22. F. J. Hawco and P. J. O'Brien, in preparation.
23. C. S. Foote, in *Free Radicals in Biology*, W. A. Pryor, Ed. (Academic Press, New York, 1976), vol. 2, p. 85.
24. Classical drug metabolic pathways, such as aniline hydroxylation and aminopyrine demethylation, are not blocked by mannitol or benzoate (*16*), two powerful ·OH scavengers. Our study shows that at least one new drug, DMSO, in addition to primary aliphatic alcohols, can be metabolized by microsomes. Methional and KTBA are also metabolized by microsomes. Therefore, further study of the role of ·OH in the metabolism of other xenobiotic agents (such as other sulfoxides) is warranted.
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Microbial Autotrophy: A Primary Source of Organic Carbon in Marine Sediments

Abstract. *The chemoautotrophic fixation of carbon dioxide by bacteria is responsible for an appreciable component of the organic carbon in a sulfide-rich marine mud. A peak of carbon dioxide fixation (at 40 centimeters subbottom) coincides with peaks in the organic carbon content, the ratio of carbon to nitrogen, and bacterial cell counts. Stimulation of fixation by thiosulfate and inhibition by anaerobic conditions implicate the chemoautotrophic sulfur bacteria as primary producers in this environment.*

The existence of autotrophic bacteria capable of synthesizing organic carbon by the reduction of CO_2 is well documented (*1*). However, their effect on the marine ecosystem is poorly understood despite their importance as the only primary producers of organic carbon other than the algae. We report here the first evidence indicating that microbial chemoautotrophs are generating appreciable quantities of organic carbon in marine sediments.

We analyzed acidified and dried subsamples of a diver-collected core from the sulfide-rich muds of Halifax Harbor, Nova Scotia, for their organic carbon content and their ratio of carbon to nitrogen, using a carbon-hydrogen-nitrogen analyzer (Hewlett-Packard 185) (precision of the carbon determination, ± 0.005 percent of the dry sediment weight). A peak of organic carbon is evident at 40 cm subbottom (Fig. 1), and a corresponding peak in the carbon/nitrogen ratio indicates that the organic material at this depth is made up of carbon-rich compounds. In addition, direct counts of bacteria made with an epifluorescent staining method (*2*) show a peak of total (living and dead) cell numbers at the same depth (Fig. 1).

A separate set of samples from the same depth intervals in the core were incubated aerobically, in darkness and at in situ temperature (2°C) for 15 days with 0.11 μM $NaH^{14}CO_3$ (New England Nuclear). The macromolecular products of the incubation were precipitated with trichloroacetic acid, and the acidified slurry was bubbled at *p*H 2.0 to ensure the removal of any residual $^{14}CO_2$. The macromolecular and low-molecular-weight fractions were then separated by filtration (*3*), and the amount of ^{14}C incorporated into both organic fractions was determined by liquid scintillation counting (*4*). We determined the total unlabeled carbonate component of the pore water used in the incubations (4.49 mM CO_2 per liter) by bubble-stripping an acidified sample (*5*) and measuring the total CO_2 on an infrared analyzer (precision, ± 0.03 mM). The amount of CO_2 taken up from the unlabeled carbonate pool is shown as a profile in Fig. 1, and a maximum is evident at the 40-cm horizon. The problem of distinguishing between autotrophic and heterotrophic uptake of CO_2 (*6*) is not a major one at this horizon. Heterotrophic fixation of CO_2 requires energy from the oxidation of organic carbon and would not produce simultaneously the peaks of organic carbon and CO_2 fixation observed.

The chemoautotrophic sulfur bacteria (*7*) play at least a partial role in the uptake of CO_2, as shown in the results of additional incubations of sediment from 40 cm subbottom (Fig. 2). Uptake is enhanced by incubation with 1 mM sodium thiosulfate ($Na_2S_2O_3$), which indicates the presence of bacteria that are capable of oxidizing reduced forms of sulfur as an energy source for biosynthesis. In addition, the inhibition of CO_2 uptake in samples incubated under N_2 shows that O_2 is required, although possibly at low concentrations (*6*).

