Lewis rat lymphocytes may recognize all three fragments equally well, but those sensitized by the bovine fragment may lack the capacity to interact with basic protein in the CNS because the sequence comprising the immunological determinant against which the lymphocytes are sensitized is not sufficiently similar to the sequence present in Lewis rat CNS. This latter concept is supported by the demonstration by Bergstrand and Kallén (*19*) that portions of xenogeneic (bovine) basic protein do not produce EAE in the guinea pig but have the capacity to induce delayed hypersensitivity. If the latter explanation were the only factor, however, one would expect the Lewis rat fragment to be at least as active as the guinea pig fragment. To account for the greater activity of the latter, the first explanation must be valid, and the possiblity of another variable must be considered. For instance, guinea pig fragment may contain a helper determinant that results in the stimulation of more lymphocytes than does the equivalent dose of the Lewis rat fragment. Further characterization and elucidation of the mechanisms responsible for the variation in the production of EAE are important, particularly because immunological mechanisms have been postulated to be operative in human demyelinating disease.

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Lower pH Limit for the Existence of Blue-Green Algae: Evolutionary and Ecological Implications

Abstract. *Observations on a wide variety of acidic environments, both natural and man-made, reveal that blue-green algae (Cyanophyta) are completely absent from habitats in which the* p*H is less than 4 or 5, whereas eukaryotic algae flourish. By using enrichment cultures with inocula from habitats of various* p*H values, the absence of blue-green algae at low* p*H was confirmed.*

The blue-green algae are of evolutionary interest, as they are the only prokaryotic organisms carrying out an oxygen-evolving photosynthesis. Their origin in the Precambrian is well substantiated, and it is likely that these algae were responsible for the initial rise in the oxygen level of the atmosphere near the end of the Precambrian (*1*). Eukaryotic algae, which arose later than the blue-green algae, differ in that their photosynthetic apparatus is present within a distinct cellular organelle, the chloroplast, whereas in the blue-green algae the photosynthetic apparatus exists as a series of cytoplasmic membrane systems perhaps connected to the plasma membrane (*2*). The blue-green algae are of considerable ecological interest since they often form massive blooms in polluted waters and since a number of species of blue-green algae produce toxins active against fish and mammals (*3*).

It is widely assumed that the blue-green algae are highly adaptable and are very tolerant of environmental extremes (*4*). However, the environmental tolerance of blue-green algae is, in many respects, not well substantiated. Only with respect to high temperatures is it clear that these algae are considerably more tolerant and adaptable than eukaryotic algae (*5*). In this report evidence is presented that in very acidic environments blue-green algae are completely absent, whereas eukaryotic algae often proliferate exceedingly well.

The data presented here are based on extensive observations of acidic habitats, both natural and man-made, throughout the world; detailed studies of acidic habitats in Yellowstone National Park; observations of algal distributions in natural *p*H gradients; and enrichment culture studies with media of various *p*H values and inocula from habitats of various *p*H values. The *p*H values were determined either directly at the site or soon after collection, by using an Orion battery-operated *p*H meter and combination glass electrode. The *p*H meter was always standardized with a buffer of *p*H near that of the sample.

Both thermal and nonthermal habitats were studied. Thermal habitats are particularly favorable for determining the lowest *p*H limit for blue-green algae because at temperatures above about 56° to 60°C eukaryotic organisms (both photosynthetic and nonphotosynthetic) are always absent and only prokaryotic organisms are present (*6*). Since some species of blue-green algae are able to grow at temperatures up to 70° to 73°C (*7*), the only oxygen-producing photosynthetic organisms at temperatures above 60°C would be blue-green algae (*8*). Observations in over 200 habitats of *p*H less than 4 throughout the world revealed that at temperatures above 56°C no photosynthetic organisms at all are found, and at temperatures from 40° to 56°C only the eukaryotic alga *Cyanidium caldarium* is found (*9*). At temperatures below 40°C in these thermal effluents a wide variety of other eukaryotic algae were found, but blue-green algae were absent.

