carrier adults (*11*). (ii) Parotid saliva from subjects with CF and their heterozygous parents contains a greater number of "fast isoamylases" than do controls (*12*). It is thought that these isozymes of amylase migrate faster in electrophoretic fields because of deamidation of asparagine and glutamine residues (*13*). Deamidation of proteins in general (*14*) and parotid salivary amylase specifically (*15*) have been related to aging. (iii) Eosinophilic plugs were shown to occur frequently in the acini of the labial mucous salivary glands of children with CF. These plugs were frequently found in minor salivary glands of normal adults but were rarely observed in normal children less than 19 years old (*16*). It appears, therefore, that premature senescence in cultured skin fibroblasts from subjects with CF is not an isolated aging phenomenon in this disease. The abnormal gene product or products responsible for CF may have a specific effect on one or more factors involved in aging. It is of some interest that accumulation of cellular calcium has been discussed as a factor in aging (*17*). Intracellular calcium pool size is greater by about 30 percent in skin fibroblasts from CF and CF carriers in comparison with their respective controls (*5*, *18*). The reported calcium differences and the premature senescence in cultured skin fibroblasts from CF subjects may have implications for aging studies as well as for CF.

Our findings suggest that CF be added to that group of human diseases in which skin fibroblasts in culture express premature aging (*19*). Furthermore, our data reconcile controversial PDT findings by showing that at early passages no PDT differences exist between CF and controls and that at late passages CF cells do indeed replicate more slowly. Also, experiments with late-passage fibroblasts may generate misleading results in that observed differences between CF and control cell lines may be ascribed to generalized senile changes rather than to specific results of the CF genotype. Skin fibroblasts are a useful model for the study of CF. In view of the data presented here, however, we caution that interesting findings concerned with fibroblasts in CF be repeated with early-passage cells to avoid potentially misleading conclusions.

Note added in proof: Since this report was accepted, we have found that incorporation of tritiated thymidine into DNA and plating efficiency of cells support the cell population kinetic data. Incorporation of tritiated thymidine into DNA was significantly decreased in cells from CF subjects in comparison with controls at passages 10 to 12; no differences occurred at passages 4 to 7. Plating efficiency was no different at passages 5 to 7; but at passages 11 to 13, significantly fewer clones were formed from CF strains in comparison with controls.

