Comp. Biochem. Physiol. Vol. 114B, No. 1, pp. 69–76, 1996

ISSN 0305-0491/96/$15.00
SSDI 0305-0491(95)02134-5

Pressure Effects on Metabolism in Tissues from Mice (*Mus Muscalis*) and Freshwater Mussel (*Elliptio complanata*)

M. A. Cheney, L. D. Hansen,† R. W. Breidenbach,‡ E. Wilhelmsen and R.S. Criddle*

*Department of Environmental Sciences, Cook College, Rutgers University, New Brunswick, NJ 08903; †Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602; ‡Thermalytics, Davis, Ca 95616; Dole Food Co, San Jose, CA 95131 and Section of Molecular and Cellular Biology, University of California, Davis, CA 95616

ABSTRACT. Metabolic rates of tissue sections from freshwater mussel gills and mouse brain and lung tissue were measured by calorimetry in ampules pressurized with gas mixtures. Increasing partial pressure of oxygen or total pressure with constant partial pressure of O_2 does not affect the respiratory quotient but increases rates of tissue metabolism. Changes in metabolic activity occur over pressure and Po_2 ranges commonly encountered by humans engaged in SCUBA diving. COMP BIOCHEM PHYSIOL 114B, 69–76, 1996.

KEY WORDS. Calorimetry, mussel, mouse, metabolism, respirometry, pressure

INTRODUCTION

Pressures above atmospheric are a significant environmental variable affecting the physiology of marine animals and also of humans engaged in ambient pressure diving. Variations in hydrostatic pressure on marine organisms significantly affect a wide range of metabolic and physical processes (2). Pressure changes as small as 1–3 atm can alter developmental morphology in aquatic angiosperms (9). Marine animals respond to increases in pressure with changed respiratory rates and altered physiological responses. Pressures in excess of 200 atm are almost universally lethal to shallow water-adapted fish and invertebrates (14). Pressure tolerance is altered by temperature, salinity and the rate of pressure change. The partial pressure of O_2 (Po_2) also plays an important role in survivability. For example, gill tissues of *Mytilus edulis* are more stable at high pressures when Po_2 is low (15).

Many studies of pressure effects on marine organisms have examined responses to pressure in the range of hundreds of atmospheres. Studies on humans have, of necessity, been done at lower pressures from 1 to 10 atm. Effects of pressure on humans have commonly focused on tissue solubilities of gases and the subsequent narcotic and toxic effects of dissolved gases, particularly nitrogen, on the central nervous system (1,3). Examinations of changes in respiration rates or pathways related to pressure change are less common. Lanphier and Camporesi (13) proposed a possible effect of pressure *per se* on respiration control and metabolic pathways, but these have been difficult to demonstrate. Human respiration rate measurements generally have been whole body determinations, measuring gas fluxes during various physiological activities or exercises at increased pressures. Because numerous neurological or humoral mechanisms can affect ventilation rates and are difficult to separate from primary effects on metabolic rate, the direct effects of increased pressure on resting metabolism of humans and other animals remain largely undefined.

Some conclusions seem clear, however. Significant changes in the rate of O_2 consumption are associated with changes in atmospheric pressure. At relatively low pressures (<10 atm), respiratory rates (measured as O_2 rates) of mouse and monkey increase approximately linearly with increased air pressure or Po_2 up to pressures at which significant neurological effects are noted and respiration rates cease to increase (4). Brauer *et al.* (4) concluded that most if not all of the increase in metabolic rate is attributable to factors other than extrathermogenesis or increased respiratory work in high-density atmospheres. They find "the increase in metabolic rate observed as a result of compression reflects some basic effect of pressure on metabolic rate rather than being secondary to any response to the environment as such." These findings indicate a lack of homeostatic control over metabolic rates as pressure changes.

In this study, calorimetric methods were used to directly examine effects of pressure change on respiration in small

Correspondence to: M. A. Cheney, Department of Environmental Sciences, Cook College, Rutgers University, New Brunswick, NJ 08903. Tel: 908-932-9094; Fax: 908-932-8644; E-mail cheney@aesop.rutgers.edu.
Received 27 July 1995; revised and accepted 21 November 1995.

excised tissue sections. The results from these studies are free from questions of neurological stimulation and altered ventilation rates and, accordingly, examine events associated with primary pressure–gas-composition–metabolic rate relationships. We find that freshwater mussel gill and mouse lung and brain tissue sections subjected to pressure changes and altered atmospheric gases show significant alterations in respiration that are dependent on the total pressure and the partial pressure of oxygen. These changes occur over the range of pressures encountered within 50 m of water depth.