Observations were also made on natural *p*H gradients in thermal habitats. One *p*H gradient studied in some detail (*10*) is in Waimangu Cauldron, New

Zealand, where the alkaline waters of Trinity Terrace enter the acidic waters of a hot lake. Since the lake is too hot for *Cyanidium caldarium*, it is devoid of algae where the *p*H is too low for blue-green algae, and the distribution of blue-green algae in relation to the *p*H gradient can be observed by directly measuring the *p*H where algal mats are seen. The lowest *p*H at which blue-green algae were found was about 4.8 to 5.0 (*10*). Another *p*H gradient was examined in the Semi-Centennial Geyser–Obsidian Creek area of Yellowstone National Park. In the mixing zone where alkaline springs enter Obsidian Creek a small *p*H gradient develops; at *p*H values over 5 only blue-green algae were seen, and at *p*H values below 4 only eukaryotic algae were seen. In the *p*H range between 4 and 5 both blue-green algae and eukaryotic algae were present.

To extend observations to nonthermal environments, studies were made on a large number of lakes of various *p*H values in Yellowstone National Park. Acid lakes are common throughout the regions of the park where geothermal activity exists, and many of these lakes have normal temperature regimes. The acidity of these nonthermal habitats is due to the oxidation of sulfide, which enters these lakes through underwater springs and gas vents, or to the drainage from acidic springs, for which the lakes serve as catchment basins. The acidities, temperatures, and hydrography of the Yellowstone lakes appear to be stable, in contrast to the properties of volcanic crater lakes in many parts of the world, which often fluctuate because of eruptions. Thus, the Yellowstone lakes provide favorable environments for observing the colonization of algae as a function of *p*H. Twenty-two lakes with *p*H values ranging from 1.9 to 8.6 were studied. Sampling of benthic algal populations was done from the shoreline. If visible algal mats were present these were sampled; but if no mat was visible, samples of mud, sand, or gravel were taken. Detailed samplings were also taken of benthic algae from Obsidian Creek, since this creek is very acidic (*p*H 2.35) at its source near Roaring Mountain but becomes progressively alkaline as it flows toward the Gardner River due to inflow of waters from alkaline geysers and hot springs or from nonthermal creeks.

Every sample except one had microscopically visible algae, usually in relatively high density. The results presented in Table 1 show that although all samples containing algae had eukaryotic algae, blue-green algae were not found in samples from any waters with *p*H 4.8 or less. To increase the sensitivity of the observations, enrichment cultures were set up with an inoculum from each sample; the medium of Allen (*11*) was used, adjusted to the *p*H of the water from which each sample was taken. Cultures were incubated in the light at room temperature and at 35° to 37°C, the latter being used because Stanier *et al.* (*12*) had stated that 37°C was selective for blue-green algae. All cultures yielded good to excellent algal growth. After 2 weeks incubation, all cultures were examined microscopically for the presence of eukaryotic and prokaryotic algae; the results are given in Table 1. (The *p*H of each culture was measured at the end of the 2-week incubation and it was usually within a few tenths of the original *p*H.) As can be seen in Table 1, growth of blue-green algae occurred in culture at *p*H values down to 4.1, but at no *p*H below 4 were any blue-green algae seen.

In a further attempt to enrich for blue-green algae at low *p*H, samples from a number of waters were pooled and the mixtures were used to inoculate culture media of various *p*H values. Again, incubations were at both room temperature and approximately 37°C. In these enrichment cultures, no blue-green algae were obtained at *p*H 5 or below, although excellent growth of blue-green algae occurred at *p*H 6 or higher.

Some studies have also been done

Table 1. Presence of eukaryotic and blue-green (prokaryotic) algae in lakes and streams of various *p*H values in Yellowstone National Park. Cultures were set up in duplicate and incubated at both room temperature and approximately 37°C. A plus sign indicates that at least one culture was positive, at either temperature; ng indicates no growth.