BURTON L. SHAPIRO
LOUIS F.-H. LAM
LORRAINE H. FAST

Division of Oral Biology, University of Minnesota, Minneapolis 55455

References and Notes

1. Cystic fibrosis is characterized clinically by chronic obstructive lung disease and generalized gastrointestinal malabsorption and their sequelae, and increased sweat salt.
2. "Tissue culture approaches to the study of cystic fibrosis," *Cystic Fibrosis Conference Reports* (Cystic Fibrosis Foundation, Atlanta, Ga., 1978), vol. 2, No. 1.
3. E. C. Raff and J. C. Houck, *J. Cell. Physiol.* **74**, 235 (1963); J. C. Houck and V. K. Sharma, *Proc. Soc. Exp. Biol. Med.* **135**, 369 (1970); J. C. Houck and F. L. Cheng, *ibid.* **147**, 167 (1974); D. S. Fletcher and T.-Y. Lin, *Clin. Chim. Acta* **44**, 5 (1973); D. W. Welch and R. M. Roberts, *Pediatr. Res.* **9**, 698 (1975).
4. P. M. Farrell, J. C. Pallavicini, M. M. Ulane, *Proc. Soc. Exp. Biol. Med.* **149**, 340 (1975); W. E. Bolton and S. C. Barranco, *Am. J. Hum. Genet.* **27**, 394 (1975); S. C. Barranco, W. E. Bolton, B. R. Haenet, *J. Cell. Physiol.* **83**, 33 (1976).
5. B. L. Shapiro, R. J. Feigal, N. J. Laible, M. H. Biros, W. J. Warwick, *Clin. Chim. Acta* **82**, 125 (1978).
6. Least-squares lines were calculated for the number of weekly doublings in each line plotted against week in culture. In this and the experiment shown in Fig. 2, weekly cell doubling did not decline and in fact increased during the first portion of the study. Therefore, the regressions were calculated from data from 7 weeks (the time of maximum average doublings) until 18 weeks in culture (the time of termination of the experiment). Regressions in Fig. 2 were calculated from 11 weeks of subculture. Estimates of onset of senescence from the regression lines were based on the projected (Fig. 1) or actual (Fig. 2) intersect of the regression lines with 1.0 doubling.
7. Findings with cell lines from the 24-year-old CF subject were almost identical to results in lines from the younger CF subjects. Accordingly, cells from this older CF subject for whom there was no age-matched control did not account for the differences between CF and control strains.
8. L. Hayflick and P. S. Moorhead, *Exp. Cell Res.* **25**, 585 (1961); L. Hayflick, *ibid.* **37**, 614 (1965); E. L. Schneider and C. J. Epstein, *Proc. Soc. Exp. Biol. Med.* **141**, 1092 (1972); S. Goldstein, E. J. Moerman, J. S. Soeldner, R. E. Gleason, D. M. Barnett, *Science* **199**, 781 (1978).
9. A possible explanation for the earlier onset of declining cell population doubling in the experiment shown in Fig. 1 in comparison with the experiment in Fig. 2 may be related to cell density at the time of seeding each week. In the first experiment, 6.5×10^5 cells were seeded in 75-cm^2 flasks and were, therefore, about 35 percent more dense than in the second experiment in which 1.6×10^5 cells were seeded in 25-cm^2 flasks.
10. H. S. Nadler, G. J. S. Rao, L. M. Taussig, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, Eds. (McGraw-Hill, New York, ed. 4, 1978).
11. C. C. Lobeck, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, Eds. (McGraw-Hill, New York, ed. 3, 1972).
12. K.-M. Doering, *Eur. J. Pediatr.* **126**, 185 (1977).
13. P. J. Keller, D. L. Kauffman, B. J. Allan, B. L. Williams, *Biochemistry* **10**, 4867 (1971).
14. A. B. Robinson, J. H. McKerrow, P. Cary, *Proc. Natl. Acad. Sci. U.S.A.* **66**, 653 (1970).
15. C. Arglebe, R. Chilla, M. Opaitz, *Clin. Otolaryngol.* **1**, 249 (1976); R. Chilla, K.-M. Doering, H. Lubahn, C. Arglebe, *Arch. Oto-Rhino-Laryngol.* **214**, 367 (1977).
16. L. R. Sweney, M. C. Hedrick, L. H. Meskin, W. J. Warwick, *Pediatrics* **40**, 421 (1967); L. R. Sweney and W. J. Warwick, *Arch. Pathol.* **86**, 413 (1968).
17. B. L. Strehler, *Time, Cells, and Aging* (Academic Press, New York, ed. 2, 1977).
18. R. J. Feigal and B. L. Shapiro, *Pediatr. Res.*, in press.
19. G. M. Martin, in *Genetic Effects on Aging*, D. Bergsma and D. E. Harrison, Eds. (Liss, New York, 1978).
20. Supported by a State of Minnesota Special Allocation for Cystic Fibrosis Research and the Cystic Fibrosis Foundation. We thank W. J. Warwick and N. J. Laible for assistance.

25 August 1978; revised 17 October 1978

Methane Efflux from Lake Sediments Through Water Lilies

Abstract. *During winter, when water lilies have no surface leaves, the gases in the rhizome lacunae approach equilibrium with the gases of the sediment water. The resulting increase of internal pressure is manifested by the sustained streams of bubbles (up to 37 percent methane and 6 percent carbon dioxide) that escape when emerging leaves are torn in the spring. Methane continues to enter the roots and rhizome during summer, rapidly moves up the petioles, and passes out through the emergent leaves into the atmosphere.*