Our experiments were designed to simulate the natural microbial environment in that we allowed mixed bacterial populations to develop without applying the pure culture technique of media enrichment to favor the autotrophs. The experiments demonstrate that a microbial mechanism of organic carbon enrichment is present in the sediment examined. Jørgensen (*8*) has stated that many of the sulfur-oxidizing autotrophs

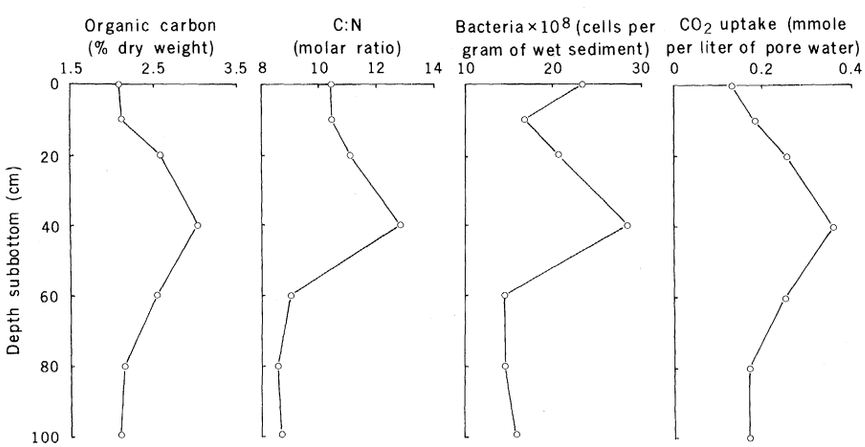

Fig. 1 (left). Distribution of organic carbon, the ratio of carbon to nitrogen, total bacterial cell counts, and CO_2 uptake with depth in a core from sulfide-rich marine muds.

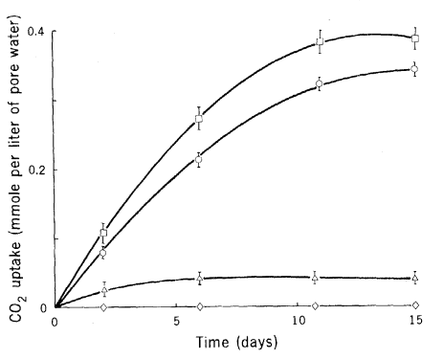

Fig. 2 (right). Uptake of CO_2 as a function of time in samples incubated aerobically without $Na_2S_2O_3$ (○), incubated aerobically with 1 mM $Na_2S_2O_3$ (□), incubated anaerobically (△), or killed and fixed with 3 percent gluteraldehyde at time zero and incubated aerobically (◇). Error bars delineate the standard deviation around the mean uptake values of triplicate incubations.

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are best adapted to an environment requiring the O_2 of aerobic conditions and the reduced forms of sulfur present only in anoxic conditions. The horizon of maximum CO_2 uptake that we have described represents this transition from oxygenated to underlying anoxic conditions (*9*).

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References and Notes

1. R. Y. Stanier, E. A. Adelberg, J. Ingraham, *The Microbial World* (Prentice-Hall, Englewood Cliffs, N.J., 1976).
2. J. E. Hobbie, R. J. Daley, S. Jasper, *Appl. Environ. Microbiol.* **33**, 1225 (1977).
3. D. Kennell, in *Methods in Enzymology*, C. Grossman and K. Moldave, Eds. (Academic Press, New York, 1967), vol. 12, p. 686.
4. R. A. Vollenwieder, *A Manual on Methods for Measuring Primary Production in Aquatic Environments* (Blackwell Scientific, Oxford, 1974).
5. R. C. Cooke, *Mar. Chem.* **5**, 75 (1977).
6. J. G. Kuenen, *Plant Soil* **43**, 49 (1975).
7. W. Vishniac and M. Santer, *Bacteriol. Rev.* **21**, 195 (1957).
8. B. B. Jørgensen, *Mar. Biol.* **41**, 19 (1977).
9. P. E. Kepkay, J. A. Novitsky, R. C. Cooke, in preparation.
10. We thank Dr. R. M. Moore for critically reading the manuscript. The work was supported by the National Research Council of Canada (grants A6508, A8358, and G0078).

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Genetic Mapping of the Ecotropic Murine Leukemia Virus–Inducing Locus of BALB/c Mouse to Chromosome 5

Abstract. *By means of an approach that combined the techniques of somatic cell genetics and Mendelian breeding studies, the inducibility locus, designated* Cv, *for ecotropic murine leukemia virus in BALB/c mice, was mapped to chromosome 5, 23 units from the locus for phosphoglucomutase-1, with gene order* Cv-Pgm-1-Gus. *This low-efficiency inducibility locus is therefore not allelic with the chromosome 7 loci previously described for two other mouse strains with high virus inducibility. These studies provide further evidence that endogenous ecotropic viruses represent viral genomes inserted at different chromosomal sites in the various mouse strains.*