Location	*p*H	Temp. (°C)	Natural samples		Cultures	
			Eukaryotic	Prokaryotic	Eukaryotic	Prokaryotic
Sour Lake	1.9	27	+	−	+	−
Unnamed lake*	2.1	22	+	−	+	−
Unnamed pond†	2.3	29	+	−	+	−
Obsidian Creek‡	2.35	23	+	−	+	−
Clear Lake	2.5	17	+	−	+	−
Sieve Lake	2.5	40	+	−	+	−
Obsidian Creek	2.7	18	+	−	+	−
Beaver Lake	2.7	16	+	−	+	−
Beaver Lake	2.8	14	+	−	+	−
Obsidian Creek	2.85	24	+	−	+	−
Nymph Lake‡	2.9	22	+	−	+	−
Obsidian Creek	3.2	17	+	−	+	−
Turbid Lake	3.25	25	+	−	+	−
Sedge Creek	3.4	17	+	−	+	−
Nuphar Lake	3.8	12	+	−	+	−
North Twin Lake	4.1	17	+	−	+	+
Obsidian Creek	4.5	14	+	−	+	−
Nymph Lake	4.8	22	+	−	+	+
Lake 7847§	5.0	30.5	+	+	+	+
Obsidian Creek	5.5	10	+	−	+	+
South Twin Lake	5.5	12	+	+	+	+
Nymph Lake	5.7	22	+	+	+	+
Nymph Lake	5.8	32	+	+	+	+
Obsidian Creek	5.9	16	+	+	+	+
Obsidian Creek	5.95	16	+	+	+	+
Obsidian Lake	6.1	20	+	+	+	−
Obsidian Creek	6.3	12	−	−	+	+
Scaup Lake	6.4	17	+	+	+	+
Beach Lake	6.6	20	+	+	+	+
Harlequin Lake	6.7	17	+	+	+	+
Yellowstone Lake	6.7	16	+	−	+	+
Obsidian Creek	6.9	15	+	+	+	+
Squaw Lake	7.7	17	+	−	+	+
Rush Lake	7.7	17	+	+	+	+
Goose Lake	7.85	17	+	+	+	+
Feather Lake	8.35	17	+	+	+	+
Swan Lake	8.65	20	+	+	ng	ng

* Small lake with no outlet south of the Mud Volcano area, near Lake 7847 (U.S. Geological Survey Quadrangle, Canyon Village, Wyoming). † Small turbid pond at Mary Bay, Yellowstone Lake, north of East Entrance Road. ‡ At Nymph Lake and Obsidian Creek various *p*H values were found at different sites. § Lake 7847 is south of the Mud Volcano area and is at an elevation of 7847 feet (2392 m) (U.S. Geological Survey Quadrangle, Canyon Village, Wyoming).

on soil algae, since soils of a wide variety of *p*H values occur in Yellowstone Park. Although blue-green algae are widespread in soils of neutral and alkaline *p*H, in soils with a *p*H of 5 or less only eukaryotic algae have been found.

In addition to observations of naturally acidic habitats, a number of observations were made in streams in southern Indiana subject to acid pollution due to drainage from coal refuse piles or spoil banks. In many of these streams, extensive algal growth occurs at *p*H values of 4 or less, but only eukaryotic algae were present. Observations were also carried out on the acid waters issuing from the leaching dumps used for the beneficiation of low grade copper ores in Montana (Anaconda Co.) and Arizona (Duval Corp.). Extensive benthic algal populations live in the effluent channels of such dumps, where the water has a *p*H of 2 to 2.5 and copper concentrations of 750 to 1000 μg/ml. Blue-green algae were never observed in these channels, although a variety of eukaryotic algae were present.

The observations reported here are consistent with other reports of the rarity of blue-green algae in environments of acid *p*H. For instance, Fogg (*13*), in his review on the physiology and biochemistry of the blue-green algae, concluded that the blue-green algae show a preference for alkaline conditions. Lund (*14*), in his two reviews on soil algae, noted that blue-green algae prefer neutral or alkaline soils and are absent from acid soils. Prescott (*15*) made an extensive survey of lakes of various *p*H values in Michigan and Wisconsin and concluded that blue-green algae were rare or absent in lakes with *p*H as low as 5, and green algae were almost the exclusive components. Gessner (*16*), in his review of the algal flora of acidic habitats, concluded that the Cyanophyceae were the rarest algal class in acid bogs. Rosa and Lhotsky (*17*) did an extensive microscopic and cultural study of algae from acid soils (*p*H 3.2 to 4.4) of the Iser Mountains, Czechoslovakia, and found no blue-green algae, although eukaryotic algae were widespread. Negoro (*18*) reported a few blue-green algae from acid waters in Japan, although these were in the minority and it is not clear that Negoro distinguished these reputed blue-green algae from eukaryotes. The main phycocyanin-containing alga found by Negoro was the eukaryote *Cyanidium caldarium*, which he erroneously classified as a blue-green alga. In Ueno's study of the acid lakes of Japan (*19*) blue-green algae were never found. In his study of the algae of acid mine waters, Bennett (*20*) found blue-green algae to occur only rarely and never abundantly.