The importance of methane to the carbon cycle of lake ecosystems is beginning to be understood. It has been estimated that nearly half the organic carbon reaching the sediment of eutrophic lakes leaves the sediment as CH_4, either in dissolved form or as bubbles (*1*-*3*). In establishing the CH_4 budgets for lakes, investigators have thought that the only escape route from the lake is across the air-water interface, either as bubbles originating in the sediment or through limited surface exchange (*1*-*4*). We report here on another interface of potential significance in the loss of CH_4 to the atmosphere, the interface between the sediment water and the internal gas phase of rooted aquatic plants. We have found that a high percentage of the CH_4 leaving the littoral zone of eutrophic Duck Lake (*5*) during August escapes through the gas passages of the water lily *Nuphar luteum*.

Nuphar luteum, a yellow water lily, perennates in lakes by means of a horizontal creeping rhizome at or just below the surface of the lake sediment. The rhizome may be up to 10 cm in diameter and

0036-8075/79/0323-1253$00.50/0

several meters long, accounting for roughly 80 percent of the plant's biomass during the summer growth period. The rhizomes often branch, producing several growing apices. Each growing apex bears a rosette of leaves that rise up through the water on long petioles.

A striking feature of the internal anatomy of aquatic plants is an extensive system of gas spaces or lacunae. In *Nuphar* the lacunae constitute between 20 and 40 percent of the root and rhizome volume and about 50 percent of the petiole volume. These lacunae facilitate oxygen supply to the plant parts deep in the anaerobic sediment (*6*). Furthermore, our studies indicate that gases dissolved in the interstitial water of the sediment freely diffuse into these gas spaces.

During winter, when water lilies have no surface leaves, the percentage composition of the internal gases in the rhizome (CH_4, 37; N_2, 54; CO_2, 6; O_2, 1; H_2O, 1; and Ar, < 1) approaches equilibrium with the gases in the interstitial water of the sediment (CH_4, 40; N_2, 54; CO_2, 4; H_2O, 1; and Ar, < 1) (*7*). Furthermore, the total pressure of the gas in the rhizome is considerably higher than atmospheric. We have repeatedly measured internal gas pressures which approach the hydrostatic pressure at rhizome depths. This internal pressure is further illustrated in the spring when the first leaves that emerge are often seen to be bubbling or can be torn to initiate sustained streams of bubbles (Fig. 1). The composition of the bubbles reflects the composition of the rhizome gases (up to 37 percent CH_4). Once the leaves reach the surface, the internal pressure dissipates and the pressure and gas composition in the rhizome closely resemble those of the atmosphere. Within 2 days after the removal of these surface leaves from an isolated section of the rhizome, the pressure and composition once again approach the values of the interstitial water of the sediment. Thus leaves appear to operate as efficient gas exchangers between the rhizome and the atmosphere. This exchange results in a flux of gases from the interstitial water of the sediment to the atmosphere.

Methane has been shown to be a constituent of the gas phase of other aquatic plants during the summer (*8*), and we have found it in nearly all parts of the water lilies. The CH_4 concentration in the plant during the summer growth period ranges from 0 to 10 percent. The CH_4 composition in the rhizome varies diurnally with the highest concentrations found at daybreak and the lowest at midday. The low values at midday indicate an outward flux of CH_4 from the plant.

Table 1. Rates of efflux of CH_4 through the water lily leaves (in milliliters per leaf per hour). At each depth sets of six emergent leaves were bagged over time intervals of at least 30 hours. The bags were sampled at roughly 6-hour intervals. The increase in the leaf-specific efflux rate with increasing depth may be due to a corresponding decrease in the aerial density of leaves (that is, the effect of depth is greatly diminished when leaf efflux rates are expressed as milliliters of CH_4 per square meter of lake surface per hour). The diurnal pattern of efflux is thought to reflect the pattern of transpiration of the plants.