MATERIALS AND METHODS

Freshwater mussels (*Elliptio complanata*), purchased from Connecticut Valley Supply Co., (Southampton, MA) were used in all studies. Mussels were maintained in an aerated aquarium at 19°C until just before use and then were acclimated in water at room temperature for 30 min before removing test tissue samples. Gills were excised from the mussel and incubated 15 min with shaking in room-temperature distilled water. Then, ~1-cm^2 (10–14 mg dry weight) sections from the uncut edge of the gill tissue were removed, rinsed in the same solution and placed on wet filter paper disks in a hastelloy calorimeter ampule for heat rate measurement. The gill tissue samples maintained nearly constant metabolic heat rates for several hours at 25°C in the water environment, with no requirement for supply of additional nutrients. After removal of gill tissue sections for a set of metabolic tests, unused gill tissue was stored for up to 4 hr at 5°C for subsequent tests of metabolic rates. These samples were not maintained in sterile conditions, but microbial contamination did not contribute significantly to measured metabolic heat under our experimental conditions. When present at significant levels, microbial contamination is evident from exponentially increasing heat rates and samples are discarded. At the end of each experiment measuring metabolic rate, tissue samples were blotted lightly, weighed, heated in a vacuum oven at 90°C for 24 hr and reweighed for dry weight.

Brain and lung tissues were obtained by dissection of mice immediately after they had been killed by cervical dislocation. Tissues were stored on ice for a few minutes during preparation and loading of the calorimeter ampules. Approximately 150 mg wet weight tissue was sealed directly in calorimeter ampules for measurement.

Metabolic heat rates of the tissue sections were measured in a Hart (Pleasant Grove, UT) Model 7707 differential scanning heat conduction calorimeter operated in the isothermal mode (7). Both 1-cm^3 and high-pressure ampules were used. Pressures up to 180 atm were maintained with selected pure or mixed gasses in specially constructed 0.7-cm^3 high-pressure ampules while monitoring heat rates (Fig. 1) (6). High-pressure gas was introduced into sample ampules through glass microcapillary columns connected to

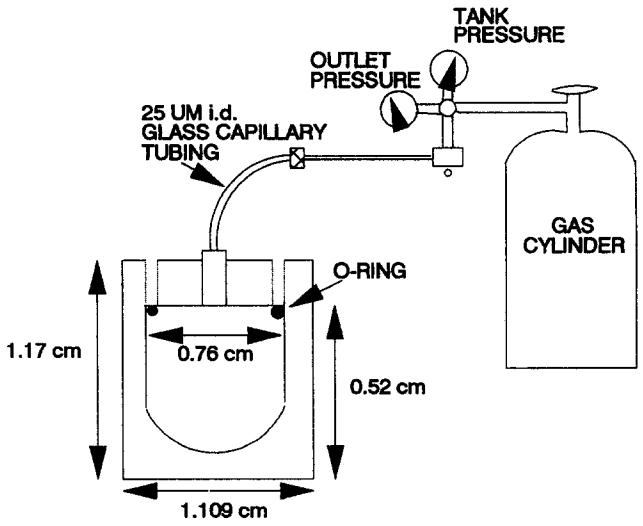

FIG. 1. Diagram of high-pressure calorimeter ampule and gas system.

pressurized cylinders through a two-stage high-pressure regulator. Pressure was read from the regulator gauges. Pressure effects were examined from 1 to 5 atm. Standard commercial pressurized cylinders of air, N_2, He, or O_2 were used for increasing ampule pressures above ambient. Ampules were generally closed and sealed in air. Repeated pressurization of ampules to 2 or 3 atm with a pure gas followed by bleeding off pressure allowed replacement of initial air with pure gas when necessary. Similar procedures allowed adjustment of starting gas to any desired composition. Pressure increases with pure gas or air were made stepwise by addition of gas from the cylinders.

Isothermal heat rate measurements yield metabolic heat rates directly in microwatts. Specific heat rates (μW/mg) were obtained by division by wet or dry weights. All experiments were replicated at least three times. Figures show data for typical experiments. Repeated measurements of the same sample with this calorimeter are precise to ± 3 μW. Metabolic heat rates measured ranged from 150 to 350 μW for most studies. Because of the nonreproducibility of tissue sample sizes, to facilitate comparisons, most data on effects of pressure are presented as percent change in metabolic rates rather than as absolute values of metabolic heat rates.

The lids of some 1-cm^3 (low-pressure) calorimeter ampules used in this study were modified with a small hole that was sealed with a silicon rubber septum. This allowed periodic sampling of headspace gasses with a microsyringe for determination of respiratory rates of gaseous metabolites. Analyses of CO_2, N_2, and O_2 from the headspace gasses used a Carle (Fullerton, CA) AGC series 100 gas chromatograph. Gas samples were passed through a 10-m, 0.18-mm ID, Alltech (Deerfield, IL) QC 345 undeactivated fused silica column, into a thermal conductivity detector and then through a 1.8-m, 80–100-mesh, 5 AMS column and a sec-

ond thermal conductivity detector. The first column separated CO_2 from O_2 and N_2; the second separated O_2 and N_2. Peak areas were determined using a Model 3394A Hewlett-Packard integrator. As withdrawal of gas sample volumes from the calorimeter ampules was not precise, O_2 and CO_2 concentration changes during metabolism were calculated relative to N_2 as a metabolically unaltered internal standard.

Comparative non-linear regression analysis was done using the Marquardt least-square technique as implemented in Statmost by Datamost Corporation. This algorithm is widely used and is readily available in many statistical packages.

