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8. The boundary layer is a region of fluid near a surface where a gradient in momentum (flow speed) exists because of fluid viscosity. Viscous dissipation robs momentum from a moving fluid, converting kinetic energy into heat. A concentration gradient (diffusional boundary layer) also exists near a surface surrounded by a fluid that is absorbing or releasing dissolved materials. For biologically important molecules (for example, oxygen) diffusing in water, the ratio of momentum boundary layer thickness to diffusional boundary layer thickness is about 8. For both types of boundary layers, as flow speed increases, boundary layer thickness decreases.
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10. A flux of dissolved material in a convecting fluid to an organism can be generally given by F_c (mol T^{-1}) = $h_m \Delta C A$, where ΔC is the concentration difference between the organism and the free-stream environment (mol L^{-3}), and A is surface area available for uptake (L^2). In a stagnant fluid, delivery will be by diffusion, and the flux is thus: $F_s = D \Delta C A W^{-1}$, assuming the boundary layer thickness is proportional to organism size. Sh is the dimensionless ratio, F_c/F_s.
11. F. M. White, *Heat and Mass Transfer* (Addison-Wesley, New York, 1988), pp. 270–367 and 643. These indices follow directly from a dimensional analysis of convective mass transfer using the Buckingham Pi theorem.
12. Algebraic solutions to scaling exponents for various assumptions of allometry and flow regime were explored with the use of the computer language Mathematica, running under a local kernel on an Apple Macintosh IIcx.
13. The surface area available for uptake is assumed $\propto W^2$.
14. For a sphere, $M \propto W^3$. For a cylinder, $M \propto r^2 W$, where $r \propto W^{\text{radial exponent}}$. For geometric scaling, radial exponent = 1. For a cylinder that gets proportionately thinner (thicker) as it grows, radial exponent <1 (>1), respectively. For a plate that does not get appreciably thicker as it lengthens (for example, an encrusting invertebrate or algal blade), $M \propto W^2$.
15. The relation between flow speed and height above the bottom is often logarithmic in benthic environments [P. A. Jumars and A. R. M. Nowell, *Am. Zool.* **24**, 45 (1984)]. Thus the average flow speed experienced by an organism growing up into the substrate's boundary layer will scale as the logarithm of its own characteristic dimension, W. Dimensional analysis of the Sh-Re relation now yields $h_m \propto W^{d-1}$ $[\log(W)]^d$. This relation forces almost independent scaling between the mass transfer coefficient and size; we can thus approximate this relation as $h_m \propto W^0$. T. Daniel (personal communication) derived this effect in the context of heat transfer to intertidal organisms.

16. I thank T. Daniel, A. Heusner, P. Jokiel, C. Jordan J. Kingsolver, M. Lesser, W. S. Price, L. Sanderson, K. Sebens, V. Weis, S. Wing, and the students of the 1990 Physical Biology course, Friday Harbor Laboratories, University of Washington, for helpful discussion. P. Basser gave me the initial inspiration to explore the chemical engineering literature and technical advice concerning *Mathematica*. Supported by NSF grants OCE-87-16427 and OCE 90-16721 (GLOBEC), National Oceanic and Atmospheric Administration National Undersea Research Program Aquarius habitat mission 88-6, and Nitrox SCUBA research cruise to the Florida Keys, and a Faculty Research Award from the University of California, Davis.

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Behavioral Hypothermia and Survival of Hypoxic Protozoans *Paramecium caudatum*

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Hypoxia has been shown to elicit behavioral hypothermia in a number of different metazoan species, all with nervous systems. The protozoan, *Paramecium caudatum*, has no nervous system and was not expected to display behavioral hypothermia. However, this species was also found to select a lower temperature in a thermal gradient under hypoxic conditions. This response proved to be beneficial as survival of hypoxic paramecia was greatly increased at lower temperatures. This unicellular species may provide a useful model to investigate the cellular and molecular basis of adaptive thermoregulatory behavior.