Water column depth (cm)	Time	
	0600 to 1800 hours	1800 to 0600 hours
40 to 50	1.70 ± 0.25	0.34 ± 0.07
50 to 70	2.57 ± 0.63	0.54 ± 0.11
70 to 120	3.69 ± 0.86	0.68 ± 0.21

We measured the rates of CH_4 efflux from emergent leaves enclosed in Saran bags (*9*). The efflux rates ranged from zero to more than 10 ml of CH_4 per leaf per hour (mean, 1.6). The highest rates of CH_4 efflux for individual leaves were observed during midday, and they fell to roughly one-fifth that rate at night (Table 1). A similar diurnal pattern has been observed in a related process involving the escape of Hg vapor from sediments through the leaves of the reed grass *Phragmites communis* (*10*). Kozuchowski and Johnson accounted for the pattern of efflux largely on the basis of transpiration rates.

Fig. 1. Bubbles containing up to 35 percent CH_4 streaming from an injured leaf of *Nuphar luteum* as it emerges during April. The accumulation of sediment gases in the rhizome during winter pressurizes the internal gas phase until the spring emergence of leaves allows these gases to escape to the atmosphere. This leaf was situated about 15 cm below the lake surface when it was torn intentionally to demonstrate the positive pressure of the internal gas phase.

In order to ascertain the limnological significance of the observed CH_4 fluxes through the plants, we used surface traps to determine the rates of CH_4 loss across the lake surface independent of the plant efflux (*11*). The observed rates are similar to those measured in other eutrophic lakes by Barber (*12*) for Lake Wingra, Wisconsin, and Robertson (*2*) for Frains Lake, Michigan.

Comparison of the rates of efflux across the lake surface with the rates of leaf efflux through *Nuphar* leaves (*13*) indicates that 75 percent of the CH_4 leaving the littoral zone escapes through the plants. On the basis of estimates of the density distribution of emergent *Nuphar* leaves on Duck Lake (*5*), the average rate of CH_4 efflux from the entire lake during 2 weeks in August is calculated to be 22 mmole m^{-2} day^{-1} (roughly 500 ml m^{-2} day^{-1}); 46 percent of that flux flows through *Nuphar* (*14*).

Methane is an important product of anaerobic decomposition in the sediments of eutrophic lakes, and its escape to the atmosphere represents a loss of energy and carbon from the lake system. Although CH_4 has been reported in a number of aquatic plants, the ecological significance of its presence has not been recognized. In essence, the lacunae in emergent aquatic plants represent an extension of the atmosphere into the sediment water, and a significant exchange of gases occurs across this interface. In the areas of Duck Lake inhabited by water lilies, most of the CH_4 escaping to the atmosphere passes from the sediment water into the lacunae of the roots and rhizomes and rapidly escapes to the atmosphere through the petioles and leaves.

J. W. H. Dacey
M. J. Klug

W. K. Kellogg Biological Station,
Michigan State University,
Hickory Corners 49060

References and Notes

1. J. W. M. Rudd and R. D. Hamilton, *Limnol. Oceanogr.* **23**, 337 (1978).
2. C. K. Robertson, *Arch. Hydrobiol.*, in press.
3. J. J. Molongoski and M. J. Klug, in preparation.
4. Most of the CH_4 dissolved in the water column is oxidized by bacteria in the interface between the oxygenated surface water or epilimnion and the anoxic CH_4-bearing hypolimnion (*1*); thus, the diffusive loss of CH_4 across the air-water interface is limited while the lake is thermally stratified.

