REFERENCES AND NOTES

1. A. G. Knudson, *Cancer Res.* **45**, 1437 (1985); A. L. Murphree and W. F. Benedict, *Science* **223**, 1028 (1984).
2. R. S. Sparkes *et al.*, *Science* **208**, 1042 (1980); W. K. Cavenee *et al.*, *Nature (London)* **305**, 779 (1983).
3. S. H. Friend *et al.*, *Nature (London)* **323**, 643 (1986); W.-H. Lee *et al.*, *Science* **235**, 1394 (1987); Y-K. T. Fung *et al.*, *ibid.* **236**, 1657 (1987).
4. M. G. M. Ts'o, L. E. Zimmerman, B. S. Fine, *Am. J. Ophthalmol.* **69**, 339 (1970).
5. A. P. Kyritsis, M. Tsokos, T. J. Triche, G. J. Chader, *Nature (London)* **307**, 471 (1984).
6. D. Bok, J. F. Basinger, M. O. Hall, *Exp. Eye Res.* **18**, 225 (1974); D. S. Papermaster, B. G. Schneider, M. A. Zorn, J. P. Kraehenbuhl, *J. Cell Biol.* **77**, 196 (1978); R. Boynton, *Human Color Vision* (Holt, Rinehart and Winston, New York, 1979).
7. J. Nathans and D. S. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4851 (1984).
8. J. Nathans *et al.*, *Science* **232**, 193 (1986).
9. G. B. Grunwald, P. Gierschik, M. Nirenberg, A. Spiegel, *ibid.* **231**, 856 (1986).
10. C. L. Lerea, D. E. Somers, J. B. Hurley, I. B. Klock, A. H. Bunt-Milam, *ibid.* **234**, 77 (1986).
11. A. G. Gilman, *Annu. Rev. Biochem.* **56**, 615 (1987).
12. B. K-K. Fung, J. B. Hurley, L. Stryer, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 152 (1981).
13. E. E. Fesenko, S. S. Kolesnikov, A. L. Lynbarsky, *Nature (London)* **313**, 310 (1985); K.-W. Yau and K. Nakatani, *ibid.* **317**, 252 (1985).
14. D. C. Medynski *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4311 (1985); T. Tanabe *et al.*, *Nature (London)* **315**, 242 (1985); K. Yatsunami and H. G. Khorana, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4316 (1985).
15. M. A. Lochrie, J. B. Hurley, M. I. Simon, *Science* **228**, 96 (1985).
16. E. Bogenmann and C. Mark, *J. Natl. Cancer Inst.* **70**, 95 (1983); E. Bogenmann, *Int. J. Cancer* **38**, 883 (1986).
17. E. Bogenmann, H. Moghadam, Y. A. DeClerck, A. Mock, *Cancer Res.* **47**, 3808 (1987).
18. H. K. W. Fong, T. T. Amatruda III, B. W. Birren, M. I. Simon, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3792 (1987).
19. J. B. Hurley, H. K. W. Fong, D. B. Teplow, W. J. Dreyer, M. I. Simon, *ibid.* **81**, 6948 (1984).
20. C. Van Dop *et al.*, *Biochem. Biophys. Res. Commun.* **124**, 250 (1984).
21. J. Nathans and D. S. Hogness, *Cell* **34**, 807 (1983).
22. R. Adler, J. D. Lindsey, C. L. Elsner, *J. Cell Biol.* **99**, 1173 (1984).
23. H. J. A. Dartnall, J. K. Bowmaker, J. D. Mollon, in *Color Vision*, J. D. Mollon and L. T. Sharpe, Eds. (Academic Press, New York, 1983), p. 69.
24. A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983); addendum, *ibid.* **137**, 266 (1984).
25. We thank J. Nathans for providing the photopigment cDNA clones, A. Mock and H. Moghadam for technical assistance, and C. Stutters for secretarial assistance. Supported by PHS grant EY04950 from the National Eye Institute (E.B.) and by a grant from the National Science Foundation (M.S.).

