increased about 12 percent when 1 m*M* sulfate was added (*3*). This finding suggests a small change in the environment of one or more tryptophan residues. Changes in the polarization of fluorescence spectra were at the limit of significance, an indication that the molecule undergoes no serious rearrangement involving tryptophan residues.

Nuclear magnetic resonance measurements were made with binding protein whose exchangeable groups were deuterated (*7*). No discernible separate peak was detected, and there was no change when sulfate was added.

Optical rotary dispersion (ORD) measurements (Cary Model 60) were made on protein (1 mg/ml) in 10 m*M* phosphate buffer, *p*H 6.9. The spectrum between 220 and 310 nm was characteristic of protein with the α-helix structure, having a trough at 233 nm, $[\alpha] = -5800$ or $[m'] = -5900$, and with a crossover point at 225 nm. The α-helix content calculated from the trough, assuming $[m']$ at 233 nm $= -13{,}000$ for a complete helix and -2000 for a random coil, gives an α-helix content of 35 percent (*8*). From the equation of Shechter and Blout (*9*) the percentage of α-helix was calculated at 193 and 225 nm to be 37.0 and 33.5 percent, respectively. A minimum decrease of 6 percent, at the 233 nm minimum was observed when 1 m*M* sulfate was added.

Circular dichroism was measured on the samples that were used for ORD. A minimum reading of -36×10^5 deg cm^2/dmole was found at 223 nm. It was reduced by 3 percent when 1 m*M* sulfate was added. There was a smaller minimum, -4.8×10^4 deg cm^2/dmole at 273 nm. It was reduced about 6 percent when sulfate was added. This must be caused by minor changes in the aromatic residues. The α-helix content calculated (*10*) from the trough at 233 nm was 38 percent.

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Biological Nitrogen Fixation in Lake Erie

Abstract. *Biological nitrogen fixation, as determined by acetylene reduction, occurs in Lake Erie. Fixation potential by blue-green algae in situ in water and by bacteria in collected sediments was demonstrated. Nitrogen-fixing activity occurred from June through November suggesting that it is significant over the extremes of seasonal variation in light, temperature, and nutrients.*

Biological nitrogen fixation by blue-green algae and bacteria occurs in freshwater lakes (*1–3*). We report here studies on the nitrogen-fixing activity in the water and sediments of Lake Erie during the period from 1 June through 22 November 1969. Samples were taken at various locations in the vicinity of the Bass Islands, which lie at the eastern edge of the shallow, rapidly eutrophying western basin of the lake. The sample sites included shorelines, bay areas, and open lake waters.

Nitrogen-fixing activity was determined by the method employed in the study of other freshwater environments (*1, 3*) which measures acetylene reduction to ethylene by nitrogen-fixing organisms. For measurements of algal fixation in situ, vaccine bottles (72 ml), capped with serum stoppers, were evacuated and filled to atmospheric pressure with a gas mixture containing acetylene, argon, and oxygen (5 : 75 : 20) (*4*). Either water containing algae or algae concentrated by plankton net and resuspended in water was added at the rate of 50 ml per sample to the gas-filled bottles by injection. Another needle was introduced through the serum stopper to relieve the gas pressure to atmospheric as the algal sample was injected. Immediately after the injection was completed, the bottles were placed back in the lake to permit incubation in situ. Samples from each site were incubated for 0, 30, 60, and 90 minutes in Lake Erie and for 5 days at 22° to 24°C under an incandescent light in the laboratory. At the end of the desired incubation time, fixation was stopped by the addition of 5 ml of 50 percent trichloroacetic acid (TCA) solution. Three controls were used for each sample. One control was the sample to which TCA had been added immediately (0 minutes); the second was a gas-filled bottle to which only TCA had been added; and the third was an untreated gas-filled bottle. Nitrogenase activity was estimated by determination of both acetylene loss and ethylene production. Acetylene and ethylene were quantitated by gas-liquid chromatography (GLC) (*5*).

Nitrogen-fixing activity in the bottom sediments was estimated in undisturbed sediments collected either by dredging or by manually collecting the samples. Sediment samples consisting of undisturbed cores (1 cm in diameter to a depth of about 6 cm) were placed in vaccine bottles (72 ml) immediately after they were collected. The bottles were capped, evacuated, and filled with a gas mixture of 6.56 percent acetylene and 93.44 percent argon (*4*). The filled sample bottles were incubated in the dark at 22° to 24°C for 5 days; the gas in the bottles was then analyzed by GLC (*5*). Nitrogenase activity was determined as described for algal samples.

Nitrogen-fixing activity of the blue-green algae was expressed as a rate based on total biuret detectable protein in all algae (Table 1). Therefore the total indicated protein is higher and, consequently, the apparent specific nitrogenase activity lower than would be expected if all activity were based on the protein from nitrogen-fixing algae only. All ethylene produced could be accounted for by a corresponding decrease of acetylene in the sample bottles.