HYPOXIA CAUSES BODY TEMPERATURE to fall in a number of different animals, from crayfish to mammals (*1*). In all the animals previously studied, thermoregulation is controlled by a nervous system. In mammals, hypoxia induces hypothermia by decreasing heat production and increasing heat loss. In ectotherms, hypoxia induces behavioral selection of a lower ambient temperature. Hypoxia-induced hypothermia is beneficial, primarily because it lowers metabolic rate, and thus O_2 need, when O_2 availability is limited. Hypothermia should enhance survival, but this has never been directly tested. The purpose of our study was to determine whether hypoxia-induced hypothermia can occur in an organism without a nervous system (for example, the protozoan *Paramecium caudatum*) and whether the response would enhance survival. Two specific hypotheses were tested: (i) Hypoxia causes paramecia to select a lower temperature in a thermal gradient. (ii) Survival of hypoxic paramecia is increased at lower temperatures.

To test the first hypothesis, selected temperature (T_s) of paramecia was determined in an aquatic thermal gradient (0.4° ± 0.6° to 34.6° ± 1.4°C) placed in a petri dish at different ambient oxygen pressures (P_{O_2}) (*2*). Thermocouples were placed at each end of the gradient and ten intermediate locations for measuring gradient temperatures.

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A microscope was positioned to visualize the paramecia in the gradient, and magnified images were recorded by video microscopy.

P_{O_2} within the gradient was adjusted by covering the petri dish with glass and flowing humidified gas mixtures through it. Six different O_2-N_2 mixtures were tested having P_{O_2}'s of 127, 2.5, 1.3, 0.6, 0.3, and 0.0 torr. The rate of P_{O_2} equilibration between the gas and the medium inside the gradient was measured in preliminary experiments by placing in the gradient an aquatic gas probe connected to a mass spectrometer (*3*). Gradient P_{O_2} was monitored continuously before and after the P_{O_2} of the gas flowing into the petri dish was lowered from 127 to 0.0 torr. After 80 min, no O_2 was detected in the gradient.

To determine the effect of P_{O_2} on T_s, 250 μl of a paramecium suspension were placed in the thermal gradient, and a gas mixture flowed through the petri dish. After 2 hours the distribution of T_s's under each experimental condition was determined by video recording for 20 s the 12 gradient positions containing a thermocouple. Thermocouple temperatures were recorded immediately before video taping. From the tapes, the number of animals present at each location was determined.

Under normoxic conditions, T_s was 28.0° ± 0.1°C (Fig. 1) (*4*). Reducing ambient P_{O_2} to 2.6 torr had no statistically significant effect on T_s ($P > 0.1$) (*5*). However, reducing P_{O_2} to 1.3 torr or below caused T_s to decrease ($P < 0.01$). To test whether this hypoxia-induced hypothermia is reversible, another experiment was performed in which T_s was determined 2 hours after gas P_{O_2} was increased to 127 torr after a 2-hour exposure to 100% N_2. Normoxia after N_2 exposure caused T_s to increase from 11.7° ± 1.2° to 26.8° ± 0.7°C, which was not significantly different from T_s under normoxic conditions before N_2 exposure ($P > 0.05$).

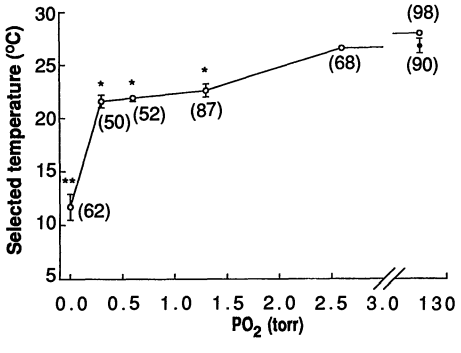

Fig. 1. The effects of gradient P_{O_2} on T_s. Values are means ± SEM. Numbers in parentheses denote the number of animals (n) observed in determining T_s at each P_{O_2}. The filled circle represents T_s at a P_{O_2} of 127 torr after N_2 exposure. Single and double asterisks indicate that the mean T_s is significantly different (*5*) from the mean T_s at a P_{O_2} of 127 torr (hollow circle). The double asterisk also indicates that the mean T_s is significantly different ($P < 0.01$) from all other values.