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Mussel Growth Supported by Methane as Sole Carbon and Energy Source

S. CRAIG CARY, CHARLES R. FISHER, HORST FELBECK

Symbioses between chemoautotrophic bacteria and several specialized marine invertebrates are well documented. However, none of these symbioses have been demonstrated to provide sufficient energy and carbon to the host to enable it to grow. Growth rates of seep mussels collected from hydrocarbon seeps off the coast of Louisiana were measured in a controlled environment where methane was the sole carbon and energy source. The growth rates increased to a maximum of 17.2 micrometers per day in response to methane and approached zero in the absence of methane. These mussels contain methanotrophic symbiotic bacteria in their gills, which suggests that these bacteria provide their hosts with a net carbon flux originating from methane.

THE OCCURRENCE OF SYMBIOTIC chemoautotrophic bacteria that reside in highly specialized tissues of certain marine invertebrates was first described as occurring in hydrothermal vent communities and since then in many other diverse reducing marine environments (*1*). These bacteria typically oxidize reduced sulfur compounds from their environment and use the energy obtained to fix carbon dioxide from the surrounding seawater (*2, 3*). These chemicals can be formed either geothermally from seawater sulfate, as at the hydrothermal vents, or biologically through sulfate-reducing bacteria in the sediments, as in mudflats or hypoxic deep-sea basins (*4, 5*). Another source of energy and carbon has recently been demonstrated for mussels associated with hydrocarbon seepage sites on the Louisiana slope in the Gulf of Mexico (*5–7*) at the base of the Florida Escarpment (*8*) and for a small pogonophoran from the Skagerrak (*9*). These animals contain methanotrophic symbionts. The symbionts in the gill cells of the mytilids (*6, 8*) and the trophosome cells of the pogonophorans (*9*) contain stacked internal membranes characteristic of type I methanotrophs. Furthermore, enzymatic tests, stable isotope determinations, net methane uptake studies, and the incorporation of ^{14}C-labeled methane indicate that these symbioses are methanotrophic (*6–8*). The methane necessary for the support of this metabolism in the mussels in situ originates either from hydrocarbon sources at the oil seeps or from biological processes at the Florida Escarpment communities (*4, 5*).

For both chemolithoautotrophic and methanotrophic associations, researchers have proposed (*2, 3, 6*) that at least part of the nutritional requirements of the respective hosts is supplied by the bacteria. Some host animals have entirely lost the ability to take up and digest external food. The vestimentiferan tube worms, the pogonophorans, many oligochaetes of the subfamily Phallodrilinae, and several bivalves of the Solemyidae have lost their digestive systems and must depend on an alternative nutritional source—most likely the symbiotic bacteria (*10*). Similarly, most other bivalves known to contain chemoautotrophic symbiotic bacteria are characterized by a reduced digestive system (*10, 11*). Other indirect evidence for the importance of bacterial carbon to the hosts includes studies in which stable isotope ratios ($^{13}C/^{12}C$) in symbiont-containing animals were measured. Comparisons of paired tissues from individual animals of a variety of symbiont-containing species, including the mussel species used in our study, showed little variation, indicating the importance of symbiont carbon to the host (*5, 6, 12*). This finding is especially convincing in the case of animals containing methanotrophic symbionts because of the negative $^{13}C/^{12}C$ associated with methane. Transfer of symbiont carbon to the host can be accomplished in several ways: through digestion of the bacteria by host lysosomes or through the translocation of part of the fixed carbon from the bacteria to the host. The first strategy has been proposed in the symbioses of the hydrothermal vent tube worm *Riftia pachyptila* by Bosch and Grasse (*13*), who document an intracellular degradation of symbiotic bacteria. Hand (*14*) and Giere and Langheld (*15*) observed similar phenomena in the bacteriocytes of *Riftia pachyptila* and in the oligochaete *Phallodrilus leukodermatus*, respectively. The second proposed strategy of nutrient transfer is the translocation of reduced organic material from the bacteria to the host, as was shown with radiolabeled bicarbonate in *Solemya reidi* (*16*). However, it has not been demonstrated that the hosts can grow when provided only with an inorganic chemical as a bacterial energy source. We report here that mussels harboring methanotrophic bacteria as symbionts (*17*) grow when supplied only with methane in the seawater.