Nitrogen-fixing activity in the water was not detected in samples taken in

Table 1. Acetylene reduction to ethylene by organisms in water from Lake Erie. Samples taken twice weekly during June, July, and September and on 17 October 1969 showed no detectable acetylene reduction, and samples taken twice weekly from 1 to 18 August showed only traces of ethylene production. Ethylene production is expressed as the number of nanomoles produced per milligram of protein per 30 minutes.

Sampling date	Ethylene production
August 1969	
22	34.5
25	62.6
27	97.0
29	25.2
October 1969	
31	21.3
November 1969	
22	3.8

June and July and appeared only in August when an algal bloom was visually apparent. Activity was at a minimum until late August when the rate rapidly increased. Fixation abruptly ceased during the first days of September and did not begin again until late October. During this renewed nitrogen-fixing period significant rates were detected but the activity was not as high as that attained in the late summer period. Algal growth was less dense, and it was necessary to concentrate the algae to produce a satisfactory specimen for rate determination.

During the summer period, the water temperature remained at 25° to 26°C. In the autumn, the water temperature decreased from 10°C in mid-October to 1°C in November.

Table 2. Acetylene reduction to ethylene by organisms in sediment from Lake Erie. Ethylene production is expressed as the number of nanomoles produced per gram of sediment per day.

Sampling date	Ethylene production
July 1969	
25	28.6
August 1969	
4	29.1
18	29.1
22	33.1
27	34.2
September 1969	
5	31.6
10	30.9
19	29.9
26	29.3
October 1969	
17	28.6
24	29.7
November 1969	
22	31.9

The samples collected in the late summer contained essentially equal numbers of *Microcystis* and *Aphanizomenon*, as determined by microscopic examination. While the ratio of *Microcystis* to *Aphanizomenon* remained constant, the proportion of *Anabaena* increased and reached a maximum concurrently with the peak of fixing activity. A similar pattern of algal distribution was noted during the autumn period.

The fixation occurring in the sediments is biological because activity was completely eliminated when sediment samples were autoclaved and then tested. Activity was resumed at essentially the same rate observed prior to autoclaving when the autoclaved samples were inoculated with 0.1 ml of native sediment (diluted 100-fold) per 25 ml of autoclaved sample and tested in the usual manner. This activity in the sediments was attributed to bacterial rather than algal action because of its lower rate and because of its occurrence in the dark over long incubation periods.

The values in Table 2 were obtained by incubation of the sediments at 22° to 24°C and thus probably reflect activity in the sediments during late August because the sediment temperatures in the lake at that time were close to the incubation temperature. Because lake temperatures markedly decreased in late October, the incubation of test samples at 22° to 24°C could give an erroneous value for fixation activity. Therefore, we analyzed the sediments for fixation at 2° to 4°C as well as at the higher temperature and found comparable rates at both temperatures. Thus, fixation activity in the sediments, as in the water, is insensitive to seasonal temperature variations.

The potential for significant biological nitrogen fixation exists in Lake Erie. That this potential is at least partly realized is strongly suggested by the demonstrated fixation in situ by the blue-green algae. Fixation by algae can occur in lake waters even though the amount of other available fixed forms of nitrogen (NO^-_3 and NH^+_4) in the water is high enough to support biological activities (*6*) as it is in Lake Erie. Thus, algal fixation may occur in the presence of bound nitrogen or in its absence. Therefore, elimination of bound nitrogen from influent water sources may not limit algal growth.

Although nitrogen fixation by blue-green algae does not occur at a constant rate over an extended period of time, the potential for such activity over much of the year is apparent. Algal fixation in situ occurs as late as the end of November when daylight is reaching its minimum length and intensity and when the water temperature is also reaching its minimum; therefore it is possible that some algal fixation occurs at almost any time of the year.

The potential for nitrogen fixation in the sediments is not subject to the great variation demonstrated in the water. Our data are based on composite samples of sediments obtained by coring and must therefore be considered to be lower than would be obtained if just the actively fixing segment was analyzed. The data give only an indication of the potential activity based on bottom surface area. There is no doubt, however, that nitrogen-fixing potential exists in the sediments and that it may have a significant role in the overall nitrogen economy of the lake.

A nitrogen-fixing potential exists in both the water and the sediments of Lake Erie. This potential must be considered in any analysis of eutrophication in this lake.

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5. The separation of acetylene from ethylene was accomplished by gas chromatography on a Varian Aerograph 204-1B gas chromatograph equipped with a hydrogen flame ionization detector and an A silicagel 30/60 column (0.32 cm by 24 m). Carrier gas (N_2) was run at a flow of 30 ml per minute, and the column was maintained at 85°C. The gases were quantitated by measuring with a planimeter the area under the peak formed by a Westronics recorder in response to the hydrogen flame ionization detector.
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