PSC generation is in the heart. (i) Elimination of myocardial contractility causes elimination of PSC's. (ii) In bled animals PSC's continue, ruling out the possibility that distension of the aorta triggers the tension changes. (iii) When the atrium and ventricles contract at different rates, PSC's are locked in frequency to the right atrial frequency. (iv) Excision of the right atrium, but not the left, causes elimination of aortic PSC's. Observations (iii) and (iv) suggest that the pacemaker region is in the right atrium, the site of the sinoatrial node.

A possible sequence of events can be summarized as follows. As the hydrostatic pressure in the vessel increases, nervous impulses originating from the heart initiate a contraction of the arterial smooth muscle. Since the pressure increase is very large, the muscle becomes overloaded and expands during the contraction phase of the PSC. The absence of active shortening in the intact vessel during the cardiac cycle probably explains the PSC having gone unnoticed for so long.

Because of the above lack of muscle shortening during activation, there is no incompatibility between the slow rate of smooth muscle shortening and the relatively rapid active tension oscillations. Under the conditions described here, with the muscle allowed to shorten in the absence of a hydrostatic pressure load, only a few percent of the maximal force development occurs (*9*), probably because the brief period of activation did not allow for substantial shortening. However, under conditions of increasing length the same degree of activation may represent a considerable resistance to stretch. This would prevent excessive dilatation of the arterial wall, which would otherwise, according to Laplace's law, amplify the wall stress caused by the pulse pressure wave. Possible initiation of both cardiac and arterial activity from a common pacemaker in the right atrium may ensure a high degree of coordination between these two events.

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References and Notes

1. R. F. Furchgott and S. J. Bhadrakom, *J. Pharmacol. Exp. Ther.* **108**, 129 (1953); R. F. Furchgott, S. J. Elrreich, E. Greenblatt, *J. Gen. Physiol.* **44**, 499 (1961); R. Deth and C. van Breemen, *J. Membr. Biol.* **30**, 363 (1977); C. van Breemen, P. Aaronson, R. Loutzenhiser, *Pharm. Rev.* **30**, 167 (1979).
2. O. Frank, Z. *Biol. (Munich)* **37**, 483 (1899); L. H. Peterson, R. E. Jensen, J. Purnell, *Circ. Res.* **8**, 622 (1969).
3. R. F. Rushmer, *Am. J. Physiol.* **183**, 545 (1955).
4. H. P. Pieper and L. T. Paul, *ibid.* **217**, 154 (1969); G. R. Nicolos and H. P. Pieper, *ibid.* **221**, 1209 (1971); *ibid.* **228**, 518 (1975).
5. F. Heyman, *Acta Med. Scand.* **157**, 503 (1957); *ibid.* **163**, 473 (1959); ——— and K. Stenberg, *ibid.* **171**, 9 (1962); F. Heyman, *Nature (London)* **216**, 402 (1967).
6. A. Mangel, M. Fahim, C. van Breemen, *Nature (London)* **289**, 692 (1981).
7. ———, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 409 (1981).
8. ———, R. Loutzenhiser, in *Eighth International Symposium on Vascular Neuroeffector Mechanisms*, J. A. Bevan, Ed. (Raven, New York, in press).
9. B. Gow, in *Handbook of Physiology*, vol. 2, *Vascular Smooth Muscle*, D. F. Bohr, A. P. Somlyo, H. V. Sparks, Eds. (American Physiological Society, Washington, D.C., 1980), p. 353.
10. Support from University of Miami biomedical grant HR 7198 and American Heart Association/Florida Affiliate grants 7/81 AG 610 and RF 2/81 is acknowledged.

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Cyanobacterial Blooms: Carbon and Nitrogen Limitation Have Opposite Effects on the Buoyancy of *Oscillatoria*

Abstract. *Gas vacuolation in* Oscillatoria rubescens *decreased with increased nitrogen limitation and increased with transitions from nitrogen to inorganic carbon limitation. Gas vacuoles consist of protein vesicles that can accumulate in carbon-limited but not in unenriched nitrogen-limited cells. Nitrogen limitation is a factor in the formation of deep population maxima; carbon limitation can promote surface blooms.*