Growth has been considered an excellent

S. C. Cary and H. Felbeck, Scripps Institution of Oceanography, Marine Biology Research Division, University of California San Diego, La Jolla, CA 92093.
C. R. Fisher, Marine Science Institute and Department of Biological Science, University of California Santa Barbara, Santa Barbara, CA 93106.

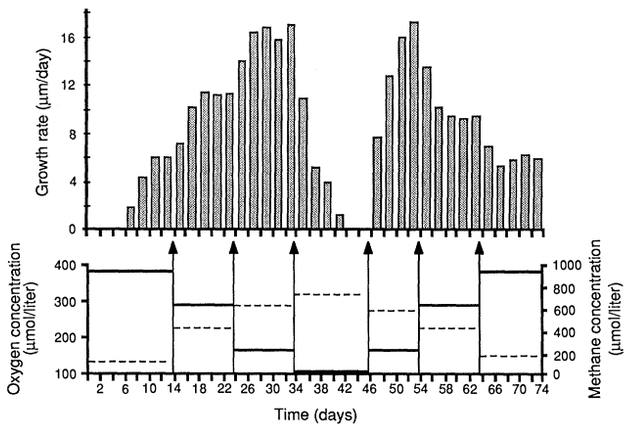

Fig. 1. Growth rates of a symbiont-containing mytilid bivalve species from oil seeps off the coast of Louisiana at various concentrations of methane and oxygen. The vertical bars illustrate the mean growth rate ($n = 8$) averaged over 2 days. The standard deviations for each growth period never exceeded ±1 µm/day. The horizontal solid lines in the lower section indicate the methane, and dotted lines are the oxygen concentrations during the respective periods. The arrows show the times when experimental conditions were changed.

indicator of overall health and vitality of a bivalve (*18*). Under stress, shell growth is one of the first factors to change. To avoid the typically long-term growth measurements, that is, measurement of length, width, weight, volume, or others in which it usually takes months to demonstrate significant changes, we chose a more sensitive technique. By means of laser diffraction, we determined the growth rate by measuring the change of the width of a gap created by the edge of the mussels and a fixed plastic tab. The projected pattern was sensed by a photodiode array, the output of which was fed into a computer for analysis. This technique has been used to measure short-term changes in the growth rate of the shallow water mytilid *Mytilus edulis* that occurred as a result of varying phytoplankton concentrations (*19*). Width changes of the gap as small as 3 µm can be observed. The growth of the mussels was determined every 2 days until a plateau was reached (*20*). The ratio of methane to air bubbled through the incubation water was then altered (*21*).

The growth rates demonstrate a clear dependence on the availability of methane in the water (Fig. 1). After an initial period of no growth when no methane was supplied, the growth rates increased to a maximum of 17.2 µm/day in the presence of methane. Without methane, growth stopped after a short period and quickly increased again when the methane supply was resumed. A response could be measured in each case after an initial lag of 2 days. This response time is comparable to that of *M. edulis* when fed different concentrations of cultured phytoplankton. To ensure that the growth was actually dependent on methane, the stepwise changes were repeated in reverse order after the period without methane and without growth. The growth rates increased again after methane was added to the seawater to approximately the same level as recorded earlier in the presence of methane.

The mussel growth rates also show a correlation to the methane and oxygen concentrations. The highest growth rates were obtained at the lowest measured methane concentration (245 µmol/liter) and a high oxygen concentration (290 µmol/liter). The lowest growth rates were observed at the highest methane (980 µmol/liter) and lowest oxygen concentration (140 µmol/liter). Methanotrophic bacteria grown in culture normally show increased yields at higher methane levels, limited only by very low oxygen concentrations (*22*). The different response reported here for an intact symbiotic association could be due to several factors. First, at high levels of methane the oxygen concentrations could be limiting for the host. Mussels in general are poor regulators of oxygen consumption, and their metabolic rates are dependent on the oxygen concentration. The lower growth rates could be due to the lower metabolic rates of the host. Second, the symbiotic bacteria in the presence of methane could be limited by the oxygen diffusing into the bacteriocytes; therefore, the bacteria respond to any changes of the oxygen concentration with an altered supply of organic carbon to the host. Third, the reduced growth could be due to an unidentified growth inhibitor in natural gas. However, the possibility that the trace hydrocarbon contaminants present in natural gas (*21*) would be deleterious to a mussel collected from a site characterized by crude oil and natural gas seeping from the sediments (*23*) is unlikely.