A series of observations on the distribution of blue-green algae with respect to *p*H is included in the extensive survey of the limits of the natural environment by Baas-Becking *et al.* (*21*). These workers report blue-green algae at *p*H values as low as 2, the data apparently coming from the paper by Kaplan (*22*) on the flora of New Zealand geothermal regions. Unfortunately, Kaplan did not present any details of any of the algae observed at acid *p*H values, but it seems likely that he may have been observing *Cyanidium caldarium*. In our extensive studies in the same parts of New Zealand visited by Kaplan, we observed *C. caldarium* commonly in acidic habitats but never observed blue-green algae (*23*).

From the results described above, I conclude that blue-green algae do not exist at *p*H values below 4, although eukaryotic algae not only exist but grow profusely. Thus, there seems to be an acid barrier which eukaryotic algae, but not blue-green algae, have been able to overcome. It should be emphasized that the eukaryotic algae present in acid habitats are both profuse in numbers and taxonomically diverse. Algae from four major divisions are found: Rhodophyta (*Cyanidium caldarium*), Bacillariophyta (diatoms, *Pinnularia, Eunotia*), Euglenophyta (*Euglena mutabilis*), and Chlorophyta (many species). Among the Chlorophyta, algae from four orders are found—Volvocales (*Chlamydomonas*), Chlorococcales (*Chlorella*), Ulotrichales (*Ulothrix*), and Zygnematales (*Zygogonium*). Thus, it seems that eukaryotic algae have been evolutionarily successful in acidic environments, whereas prokaryotic algae have been excluded.

The evolutionary implications of this conclusion seem vast. If we assume that blue-green algae have never been able to live in acidic environments, it seems evident that in the portion of the Precambrian when blue-green algae existed and eukaryotic algae did not, acidic habitats would have represented uncolonized niches within which eukaryotic algae, if they evolved, could have reproduced without competition from blue-green algae. It might have been in acidic environments that the first eukaryotic algae arose. Although it is not known with certainty why acid is detrimental to blue-green algae, one possibility is that it affects the photosynthetic apparatus, which in these algae is usually at the periphery of the cell adjacent to the plasma membrane. Chlorophyll especially is very acid labile and decomposes to pheophytin under mildly acidic conditions. In eukaryotic algae, chlorophyll and the photosynthetic apparatus are segregated in chloroplasts, which are membrane-bounded structures surrounded by cytoplasm. Since the cytoplasm is probably of neutral *p*H even in eukaryotic algae living at acid *p*H, the cytoplasm provides an environment of neutral *p*H within which the chloroplast can exist. I suggest that if eukaryotic algae evolved they could have invaded acidic environments, where they would be free from competition, so that the evolution of the chloroplast would have immediately provided a selective advantage. It is then conceivable that eukaryotic algae could have radiated from acidic environments into the wide variety of other environments in which they exist today. It is irrelevant for this hypothesis whether eukaryotic algae first arose from a symbiotic association of a blue-green alga with a nonphotosynthetic organism, as advanced by Margulis (*24*), or whether a de novo origin of the eukaryotic algae occurred.

The ecological implications of the above conclusions are also clear. Blue-green algal blooms should never occur in acid lakes, and the pollution of lakes and streams with acid mine drainage should eliminate blue-green algae from these waters. Since even in mildly acidic waters (*p*H 5 to 6) blue-green algae are uncommon, mild acidification of lakes may control or eliminate blue-green algal blooms. Since in rice cultivation nitrogen fixation by blue-green algae may be beneficial, rice culture in acidic soils may be less favorable than in neutral and alkaline soils, and liming of such soils may considerably improve rice yields.

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Struvite and Prebiotic Phosphorylation

Abstract. *Struvite, $MgNH_4PO_4 \cdot 6H_2O$, rather than apatite or amorphous calcium phosphate is precipitated when phosphate is added to seawater containing more than 0.01M NH_4^+ ions. Struvite may have precipitated from evaporating seawater on the primitive earth, and may have been important for prebiotic phosphorylation.*

The concentration of dissolved inorganic phosphate in the oceans, at present, is in the range from 10^{-5} to $10^{-6}M$ (*1, 2*). It is believed that the concentration is maintained at this low value by the insolubility of minerals of the apatite group, particularly hydroxylapatite, $Ca_5(OH)(PO_4)_3$. Hydroxylapatite is a relatively inert material and cannot easily be used as a source of phosphate for the synthesis of organic phosphates of prebiological interest. A number of authors have suggested, therefore, that the insolubility and low reactivity of the most abundant phosphate mineral constitute major difficulties for theories of prebiotic synthesis (*1, 3*).

