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

$(NH_4)_2SO_4$ standard	Air	*Nostoc commune**	*Chlorella pyrenoidosa*
	Atom percent N^{15}		
0.3570	0.3626	0.4679	0.3617
	Atom percent excess N^{15}		
		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^{15}. The methods used for the incubation with N^{15} 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^{15} (the average value for the $(NH_4)_2SO_4$ standard and air), the atom percent excess of N^{15} 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^{15}, 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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"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 *p*H 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*) contining 0.3*M* sucrose, 0.1*M* Na_2HPO_4 and 0.016*M* $MgSO_4$ adjusted to *p*H 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

flattened between its surface and the underside of the cover slip. This caused some of the protoplasts to assume an appearance similar to erythrocytes, with a dark outer edge and a light central area. It was in this central area that flickering was most readily visible.

The above technique is also useful for obtaining photographs of a large number of live organisms (particularly highly motile ones) at one time, since intact cells are not flattened by this procedure.

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Thymus: Its Role in Lymphoid Recovery after Irradiation

Abstract. *Regeneration of spleen lymphoid centers after destruction of lymphoid tissues is dependent on the thymus.*

As a result of experiments with embryonic (*1*) and neonatal (*2*) thymus, a resurgence of interest has developed in the idea that the thymus represents the major primordium of the mammalian lymphoid immunological system (*3*). Experiments directed toward understanding the mechanics of thymus lymphopoesis have shown that the thymus is an autonomous lymphoid organ capable of morphogenesis in vitro as well as upon transplantation (*1*, *4*). Removal of the thymus from neonatal animals leads to depletion of lymphoid cells from various lymphoid organs as well as from the circulation (*2*).

That the thymus can play a role in adult life as well as in the embryonic and neonatal period has already been clearly demonstrated in studies of murine leukemias (*5*). A role for the thymus in normal lymphoid function has also been proposed (*6*, *7*), but the nature of this role has not been well defined. The possibility that the thymus in the adult mouse influences lymphopoeisis in a manner analogous to that seen during development was examined in the experiments reported here.

To determine optimal experimental conditions, a series of experiments were performed to establish the radiation sensitivity of thymus lymphoid differentiation. Thirty thymus rudiments were removed from 12-day-old mouse embryos. They were irradiated with 140 kv (peak), 5 ma x-rays with ½ mm Al filtration at a dose rate (in air) of 80 r/min. Doses ranged from 600 to 1500 r. The thymuses were then grown for 1 week in vitro under conditions known to promote differentiation of thymus lymphoid cells (*4*). Doses up to 900 r failed to interfere appreciably with subsequent lymphoid morphogenesis. In a series of exploratory runs 900 r was effective in destroying the lymphoid cells of the adult spleen. This dose was therefore chosen to assess the function of the adult thymus. Ten F_1(C3HxAKR) male mice were thymectomized at 3 to 4 weeks of age and irradiated with 900 r x-rays at 10 weeks of age. Ten nonthymectomized, irradiated litter-mates served as controls. Ten days after irradiation the animals were killed, and the spleens were prepared for histological examination. In nine out of ten control mice, early signs of recovery were seen in the accumulation of lymphoid cells in spleen lymphoid centers. In all ten thymectomized, irradiated mice, on the other hand, such lymphoid cell accumulations were lacking.

These experiments are particularly pertinent with regard to Miller's finding that recovery of immunological function after irradiation is impaired by thymectomy of adult animals (*8*); our results suggest the cellular basis for his observations. Neither in Miller's experiments nor in ours has it been determined whether the role of the thymus is indirect—that is, through production of a lymphocytosis stimulating factor (*7*) or by providing a tissue environment conducive to differentiation of nonthymic cells (*8*)—or, on the other hand, direct, by contribution of cells which migrate to the spleen (*3*). Indeed, the alternatives are not mutually exclusive, for a few generative cells, migrating from the thymus to the spleen, might well serve as centers for induced differentiation of lymphoid cells. Experiments involving marked cells, extracts, and transplants are necessary before merits of various alternatives can be assessed critically (*9*).

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Antigenicity of Polypeptides: Immunological Unresponsiveness to Copolymers of α-Amino Acids

Abstract. *Immunological tolerance toward three synthetic random copolymers of the α-amino acids, glutamic, lysine, alanine, and tyrosine, was produced by a single injection of the polymers into newborn rabbits. The tolerant state could be extended by an additional intravenous injection of antigen. Repeated injections of the polymer in adjuvant mixture could "break" the tolerant state.*

The antigenicity of random copolymers of L-α-amino acids has been investigated in rabbits (*1–3*), guinea pigs (*4*, *5*), and man (*6*), with an aim of learning about the chemical basis for immunogenicity. Studies of the multichain polypeptide antigens (*2*) have also contributed to the existing knowledge on the molecular requirements for antigenicity. This study was undertaken to determine whether random copolymers could induce a state of immunological unresponsiveness in rabbits similar to that induced by other nonliving antigens, mainly serum proteins (*7*).

The percentage composition in moles of the amino acids in the random copolymers used in this study is indicated by the subscript. The polymers glu_{60} ala_{40} (GA); glu_{42} lys_{28} ala_{30} (GLA30),