To determine whether this hypoxia-induced hypothermia may facilitate survival, suspensions of paramecia were equilibrated with P_{O_2}'s of 127, 0.3, or 0.0 torr. Each gas mixture was tested at five different temperatures (34.3°, 26.6°, 22.0°, 16.3°, 11.7°C) (*6*). Samples from the paramecium suspension were analyzed to determine the paramecia concentration at 15- to 90-min intervals. Survival was assessed by plotting the percentage of initial paramecium concentration against time and calculating the time for the paramecium concentration to fall to 50% of the initial concentration (T_{50}). There was no apparent decrease in paramecium concentration under normoxic conditions at any temperature (Fig. 2A). In contrast, hypoxic conditions caused paramecium concentration to decrease with time (Fig. 2, B and C). This decrease varied inversely with temperature. At a P_{O_2} of 0.3 torr, no decrease in paramecium concentration occurred after 7 hours at 11.6°C. However, at higher temperatures, T_{50}'s were 353, 280, 120, and 47 min at temperatures of 16.3°, 22.0°, 26.6°, and 34.3°C, respectively. In anoxia, paramecium concentration decreased at all temperatures tested. The T_{50}'s were 168, 65, 45, 38, and 23 min at temperatures of 11.6°, 16.3°, 22.0°, 26.6°, and 34.3°C, respectively.

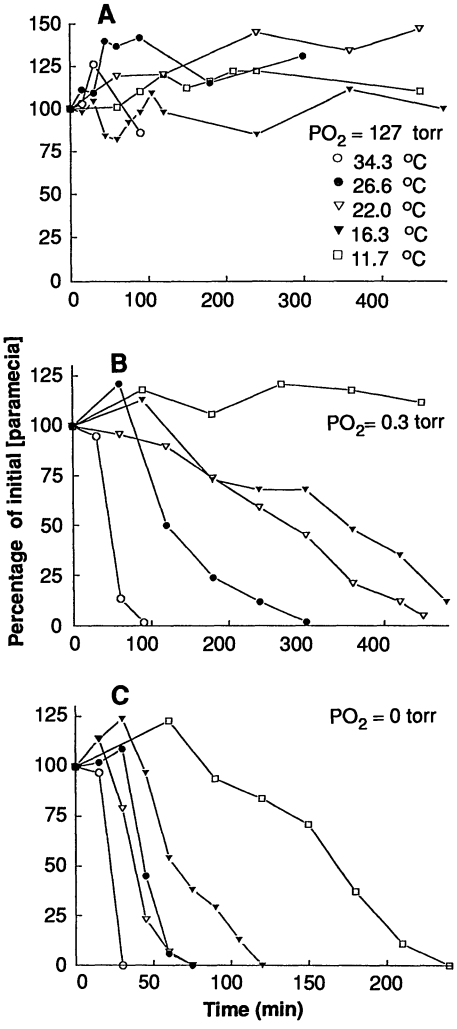

Fig. 2. The effects of temperature on paramecia survival at ambient P_{O_2}'s of 127, 0.3, and 0.0 torr.

Our results indicate that hypoxia-induced hypothermia is extremely widespread among taxa, occurring even in a single-celled organism, *Paramecium caudatum*. This response is clearly beneficial to this animal because decreasing temperature increases survival under hypoxic conditions. Thus, if T_s were not affected by hypoxia, paramecia would rapidly die.

The threshold of the hypoxia-induced hypothermia is between 1.3 and 2.6 torr. A previous study of the effects of hypoxia on *Paramecium caudatum* showed a decrease in swimming speed at a threshold P_{O_2} of about 1 torr (*7*). The stimulus that elicits the change in T_s is not known (*8*). Whether ambient P_{O_2} is directly sensed by the animal or whether hypoxia elicits a change in a cellular variable that then leads to a reduction in T_s is not known. Because the threshold P_{O_2} is so low, it is likely that, below the threshold, O_2 flux into the cell does not provide enough O_2 to meet the normal O_2 requirements of the cell. Changes in many cellular variables would probably occur under such conditions.