Since we have observed particulates in the guts of freshly collected seep mussels, it appears that additional assimilation through normal feeding may be possible. At this time, it is uncertain to what extent this feeding mode contributes to the overall nutritional requirements of the host. Methane can supply only carbon and energy to the bacteria; the essential elements phosphorus, nitrogen, and sulfur must be derived from another source. Since the natural growth rate of the mussels is unknown, it cannot be excluded that the maximal growth rate reported here is lower than the one found in situ. When fed natural concentrations of phytoplankton, *M. edulis* grew an order of magnitude faster than reported here for the seep mussels (*19*). Methane may account for only part of the growth of these animals, the remainder being supported by particulate or dissolved material. However, the experiment described here shows that mussels, known to contain methane-oxidizing bacteria, can grow with methane as their sole carbon and energy source.

REFERENCES AND NOTES

1. C. M. Cavanaugh, *Bull. Biol. Soc. Wash.* **6**, 373 (1985); H. Felbeck, M. A. Powell, S. C. Hand, G. N. Somero, *ibid.*, p. 261.
2. H. Felbeck, *Physiol. Zool.* **58**, 272 (1985).
3. A. E. Anderson, J. J. Childress, J. A. Favuzzi, *J. Exp. Biol.* **131**, 1 (1987).
4. C. K. Paull *et al.*, *Nature (London)* **317**, 709 (1985); C. K. Paull *et al.*, *Science* **226**, 965 (1984).
5. J. M. Brooks *et al.*, *Science* **238**, 1138 (1987).
6. J. J. Childress *et al.*, *ibid.* **233**, 1306 (1986).
7. C. R. Fisher, J. J. Childress, R. S. Oremland, R. R. Bidigare, *Mar. Biol.* **96**, 59 (1987).
8. C. M. Cavanaugh, P. R. Levering, J. S. Maki, R. Mitchell, M. E. Lidstrom, *Nature (London)* **325**, 346 (1987).
9. R. Schmaljohann and H. J. Flügel, *Sarsia* **72**, 91 (1987).
10. M. L. Jones, *Bull. Biol. Soc. Wash.* **6**, 117 (1985); C. Erseus, *Zool. Scr.* **13**, 239 (1984); E. C. Southward, *J. Mar. Biol. Assoc. U.K.* **62**, 889 (1982).
11. R. G. B. Reid and D. G. Brand, *Veliger* **29**, 3 (1986); M. Schweimanns and H. Felbeck, *Mar. Ecol. Prog. Ser.* **24**, 113 (1985).
12. G. H. Rau, *Science* **213**, 338 (1981); B. Spiro, P. B. Greenwood, A. J. Southward, P. R. Dando, *Mar. Ecol. Prog. Ser.* **28**, 233 (1986).
13. C. Bosch and P. P. Grasse, *C. R. Acad. Sci.* **299**, 413 (1984); *ibid.* **301**, 1 (1984).
14. S. C. Hand, *Biol. Bull. (Woods Hole)* **173**, 260 (1987).
15. O. Giere and C. Langheld, *Mar. Biol.* **93**, 641 (1987).
16. H. Felbeck, *J. Comp. Physiol.* **152**, 3 (1983); C. R. Fisher and J. J. Childress, *Mar. Biol.* **93**, 59 (1986).
17. The animals were collected at a depth of 700 m with the submersible *Johnson Sealink* on a cruise to the Louisiana oil seeps (27°41'N, 91°32'W) in the spring of 1987. They were brought to the surface in a temperature-insulated container and maintained in flowing seawater bubbled with methane at in situ temperatures.
18. C. B. Jörgensen, *Ophelia* **15**, 175 (1976); R. L. Thompson and B. L. Bayne, *Mar. Biol.* **27**, 317 (1974).
19. T. Strömgren, *Limnol. Oceanogr.* **20**, 845 (1975); ——— and S. C. Cary, *J. Exp. Mar. Biol. Ecol.* **76**, 23 (1984).
20. Intact small mussels were fixed to Lucite panels by gluing one valve to the support. A fixed gap was then created by attaching plastic tabs near the valve edge. A laser beam was projected through the gap to create a characteristic diffraction pattern. The distance between the light maxima in this diffraction pattern is directly proportional to the gap width. A panel with eight mussels was incubated in a 7-liter water tank at in situ temperature (9.0° ± 0.5°C). The incubation chamber was held in a secondary water bath to maintain constant temperature. The filtered seawater (0.45-µm mesh) was changed daily to avoid growth and accumulation of methylotrophic bacteria that could have served as an additional food source. Different ratios of natural gas and air, monitored by gas flow meters, were bubbled continuously through the water to keep methane concentrations at constant levels. The ratio was changed stepwise from 10 to 50% methane. The rate of