Blue-green algae (Cyanobacteria) can form dense surface and deepwater population maxima in lakes because their cells possess gas vacuoles. Gas vacuoles are aggregates of vesicles with gas-permeable, proteinaceous walls. In a stable water column the relative cellular volume occupied by these structures can determine whether an alga will rise to the surface or accumulate at some depth. Laboratory and field studies indicate that relative gas vacuolation (RGV) and buoyancy in blue-green algae depend on an interaction of light and limiting nutrients that affects relative rates of photosynthesis, growth, and gas-vesicle synthesis (*1*). Of particular interest are growth-limiting conditions that permit RGV to increase, as it does before or during surface blooms. Smith and Peat (*2*) reported that gas vacuolation increased in the later phases of growth by batch cultures of *Anabaena flos-aquae*. Reynolds (*3*) described similar increases in bloom-forming populations of *Anabaena* and *Microcystis* as their growth rates decreased. In these studies, one or more factors limited growth but permitted RGV to increase. Light can do this, at least in nutrient-sufficient algae. With a reduction in light intensity, RGV increased in batch cultures of *A. flos-aquae* (*4, 5*), but not in *Oscillatoria rubescens* taken from a N-limiting chemostat (*6*). As alternatives to light, Reynolds and Walsby (*7*) suggested the major growth-limiting nutrients: C, N, and P. We report that RGV in *O. rubescens* decreased with increases in the degree of N limitation and increased with transitions to inorganic carbon (C_i) limitation.

We worked with cyclostats designed specifically for use with *O. rubescens* (*8, 9*), and we measured RGV nephelometrically with a modified version of Walsby's technique (*10*). After a reduction in the dilution rate of a N-limiting cyclostat, RGV in *O. rubescens* declined along with cell N content, and then stabilized as N content continued to decline (Fig. 1A). A companion culture was supplied by the same peristaltic pump but deprived of CO_2 by a trap installed in the air line after the reduction in dilution rate (Fig. 1B). With the removal of CO_2, RGV remained stable at first and then increased as the culture became increasingly limited by C_i. (Presumably, the smaller decrease in the cell N of this culture reflected the loss of some nitrogenous compounds such as RNA that vary with growth rate.)

The effect of CO_2 deprivation was also observed in two initially N-limiting cyclostats with constant dilution rates (Fig. 2). On day 3, we installed a CO_2 trap in the air line of culture A (Fig. 2), and by day 4, RGV had increased from its previous steady-state level. On day 6, we transferred the CO_2 trap to culture B (Fig. 2), and as RGV decreased in culture A, it increased in B. Thus, the relative gas vacuole content of the cells of *O. rubescens* decreased with increased N limitation and increased with transitions to C_i limitation. The negative effect of N limitation on RGV in the axenic cultures of *O. rubescens* used in this study confirmed observations reported earlier by A.R.K. on xenic cultures of the same alga grown in a chemostat (*6*). Moreover, increases in the availability of C_i and N can also have opposite effects on blue-green algal buoyancy. Increases in ammonium-ni-

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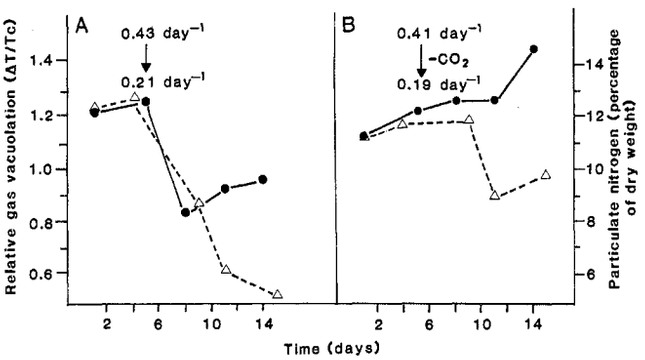

Fig. 1. (A) Relative gas vacuolation (*10*) (●) and the percentage of nitrogen content (△) in a N-limited culture of *O. rubescens* subjected to a reduction in dilution rate on day 5. The arrow indicates the time and magnitude of the change in dilution rate. (B) The same measurements in a culture subjected to CO_2 deprivation as well as a reduction in dilution rate on day 5.

trogen increased RGV in N-limited cultures of *O. rubescens* (*6*) and induced surface blooms by metalimnetic populations of *O. agardhii* var. *isothrix* (*11, 12*). Whereas with carbon, Walsby and Booker (*13*) found less surface accumulation by *A. flos-aquae* in the half of a partitioned laboratory column treated with carbonate, and Klemer and Brasino (*14*) observed a much more rapid collapse in isolated portions of a natural *Anabaena* bloom treated with bicarbonate than in similarly isolated controls. These different buoyancy responses indicate that N limitation has a role in the formation of subsurface population maxima by certain species and that C_i limitation can contribute to the formation or maintenance of surface blooms.