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2. *Paramecium caudatum* were cultured in Cerophyl medium (Ward's Natural Science Establishment) at 23°C. Animals in mid-log phase growth were isolated by centrifugation at 100*g* for 2 min and then washed three times in Cerophyl medium. The concentration of the final suspension was adjusted to about 1300 cells per milliliter. The gradient (80 mm long, 3 mm wide, and 1.5 mm deep) was constructed with Tygon tubing cut length-wise and glued to the bottom of a glass petri dish. One end was cooled by a copper tube beneath the petri dish which carried chilled polyethylene glycol. The other end was warmed by heating tape. The temperatures (mean ± SD) of the 12 thermocouples during the measurements were (in degrees Celsius): 34.6° ± 1.4°, 29.5° ± 1.0°, 28.2° ± 0.9°, 27.1° ± 0.8°, 26.1° ± 0.6°, 23.4° ± 0.4°, 20.3° ± 0.5°, 16.6° ± 0.6°, 14.0° ± 0.5°, 8.8° ± 0.6°, 2.4° ± 0.6°, and 0.4° ± 0.6°.
3. The mass spectrometer was based on a Balzers QMA 120 mass analyzer. The gas probe was manufactured by AMIS/AS, Odense, Denmark.
4. Thermoregulation in paramecia under normoxic conditions was first investigated by M. Mendelssohn [*Pflügers Arch. Ges. Physiol.* **60**, 1 (1895)] who showed that paramecia accumulate between 24° and 28°C in a thermal gradient.
5. The effect of ambient P_{O_2} on T_s was evaluated with one-way analysis of variance. Post hoc comparisons were performed with a Newman-Keuls test. Statis-

tical significance was set at a level of $P = 0.05$.

6. The paramecium suspension was placed in a glass tube submerged in a variable temperature bath. The top of the glass tube was sealed with a rubber stopper. Two pieces of stainless steel tubing were fed through the stopper. One piece of tubing was used to bubble (about 20 ml/min) the paramecium suspension with specific gas mixtures. The other tube was a gas outlet. Samples of the paramecium suspension were withdrawn for analysis of survival with a needle pushed through the rubber stopper. Approximately 150 μl of the paramecium suspension were withdrawn from the glass tube at a time. Ten 10-μl aliquots of the suspension were placed under a microscope and the number of paramecia in each aliquot were counted to determine paramecium concentration. All paramecia examined were moving. Motionless paramecia were never observed, probably because paramecia cytolyze within minutes of dying (7).
7. J. A. Kitching, *Biol. Bull.* 77, 339 (1939).
8. Despite a constant P_{O_2} throughout the gradient, $[O_2]$ was higher at the cold end of the gradient because O_2 solubility in water varies inversely with temperature. Thus, it is possible that the reduction in T_s with hypoxia was not due to a change in thermoregulatory behavior, but rather to a chemotaxis toward a higher ambient $[O_2]$. We think that this possibility is unlikely because (i) the chemical activity of dissolved O_2 is determined by P_{O_2}, not by $[O_2]$; (ii) diffusion of O_2 into cells is determined by P_{O_2} gradients, not by $[O_2]$ gradients; and (iii) under anoxic conditions when both P_{O_2} and $[O_2]$ were zero throughout the gradient, T_s was reduced (Fig. 1).
9. We thank B. Morlock for excellent technical assistance. Research supported by NIH grants HL-38942 (to G.M.M.) and HL-40537 (to S.C.W.).

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Suppression of Gene Amplification in Human Cell Hybrids

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Gene amplification, one example of genetic instability, is of prognostic and clinical importance in neoplasia. In tumorigenic cells, gene amplification occurs at a very high frequency, whereas in normal diploid fibroblasts the event is undetectable by the clonogenic assay. To investigate genetic control of gene amplification, amplification frequency was measured in hybrids of tumorigenic cells and normal diploid cells. The ability to amplify an endogenous gene behaved as a recessive genetic trait, and control of gene amplification potential segregated independently of tumorigenicity and immortality.

CURRENTLY, THE DEVELOPMENT OF multiple genetic alterations is thought to be a basis of neoplasia (*1*), and increased genetic instability might be necessary to produce these changes (*2–4*). Tumorigenic cells may form from accelerated genetic changes coupled with selection pressures exerted by the surrounding microenvironment (*2*). In such a model, the accumulation of random genetic changes would ultimately lead to neoplasia. Indeed, various karyotypic changes, including aneuploidy, deletions, inversions, translocations, amplifications, and point mutations, are apparent in tumor cell populations. Such changes may contribute to the generation of cellular and biochemical heterogeneity, a hallmark of tumor cell populations (*4*).