5. Duck Lake, Kalamazoo County, Mich., is a eutrophic kettle lake with a maximum depth of 3.8 m and a mean depth of 2.0 m [M. C. Miller, thesis, Michigan State University (1972)]. During the summer of 1977, 52 percent of the surface area was covered by water lilies at densities of 10 to 20 leaves per square meter.
6. G. E. Hutchinson, *A Treatise on Limnology*, vol. 3, *Limnological Botany* (Wiley, New York, 1975); H. S. Conard, *The Waterlilies* (Publication 4, Carnegie Institution of Washington, Washington, D.C., 1905); H. E. Laing [*Am. J. Bot.* **27**, 861 (1940)] analyzed the distribution of gases in *Nuphar* sp. but did not report the presence of CH_4, probably because of analytical problems.
7. Bubbles were collected in a funnel held immediately above the sediment surface, and the gas composition was determined by gas chromatography.
8. R. T. Hartman and D. L. Brown, *Limnol. Oceanogr.* **11**, 109 (1966); R. S. Oremland and B. F. Taylor, *ibid.* **22**, 566 (1977). We have also found CH_4 in *Nymphaea odorata*, *Brasenia schreberi*, *Ceratophyllum demersum*, *Polygonum* sp., and *Typha* sp. We sampled the gases by syringe directly from plants in situ or immediately after they had been uprooted. Samples were analyzed by gas chromatography.
9. A low-permeability Saran bag (Anspec Company, Ann Arbor, Mich.) measuring 30 cm by 60 cm was tied closed around the petiole of the leaf. The gas in the bag was sampled through a serum stopper, and the CH_4 concentration was determined by gas chromatography. The volume of the bag was determined at the end of each measurement by the addition of a CH_4 internal standard. The bags are transparent and did not appear to influence leaf behavior. The CH_4 entered the bags within the first few minutes after attachment to the leaves at rates comparable to the rates observed over longer time intervals. Moreover, there was no significant divergence in the petiole gas composition of bagged leaves and adjacent nonbagged leaves.
10. The report by J. Kozuchowski and D. L. Johnson [*Nature (London)* **274**, 468 (1978)] is the first we know of that shows that gases originating in the sediment move through plants to the atmosphere. The rates of efflux they report are very much smaller ($\sim 10^{-5}$) than ours; the difference can be accounted for largely on the basis of the difference in the vapor pressures of Hg and CH_4 in the respective sediments. They suggest that the diurnal pattern of efflux from the plants results from variation in the transpiration rate.
11. The rates were determined with surface gas traps sampled at roughly 6-hour intervals. The insides of Styrofoam cartons (used for shipping acid bottles) were coated with a polyurethane sealant and floated on the lake surface, forming four-chambered surface traps. Any CH_4 entering the traps was diluted in the 1.8-liter atmosphere of the coated chamber. Such a coated chamber lost less than 0.7 percent of its CH_4 per hour. Experiments in which oil was used to slow surface exchange within the floats has revealed that most of the CH_4 accumulating in the traps is the result of ebullition of sediment gas.
12. L. E. Barber, thesis, University of Wisconsin (1974).
13. The leaf efflux value for the littoral zone (19.7 ± 1.6 mmole m^{-2} day^{-1}) is the mean for 18 leaves weighted according to the aerial density of the leaves on the lake. Surface efflux values (littoral zone, 6.7 ± 0.7; limnetic zone, 17.7 ± 2.2 mmole m^{-2} day^{-1}) are derived from extensive data collected over several weeks by gas traps placed on the lake surface (not over emergent leaves). The efflux rate for the littoral zone is therefore the sum of the leaf and surface fluxes; this combined flux is greater than that observed for the open water of the limnetic zone.
14. The total contribution of plants to this efflux in Duck Lake is probably higher than indicated. *Nymphaea odorata*, a white water lily, is fairly abundant in the deeper regions of the littoral zone; from preliminary data on these plants it appears that the flux rates per leaf are comparable to those of *Nuphar*.
15. We thank K. E. Hogg, J. J. Molongoski, E. D. Goodman, D. J. Hall, B. Laughlin, and collaborators in our laboratory. The work was supported by National Science Foundation grant DEB-76-06884 to M.J.K. and J. M. Tiedje and by Environmental Protection Agency grant R803859 to E. D. Goodman. Kellogg Biological Station publication 369; Michigan Agricultural Experiment Station journal article 8407.