supply of the mixture never exceeded 100 cm^3/min. The growth resulting from the different methane levels was then measured every 2 days. The methane concentrations in the water were measured by gas chromatography with a Porapak Q column and a flame ionization detector. The oxygen concentrations were monitored with a polarographic oxygen electrode (Strathkelvin, U.K.). During the entire duration of this study the mussels appeared to be in good health, that is, none of the animals died, the siphons were extended, and extensive byssal threads were produced.
21. Monthly Gas Quality Report, Ehrenberg meter station, no. 32002, sampling date, 30 July 1987 (San Diego Gas and Electric, San Diego, August 1987).
22. C. Anthony, *The Biochemistry of Methylotrophs* (Academic Press, New York, 1982).
23. M. C. Kennicutt II *et al.*, *Nature (London)* **317**, 351 (1985).
24. Supported by NSF grants OCE86-10512 (H.F.) and OCE86-10514 (J. J. Childress and C.R.F.). The National Undersea Research Program of the National Oceanic and Atmospheric Administration supported the collecting cruise and the dive time with the RV *Seward Johnson* and the DSRV *Johnson Sealink*, and *NR1* submersible support was provided by the Office of Naval Research. We thank the crew and pilots of the RV *Seward Johnson* and the DSRV *Johnson Sealink* for their assistance in collecting animals, B. Ward for the use of the gas chromatograph, and J. J. Childress for critically reviewing the manuscript.

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Biologic Features of HIV-1 That Correlate with Virulence in the Host

CECILIA CHENG-MAYER, DEBORAH SETO, MASATOSHI TATENO, JAY A. LEVY

Individuals infected with the human immunodeficiency virus type 1 (HIV-1) may be asymptomatic or have AIDS-related complex or the acquired immuno deficiency syndrome (AIDS). Little is known about the factors that influence progression of infection to AIDS. In this study of isolates of HIV-1 obtained at intervals during the infection of four individuals, the development of disease was found to be correlated with the emergence of HIV-1 variants that were more cytopathic in vitro as the disease progressed and that replicated more efficiently in a wide variety of different human cells. The biologic properties of HIV-1 in vitro thus appear to reflect its virulence in the host. Further studies of such sequentially isolated viruses may lead to the identification of viral genes that govern pathogenesis.

BIOLOGIC (*1–6*), SEROLOGIC (*7*), and molecular (*8, 9*) studies of the human immunodeficiency virus type 1 (HIV-1) have indicated that this virus is highly heterogeneous. Individual isolates of HIV-1 can be distinguished by their differential capacity to infect and replicate in a wide variety of cultured human cells including T and B lymphocytes, macrophages, and brain-derived cells (*1, 4, 6, 10, 11*). Studies of the varying abilities of HIV-1 isolates to replicate to high titers and to induce cytopathic changes in infected cells have indicated that these characteristics are correlated with the efficiency of the virus to form plaques in the MT-4 cell line (*12*). Differences in the susceptibility of various HIV-1 isolates to neutralization by HIV-1–positive sera have also been observed (*6, 7, 11*).