Experiments in lakes suggest that the formation of subsurface population maxima is a buoyancy response to high light intensities and nutrient depletion near the surface (*11, 15, 16*). N-limited *O. agardhii* var. *isothrix*, for example, lost buoyancy when transferred from a deep population maximum toward the surface; Walsby and Klemer (*15*) found that this effect increased with increases in light intensity but decreased with the addition of ammonium-nitrogen. Walsby (*5*) had related such reductions in buoyancy to the collapse of gas vesicles by increased turgor pressure, and Dinsdale and Walsby (*17*) had shown that the turgor collapse mechanism is dependent on photosynthesis. They reasoned that increases in cell turgor sufficient to collapse some of the pressure-sensitive gas vesicles occur when light and nutrient conditions are such that the rate of photosynthesis exceeds the rate of nutrient-limited growth. Thus N limitation could promote increased turgor pressure and gas-vesicle collapse by contributing to the accumulation of osmotically active photosynthate, whereas the relief of N limitation would increase the assimilation of photosynthate and would reduce turgor pressure. More recently, Allison and Walsby (*18*) reported that the photosynthesis-dependent turgor rise is due to the accumulation of potassium ions as well as photosynthate.

Increases in the RGV of N-limited cells are not, however, entirely explained by changes in turgor pressure. N-limited algae tend to have large carbohydrate reserves and are capable of high rates of N uptake and protein synthesis at the expense of those reserves (*19*). Furthermore, N enrichment alters the path of newly fixed carbon in such algae, and there is an increase in amino acid synthesis relative to sugar phosphate and sucrose synthesis (*20*). Thus an increase in N availability would not only reduce turgor pressure in previously N-deficient blue-green algae, but would also promote the synthesis of protein; that this could include gas-vesicle protein was indicated by the response of N-limited *O. rubescens* to increased availability of ammonium-nitrogen in a chemostat (*6*). Another condition known to favor increases in RGV (*4, 5*) and in the synthesis of protein relative to that of other compounds (*21*) is low light intensity. Hence, when N-limited *Oscillatoria* loses buoyancy and sinks in a stratified lake, the alga approaches a combination of conditions conducive to both protein and gas vesicle accumulation. In this manner, negative buoyancy responses to surface conditions (high light–nutrient poor) and positive responses to deepwater conditions (low light–nutrient rich) tend to keep *Oscillatoria* toward the bottom of the photic zone.

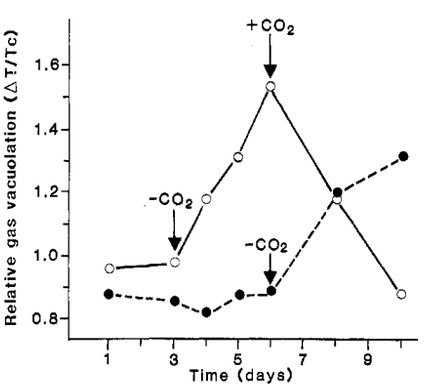

Fig. 2. Relative gas vacuolation (*10*) in two cyclostat cultures growing at constant dilution rates [0.41 day^{-1} in culture A (○) and 0.39 day^{-1} in culture B (●)]. In culture A, CO_2 deprivation began on day 3 and ended on day 6. In culture B, CO_2 deprivation began on day 6.

Whereas Walsby and Klemer (*15*) found that *O. agardhii* var. *isothrix* achieved positive buoyancy only at the base of the photic zone, Klemer, Pierson, and Whiteside (*12*) found that, when N and P were sufficient, this alga maintained its buoyancy and rose to the surface with no prior reduction in surface light intensity. In N- and P-sufficient alga, C_i limitation could promote bloom formation by preventing the depletion of nutrient reserves and the turgor collapse of gas vesicles in already buoyant alga or by permitting actual increases in gas vacuolation. In order for nutrient depletion to occur and for Walsby's turgor-collapse mechanism to operate, photosynthesis must increase with light intensity as the alga nears the surface. Dinsdale and Walsby (*17*) showed that turgor pressure does not increase with light intensity in CO_2-limited *Anabaena*. Thus, C_i limitation could prevent buoyancy reduction despite increased light intensity, and with already buoyant cells, this alone could account for a bloom. Schindler (*22*) has made a convincing case against C_i as a primary factor in the limitation of phytoplankton yields, but he and others (*23*) have reported C_i limitation of photosynthetic rates. As Talling (*24*) has indicated, the reduction of C_i to rate-limiting concentrations is most likely to occur in enriched lakes high in algal biomass and relatively low in alkalinity. Lake Oscaleta in New York is such a lake, and it was the site of the experiment by Klemer and Brasino (*14*) in which portions of an *Anabaena* bloom enriched in bicarbonate collapsed.