13 November 1978

Hydrogenase in *Rhizobium japonicum* Increases Nitrogen Fixation by Nodulated Soybeans

Abstract. *Some* Rhizobium *strains synthesize a unidirectional hydrogenase system in legume nodule bacteroids; this system participates in the recycling of hydrogen that otherwise would be lost as a by-product of the nitrogen fixation process. Soybeans inoculated with* Rhizobium japonicum *strains that synthesized the hydrogenase system fixed significantly more nitrogen and produced greater yields than plants inoculated with strains lacking hydrogen-uptake capacity.* Rhizobium *strains used as inocula for legumes should have the capability to synthesize the hydrogenase system as one of their desirable characteristics.*

The increasing demand for protein and the high cost of producing nitrogen fertilizer pose problems that may be solved in part by more extensive use of biological nitrogen fixation. Soybeans and most leguminous plants form root nodules whose associated bacteria can convert N_2 to ammonia. However, the enzyme that catalyzes the reduction of N_2 to ammonia also produces H_2 as a by-product (*1*), and H_2 production represents a loss of energy that otherwise would be available for N_2 fixation. We now show how this H_2 can be recycled with an accompanying increase in the dry weight and nitrogen content of plants.

Hoch *et al.* (*2*) were the first to observe H_2 evolution from nodules of soybeans. Dixon (*3*) reported that nodules of *Pisum sativum* formed by strain ONA 311 of *Rhizobium leguminosarum* evolved no H_2 but utilized H_2 from an external supply. We observed consistent O_2-dependent H_2 consumption by nodules from several legumes that evolved little or no H_2 under aerobic conditions. A survey (*4*) has revealed that the N_2-fixing potential of many legume-rhizobial associations may be decreased through H_2 losses. Estimates of losses from many agricultural legumes has ranged between 20 to 40 percent of the electron flow through nitrogenase (*5*). Dixon (*3*) concluded that the extent of H_2 loss was influenced by the host legume, but Carter *et al.* (*6*) observed no consistent effect of several different soybean cultivars on H_2 losses from nodules formed by selected strains of *R. japonicum*. There is evidence (*7, 8*) that environmental conditions influence the magnitude of H_2 evolution from nodules of *Pisum sativum*.

Some rhizobial bacteroids possess two enzyme-catalyzed reactions that participate in H_2 metabolism. These include the adenosine triphosphate (ATP)-dependent H_2 evolution reaction of nitrogenase and unidirectional hydrogenase which catalyzes H_2 oxidation in presence of an appropriate acceptor (*5*). Emerich *et al.* (*9*) have shown that H_2 oxidation by bacteroid suspensions increased the ATP content of bacteroids and protected nitrogenase from O_2 damage. Our objective, therefore, was to determine whether the observed physiological benefits of the hydrogenase system could be measured by increased N_2 fixation and growth of nodulated soybeans. We describe greenhouse and growth chamber experiments that compare yields and total N contents of soybean plants inoculated with H_2-uptake positive and H_2-uptake negative strains of *R. japonicum*.

In the first experiment we used five H_2-uptake positive strains and five H_2-uptake negative strains as inocula for soybeans in growth chambers. The strains included in the groups of positive and negative strains were selected on the basis of their capacities to produce reasonably comparable nodule weights and nitrogenase activities in previous experiments where 32 strains were surveyed (*6*) (legend of Table 1). We assumed random distribution of unknown genetic variability among all strains of the two groups that were compared. Capacities to form the hydrogenase system in nodules by the two groups, however, were strikingly different (Table 1).

In the first experiment (Table 1) the nodule weights of plants inoculated with the H_2-uptake negative strains were greater than those inoculated with the H_2-uptake positive strains. Relative efficiency values, which are estimates of the proportion of electron flow through nitrogenase that is utilized for N_2 reduction (*4, 5*) ranged from 0.97 to 1.00 for nodules formed by H_2-uptake positive strains and from 0.66 to 0.84 for nodules formed by the H_2-uptake negative strains. Nodules formed by the H_2-uptake negative strains (Table 1) reduced acetylene at high rates and all evolved H_2. In contrast, nodules formed by the group of H_2-uptake positive strains (Table 1) evolved little or no H_2 and thus showed relative efficiencies near 1.0. Acetylene reduction rates for the two groups of strains, however, were not appreciably different. In comparison with

0036-8075/79/0323-1255$00.50/0