In the present studies, these biologic and serologic properties of HIV-1 were used to examine the possible changes in the virus over time in the same individuals. Our results from four individuals show that the progression of disease is correlated with the emergence of HIV-1 isolates that, in comparison with the virus isolated initially, are more cytopathic and replicate to higher titers in a wide variety of different human cells. Serologic and genomic restriction analyses indicate that in each individual the sequential isolates are related. The observations suggest that certain changes in the structure of HIV-1 can influence its virulence in the host.

Isolates of HIV-1 were obtained at intervals from peripheral blood mononuclear cells (PMC) of four subjects selected randomly from a group of seropositive individuals whom we have followed for the past 4 years (Table 1). Three of the four subjects progressed to more severe disease during the time of observation; one has remained asymptomatic (Table 1, subject 4). At the time of virus isolation, we noted that HIV-1 was recovered more readily from each individual's PMC as the disease progressed. For example, in subject 2, HIV_{SF216} emerged in the patient's PMC culture within 12 days whereas the previous isolates (HIV_{SF94} and HIV_{SF118}) took close to 1 month to be detected. Similar findings on time of recovery of HIV-1 from PMC have been reported (*12, 13*).

We first determined the abilities of HIV_{SF2} and HIV_{SF13} (from subject 1) to infect established human T cell lines (HUT-78 and MT-4), the U937 monocytic cell line, primary macrophage/monocytes, and certain established B cell lines (*1, 6*). These viruses had been kept in culture for over 3 years. As shown in Table 2, both isolates replicated in HUT-78 and MT-4 cells and the monocytic line, but only HIV_{SF13} productively infected primary macrophages and both of the B cell lines. Furthermore, HIV_{SF13} was more cytopathic as reflected by syncytia formation and balloon degeneration in infected PMC, HUT-78, and MT-4 cells. It also readily induced plaques in MT-4 cells (Table 2).

Subgroups of HIV-1 isolates have been identified on the basis of their patterns of sensitivity to serum neutralization (*7*). When tested with three HIV-1 antibody positive sera, HIV_{SF2} and HIV_{SF13} were neutralized to a similar extent by all three sera at dilutions of 1:100 or greater.

We then studied the biologic and serologic properties of isolates from three other individuals (Table 1). These isolates had been in culture for only 3 to 4 weeks. As the disease progressed in subjects 2 and 3, the HIV-1 isolated had a wider host range and greater cytopathic and replicative properties. In subject 2, for example, the isolate obtained 2 months before the patient died (HIV_{SF216}) replicated quickly and to high titers in all established human cell lines and primary macrophages, and also produced

Table 1. Isolation of HIV-1 from individuals at different stages of infection. The isolates were initially recovered by cocultivation of the individual's PMC with PMC from seronegative donors for 10 to 30 days as described (*13, 16*). The isolates were subsequently passaged onto fresh PMC and when virus titers were high [reverse transcriptase (RT) activity $\geq 10^6$ cpm/ml (*17*)], aliquots of filtered virus stocks were frozen at −70°C until use. KS, Kaposi's sarcoma; PCP, *Pneumocystis carinii* pneumonia; LAN, persistent lymphadenopathy.

HIV-1 isolate	Month and year of isolation	Clinical state
	Subject 1	
SF2	11/83	Oral candidiasis
SF13	4/84	KS, PCP
	11/84	Deceased
	Subject 2	
SF94	3/85	Asymptomatic
SF118	4/85	Asymptomatic
SF216	10/85	LAN, diarrhea
	12/85	Deceased
	Subject 3	
SF73	12/84	Asymptomatic
SF328	3/86	LAN
SF665	9/87	PCP
	Subject 4	
SF341	4/86	Asymptomatic
SF488	4/87	Asymptomatic

Department of Medicine, Cancer Research Institute, University of California, School of Medicine, San Francisco, CA 94143.