In our study, we found that RGV in *O. rubescens* could increase under C_i-limiting conditions. Others have shown that, as in the case of light limitation, the protein content of blue-green algae is relatively high under C_i limitation. Konopka and Schnur (*25*) obtained ratios of carbohydrate to protein in *Merismopedia tenuissima* that were four to seven times higher in P-, N-, or S-limited continuous

cultures than in C-limited continuous cultures or in nonlimited batch cultures. In chemostat cultures of *Anacystis nidulans*, Parrott and Slater (*26*) found that protein content as a percentage of dry weight was greater under CO_2 limitation than under light limitation. As mentioned above, increases in RGV have been associated with the onset of growth limitation in laboratory cultures and in bloom-forming populations of blue-green algae. We suggest that only those growth-limiting conditions that favor relative protein synthesis are likely to permit such increases in buoyancy and to promote surface blooms. Unlike N limitation, light and C_i limitation can meet this requirement.

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References and Notes

1. A. E. Walsby, *Sci. Am.* **237**, 90 (August 1977).
2. R. V. Smith and A. Peat, *Arch. Microbiol.* **58**, 117 (1967).
3. C. S. Reynolds, *Freshwater Biol.* **2**, 87 (1972); *Proc. R. Soc. London Ser. B* **184**, 29 (1973).
4. A. E. Walsby, *Proc. R. Soc. London Ser. B* **173**, 235 (1969).
5. ———, *ibid.* **178**, 301 (1971).
6. A. R. Klemer, *Verh. Int. Ver. Theor. Angew. Limnol.* **20**, 2293 (1978).
7. C. S. Reynolds and A. E. Walsby, *Biol. Rev. Cambridge Philos. Soc.* **50**, 437 (1975).
8. J. Feuillade and M. Feuillade, *Limnol. Oceanogr.* **24**, 562 (1979).
9. The cultures were kept at 20° ± 1°C in a constant-temperature room and were illuminated on a light:dark cycle of 18:6 with 1400 lux by a bank of 20-watt fluorescent lamps. Mixing was provided by filtered, compressed air, and the N-limiting medium was delivered by peristaltic pumps. The medium, Gorham's ASM diluted four times, was autoclaved and then supplemented with 40 mg of Na_2CO_3 per liter [P. R. Gorham, J. McLachlan, U. T. Hammer, W. K. Klin, *Verh. Int. Ver. Theor. Angew. Limnol.* **15**, 796 (1964)]. The reservoir concentration of N (S_R) was 3.5 mg of NO_3^--N per liter. Algal biomass was measured as absorbancy at 650 nm and as dry weight of culture effluent collected overnight on 0.8-μm Millipore filters and dried at 80°C to constant weight. Particulate N, expressed as a percentage of dry weight, was determined according to the ultramicro method of L. Strauch (*Z. Klin. Chem.* **3**, 165 (1965).
10. A. E. Walsby, *Limnol. Oceanogr.* **18**, 653 (1973). We used a Turner 111 fluorometer to measure sample turbidity before and after we collapsed the gas vesicles, and we expressed RGV as $\Delta T/T_c$, where ΔT represents the turbidity change that occurs with gas-vesicle collapse, and T_c represents the turbidity of the nonvacuolated cells. We corrected T_c for background turbidity by subtracting the turbidity of sample filtrates obtained with 5-μm Nucleopore filters. ΔT is proportional to gas vacuolation and provides an index of RGV when divided by a measure of algal biomass such as algal turbidity (T_c), absorbancy, or dry weight. In our axenic cultures, OD and dry weight varied closely with T_c ($r > .95$) and provided equally good measures of biomass. In duplicate determinations of RGV on four different dilutions of the same sample, the coefficient of variation was less than 4 percent.
11. A. R. Klemer, thesis, University of Minnesota, (1973); *Arch. Hydrobiol.* **78**, 343 (1976).
12. ———, D. Pierson, M. C. Whiteside, in preparation.
13. A. E. Walsby and M. J. Booker, *Br. Phycol. J.* **11**, 200 (1976); M. J. Booker and A. E. Walsby, *ibid.*, **16**, 411 (1981).
14. A. R. Klemer and T. L. Brasino, in preparation.
15. A. E. Walsby and A. R. Klemer, *Arch. Hydrobiol.* **74**, 375 (1974).
16. A. E. Konopka, T. D. Brock, A. E. Walsby, *ibid.* **83**, 524 (1978).
17. M. T. Dinsdale and A. E. Walsby, *J. Exp. Bot.* **23**, 561 (1972).
18. E. M. Allison and A. E. Walsby, *ibid.* **32**, 241 (1981).
19. P. J. Syrett, *Ann. Bot. (London)* **17**, 20 (1953); in *Physiology and Biochemistry of Algae*, R. A. Lewin, Ed. (Academic Press, London, 1962), p. 171; A. E. Konopka and M. Schnur, *J. Phycol.* **17**, 118 (1981).
20. G. E. Fogg, *Ann. Bot. (London)* **20**, 265 (1956); R. A. Pelroy and J. A. Bassham, *Arch. Microbiol.* **86**, 25 (1972); A. C. Lawrie, G. A. Codd, W. D. P. Stewart, *ibid.* **107**, 15 (1976).
21. A. E. Konopka and M. Schnur, *Microb. Ecol.* **6**, 291 (1980).
22. D. W. Schindler, *J. Phycol.* **7**, 321 (1971).
23. ——— and E. J. Fee, *J. Fish. Res. Board Can.* **30**, 1501 (1973); J. E. Burris, R. Wedge, A. Lane, *J. Freshwater Ecol.* **1**, 81 (1981).
24. J. F. Talling, *J. Ecol.* **64**, 79 (1976).
25. A. E. Konopka and M. Schnur, *J. Phycol.* **17**, 118 (1981).
26. L. M. Parrott and J. H. Slater, *Arch. Microbiol.* **127**, 53 (1980).
27. We thank E. Menthon and V. Hink for assistance in the laboratory and J. Shapiro, A. E. Konopka, and A. E. Walsby for their comments on early drafts of the manuscript. This work was supported by State University of New York Research Foundation fellowships to A.R.K. during the summers of 1977 and 1979.

