remained on the glass slide. A very small drop of protoplast suspension was placed on the film and covered with a cover slip. The preparation was observed under oil-immersion phase-contrast optics. As the solution was taken up by the gelatin the protoplasts could be seen to be