One marker of genomic instability is gene amplification (*5, 6*), that is, an increase in the number of copies of a particular gene at a specific locus. Tumorigenic cells display a high rate of spontaneous gene amplification (*5*), as can be measured by their ability to become resistant to *N*-(phosphonoacetyl)-L-aspartate (PALA). PALA specifically inhibits the aspartate transcarbamylase activity of the multifunctional enzyme that contains carbamyl phosphate synthase, aspartate transcarbamylase, and dihydro-orotase (CAD) activities. The frequency of gene amplification–the proportion of surviving colonies in PALA relative to the number of surviving colonies without PALA—ranges from 10^{-3} in highly tumorigenic cell lines to 10^{-6} in nontumorigenic but immortalized cell lines (*5*). In contrast, gene amplification is undetectable ($<10^{-9}$) in primary, diploid cell populations (*7, 8*), reflected in the inability of normal cells to make drug-resistant clones.

We used somatic cell hybrids of cells that have a high frequency of amplification and those that have no detectable frequency of amplification to determine if the ability to amplify is a dominant or recessive trait (Table 1) (*9–13*). Such hybrids were suppressed in their ability to amplify the gene encoding the CAD enzyme. By measuring PALA resistance we analyzed both parental and hybrid cells for their ability to amplify the endogenous CAD gene. Every PALA-resistant subclone examined to date, both in rodent (*5, 14, 15*) and in human (*16*) cells, carried additional CAD gene copies and arose by amplification of the CAD locus (*17*). Although normal diploid fibroblasts showed no detectable gene amplification (*7*), highly tumorigenic fibrosarcoma and carcinoma cell lines amplified the CAD gene at high frequencies (Table 1). The SFTH400 hybrid cells, a fusion of a normal fibroblast and a fibrosarcoma cell line, displayed an amplification frequency several orders of magnitude lower than that measured in the tumorigenic parental cell line, HT1080 (Fig. 1 and Table 1). Similar results were seen with other nontumorigenic hybrid cell lines, SFTH300(S) and ESH5(S) (Table 1).

The expression or suppression of the ability to amplify was independent of the tumorigenic phenotype of the hybrid. Fusion of a normal (nonneoplastic) cell population with a highly tumorigenic cell line results in hybrid progeny with the malignant phenotype suppressed (*9*). As the hybrid cell line is propagated, however, chromosomes are lost, and rare tumorigenic segregants appear, which suggest that a specific chromosome involved in the suppression of malignancy is lost when the malignant phenotype reappears (*9–11, 18*). Reintroduction of the lost chromosome into the tumorigenic cell can completely suppress tumorigenicity (*19*). By comparing the amplification potential in suppressed hybrids and their tumorigenic segregants, we determined that suppression of gene amplification was not coupled to suppression of tumorigenicity (Fig. 1). Both SFTH400(S) cells and their

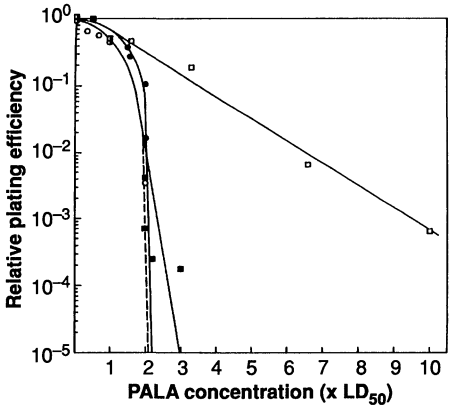

Fig. 1. Selection of somatic cell hybrids for PALA resistance. Cells were propagated and selected as in (*5*). They were plated at appropriate densities and placed in the indicated concentrations of PALA. When colony size exceeded 50 cells, plates were fixed, stained, and counted (*5*). Relative plating efficiency is the ratio of cells surviving in PALA to cells surviving in the absence of it. Amplification potential was determined at 9 × LD_{50}. Each curve is the average of a minimum of four separate determinations. Parents: open circles, GM2291 (LD_{50} = 1.5 μM); open squares, HT1080 (LD_{50} = 6 μM). Hybrids: closed circles, SFTH400(S) (LD_{50} = 10 μM); closed squares, SFTH400(T) (LD_{50} = 20 μM). Dashed line represents curve for GM2291.

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