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Fast-Growing Rhizobia Isolated from Root Nodules of Soybean

Abstract. *Fast-growing rhizobia have been isolated from soybean root nodules collected in China. These new isolates are physiologically distinct from slow-growing soybean rhizobia. They formed effective nitrogen-fixing associations with wild soybean and an unbred soybean cultivar from China, but were largely ineffective as nitrogen-fixing symbionts with common commercial cultivars of soybeans.*

Rhizobium isolates from nodules of domesticated soybeans and the wild progenitor species of soybeans (*Glycine soja* Sieb. and Zucc.) obtained from three recent expeditions to China (*1*) have been characterized. In all three collections, we found fast-growing *Rhizobium*. They were obtained from the east-central provinces of Shansi, Honan, Shandong, and Shanghai, which are included in the area considered to be the center of origin and diversity for the soybean (*2*). Presumably, the center of origin for its nitrogen-fixing microsymbiont *Rhizobium japonicum* is in the same region. These fast-growing isolates are a previously undescribed group of *Rhizobium* that may be useful in the genetic improvement of *Rhizobium* strains for soybean production.

Rhizobium are bacteria capable of forming a nitrogen-fixing symbiosis with leguminous plants. The taxonomic status of *Rhizobium* is controversial because it is based on host infectivity (*3*). For example, strains forming nodules on the soybean plant are given the species rank *R. japonicum*. Within designated species, the bacteria exhibit fairly uniform biochemical characteristics. Two important manifestations of biochemical differences, growth rate and acid production, have been used in differentiating rhizobia with different host affinities (*3, 4*).

Fast-growing species of rhizobia (*R. meliloti, R. leguminosarum, R. trifolii,* and *R. phaseoli*) usually have generation times of 2 to 4 hours, whereas the slow-growing species (*R. japonicum* and *R. lupini*) have generation times of six or more hours (*5*). A fast growth rate is associated with an acid reaction in mannitol medium, whereas slow growth is usually accompanied by an alkaline reaction. A comparison of growth rates and acid production of fast- and slow-growing *R. japonicum* and of other fast-growing species shows that the new soybean rhizobia show more resemblance to other fast-growing species of rhizobia than to typical slow-growing *R. japonicum* (Table 1).

All ten of the soybean cultivars tested formed nodules when inoculated with pure cultures of the fast-growing rhizobia (Table 2). However, only on cultivar Peking [a black-seeded genetically unimproved line from China (*6*)] did the fast-growing *R. japonicum* form effective nitrogen-fixing symbioses. On the commercial soybean cultivars, these bacteria were either completely ineffective or only poorly effective in the fixation of nitrogen. They also formed an effective symbiosis with *G. soja*, the putative wild ancestor-progenitor of the cultivated soybean (*7*). The fast-growing isolates formed ineffective nodules with two *Macroptilium* species and *Sesbania cannabina* Roxb., hosts that associate readily with fast- and slow-growing *Rhizobium*.

Cultures of the fast-growing isolates were verified as *R. japonicum* by repeated soybean (Peking) infection and isolation tests. These cultures were scrutinized for the presence of slow-growing colonies that might be masked by the fast-growing type. None were found.

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