Many inbred mouse strains carry the genetic information for production of ecotropic (mouse-infectious) murine leukemia viruses (MuLV's); however, there are marked differences among strains in the level of virus expression. Crosses between high-lymphoma, high-virus strains such as AKR and virus-negative strains have shown that high-virus mice generally carry multiple genetic loci for virus induction (*1*), and that these loci represent chromosomally integrated viral genomes (*2*). In contrast to high-virus mice, many strains with a lower incidence of leukemia, such as BALB/c, yield virus sporadically, in low titer, and relatively late in life. Similarly, virus can be induced by 5-iododeoxyuridine (IdU) with far greater efficiency from cell cultures from high-virus mice than from low-virus strains (*3*). Genetic studies with BALB/c mice have suggested that there is single gene control of ecotropic virus inducibility (*4*), and that different genes govern the inducibility of ecotropic and xenotropic virus (*5*). More recent studies with BALB/c mice have shown that embryo fibroblasts of backcross mice inducible for ecotropic virus contain the DNA sequences specific for this virus while cells of non-inducible segregants do not (*6*). These findings indicate that, as in AKR, the BALB/c viral inducibility locus contains viral structural genes.

In this report we describe the assignment of the inducibility locus for the infectious ecotropic virus of BALB/c mice to chromosome 5 using somatic cell genetics, and we confirm this assignment and establish the intrachromosomal location of this gene by Mendelian breeding studies. Our studies complement the work of Ihle *et al.* (*7*), in which the C3H/HeJ and BALB/c endogenous virus loci identified by serological assays and DNA hybridization were also mapped to chromosome 5.

Somatic cell hybrids were made between the peritoneal cells of BALB/c mice and cells of the Chinese hamster cell line, E36, by methods previously described (*8*). Thirty-nine primary hybrid clones were isolated in HAT (*9*) selective medium in two independent fusion experiments. Parallel cultures of each hybrid clone were used to test for expression of mouse isozyme phenotypes and ecotropic virus inducibility. Cell extracts were assayed for the expression of ten isozyme markers by vertical starch gel electrophoresis (*10*); dipeptidase-1 (*Dip-1*; chromosome 1), dipeptidase-2 (*Dip-2*; chromosome 18), tripeptidase-1 (*Trip-1*; chromosome 10), phosphoglucomutase-1 (*Pgm-1*; chromosome 5), phosphoglucomutase-2 (*Pgm-2*; chromosome 4), glucose phosphate isomerase (*Gpi-1*; chromosome 7), purine nucleoside phosphorylase (*Np-1*; chromosome 14), soluble malic enzyme (*Mod-1*; chromosome 9), glutathione reductase-1 (*Gr-1*; chromosome 8), and triose phosphate isomerase (*Tpi*; chromosome 6). Activity of hypoxanthine-guanine phosphoribosyl transferase (*Hprt*; X chromosome) was detected indirectly by monitoring hybrid cell growth in selective medium (*11*).

Thirty-six hybrid clones were tested for ecotropic virus induction. Subconfluent cultures were exposed to 20 μg of IdU per milliliter for 40 to 48 hours, and SC-1 cells (*12*) were then added. Two weeks later and at weekly intervals thereafter, the mixed cultures were passaged and monitored for virus production by the XC test (*13*). In positive cultures, virus generally became detectable by 3 to 4 weeks. Virus-negative clones showed no XC foci 6 weeks after induction.

Nineteen of the 36 hybrids were inducible for mouse ecotropic virus; in all cases tested the virus was N-tropic. None of the virus-inducible hybrids tested produced ecotropic virus without induction. A comparison of isozyme expression and virus induction showed that only phosphoglucomutase-1 segregated with a high degree of concordance with virus induction (Table 1). Both phenotypes were concordantly expressed in 30 of 33 primary clones tested. Of the three remaining clones, one virus-negative clone showed weak enzyme activity, and one clone lacking mouse phosphoglucomutase-1 produced only trace amounts of virus. These clones may therefore represent false discrepancies in which the chromosome frequencies were near the threshold level necessary for detectable gene expression. The other mouse enzyme phenotypes were all highly asyntenic with virus induction (31 to 50 percent discordance).

Forty-six secondary clones were derived from seven virus-inducible primary clones. Only two of these secondary clones produced detectable levels of ecotropic virus by the XC test and only those two subclones had mouse phosphoglucomutase-1 activity (Table 1). In contrast, activity for each of the other nine mouse isozymes was detected in at least 30 percent of these 46 hybrids. Thus, virus inducibility and phosphoglucomutase-1 activity were concordantly lost or retained in 76 of the 79 primary and secondary clones examined, provid-

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