There are already a number of reports in the literature which suggest that the problem may not be as severe as it seems at first. Miller and Parris have shown that pyrophosphate is formed on the surface of apatite as a result of the action of cyanate ion (*4*). Perhaps pyrophosphate could be used as a phosphorylating agent rather than the apatite itself. We have shown that, in the presence of urea and ammonium chloride, at temperatures in the range from 85° to 100°C, hydroxylapatite will phosphorylate nucleosides and nucleotides, although slowly (*5, 6*).

There are a number of reasons for doubting that hydroxylapatite was, in fact, the only phosphate mineral at the site where life began. It is possible that the crucial site for the origin of life was an inland lake, for example, in which case the state of phosphate in the oceans would have been irrelevant. Even if life began in tide pools, it is unlikely that apatite was the major phosphate mineral available. It has been shown that hydroxylapatite is precipitated from aqueous solution only if the Ca^{2+} concentration exceeds the Mg^{2+} concentration by a factor of 5; otherwise, a much more soluble amorphous calcium phosphate is obtained (*2*). In fact, the ratio of Ca^{2+} to Mg^{2+} in present-day seawater is in the range 0.2 to 0.3 (*7*). This makes it seem unlikely that the concentration of Ca^{2+} was ever greatly in excess of that of Mg^{2+} in the prebiotic ocean, and likely, therefore, that an amorphous phosphate rather than hydroxylapatite was precipitated in evaporating tide pools.

Table 1. Phosphates precipitated from artificial seawater to which ammonia has been added.

NH_4^+ concentration ($\times 10^{-2}M$)	Nitrogen in solid (%)	X-ray pattern
5	5.45	Struvite
2	5.38	Struvite
1	2.69, 4.23	Struvite
0.5	0.15	Amorphous calcium phosphate
Authentic $MgNH_4PO_4 \cdot 6H_2O$	5.42*	Struvite

* The nitrogen values obtained in the micro-Kjeldahl determinations were consistently low by 5 to 6 percent, both for the struvite precipitated in our experiments and for authentic samples of struvite treated in the same way. Calibrations with ferrous ammonium sulfate gave correct nitrogen values. We attribute this discrepancy to the loss of ammonia during the work-up procedure.

Here we wish to examine a different possibility, namely, that the major phosphorylating mineral was struvite, $MgNH_4PO_4 \cdot 6H_2O$. There are two arguments supporting this point of view. Struvite may be used with particular advantage in certain prebiotic phosphorylation reactions (*6*). When struvite is heated with urea at 65°C, it is converted into magnesium pyrophosphate (yield, 20 percent in only 10 days). When struvite is heated with urea in the presence of nucleotides, nucleoside pyrophosphates such as uridine 5′-diphosphate and the pyrophosphate P_1,P_2-diuridine 5′-pyrophosphate are formed in good yield. The second argument is of quite a different kind. Ammonia may have been abundant in the primitive ocean, and struvite, which is surprisingly insoluble in water, precipitates readily from solutions containing Mg^{2+}, NH_4^+, and PO_4^{3-} ions. In this report we show that, under the conditions prevailing on the primitive earth, struvite may well have been the major phosphate mineral deposited from evaporating tide pools.

Magnesium ammonium phosphate of unspecified purity was purchased from Pfaltz and Bauer. All other inorganic salts were analytical reagent grade. Artifical seawater was prepared from Rila Marine Mix (Rila Products, Teaneck, New Jersey). The final concentrations of Ca^{2+}, Mg^{2+}, and HPO_4^{2-} in the artificial seawater were: Ca^{2+}, $1.13 \times 10^{-2}M$; Mg^{2+}, $4.96 \times 10^{-2}M$; HPO_4^{2-}, $5.75 \times 10^{-6}M$ (*8*).

We prepared a series of solutions of artificial seawater to which we added varying amounts of ammonium chloride. One liter of each of these solutions was maintained at *p*H 8.0 ± 0.1 at room temperature in an automatic titrater, while a solution of sodium orthophosphate was added slowly. The amount of phosphate added was never enough to precipitate more than 20 percent of the NH_4^+, Ca^{2+}, or Mg^{2+} ions. Stirring was always continued for at least 18 hours after the uptake of base had stopped. Carbon dioxide was excluded from the system by means of a tube containing granules of BaO.