RESULTS

The data in Fig. 2 outline the general nature of calorimetric measurement of tissue metabolic rates, the effects of pressure and the means of treating experimental results. The experiment in Fig. 2A illustrates the nature of the data showing metabolic heat rates of mouse brain tissue as a function of pressure and time at 25°C. The initial rapidly changing heat rate at $t < 0.3$ hr represents thermal equilibration in the calorimeter. The subsequent heat rate at $0.3 < t < 0.9$ hr is proportional to the metabolic rate. The slowly decreasing heat rate in this sample is a measure of the changing metabolic rate of the tissue section in the current ampule conditions. At 0.9 hr after initiation of the measurements, the pressure in the ampule was rapidly increased from 1 to 3.5 atm with air. A rapidly decaying transient increase in heat rate is noted at $0.9 < t < 1.1$ hr due to compression heating of the gas in the ampule, after which a slowly decreasing heat rate is again observed but at a higher value. Finally, at 1.25 hr the pressure was adjusted back to 1 atm. A transient decrease in apparent heat rate is due to expansion cooling of the gas in the ampule. Thermal equilibration after each change in pressure requires about 15–20 min. The slopes of the metabolic heat rate curves after equilibration indicate the rate of loss of metabolic activity over the course of the experiment.

Figure 2A shows that the effects of increased air pressure on metabolic rate are rapidly reversed when the pressure is returned to 1 atm. The increase in metabolic rate caused by the pressure increase is calculated by joining the low-pressure segments of the curves with a line and measuring the heat rate increase from this line to a midpoint on the high-pressure segment of the curve, as shown in Fig. 2. As the tissue sections examined varied in size and metabolic activity, comparisons among samples are made in terms of the percent increase (i.e., the heat rate increase divided by the interpolated heat rate).

Figure 2B presents a control experiment illustrating the effect of pressure on a tissue unaffected by pressure change over the range of pressures tested. A section of redwood tree meristematic tissue was pressurized using the same regimen used for the brain tissue in Fig. 2A. These data show pressure increases have no measurable effect on metabolic rates in redwood tissue.

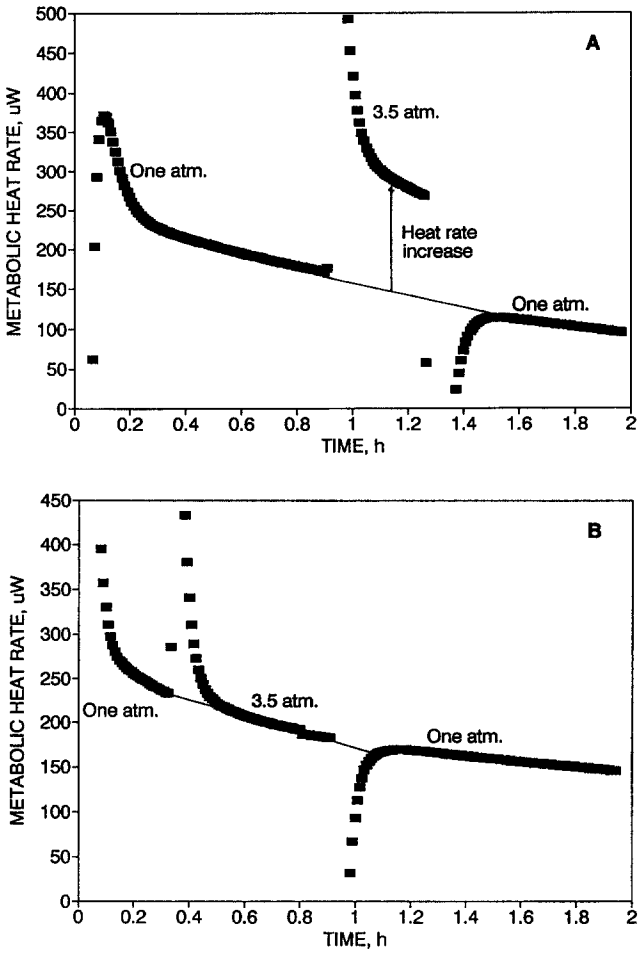

FIG. 2. Calorimetric measurement of metabolic heat rate of tissue sections vs time at 1 atm and at 3.5 atm in air. (A) Mouse brain tissue metabolic heat rate is measured at 1 atm, then rapidly increased to 3.5 atm and finally reduced again to 1 atm. The heat rate increase is measured at the midpoint of the high-pressure heat rate measurement and calculated as percent increase relative to the interpolated value of the unpressurized heat rate at this time. (B) Effect of pressure on metabolic rate in an endothelial tissue section from a redwood tree. Redwood meristematic tissue was subjected to the same pressure changes described in A.

The effects of pressure, headspace gas composition and temperature on metabolic heat rates were examined in detail using freshwater mussel gill tissue sections. Because three samples are examined simultaneously in the calorimeters used, each experiment included two test samples and a similar-sized control tissue section to monitor heat rate changes in the absence of pressure changes. Figure 3 illustrates heat rate data obtained for two mussel gill tissue samples subjected to stepwise increases in pressure with air and also for an unpressurized control gill tissue sample excised from the same test animal. Activity changes are reported as percent change relative to the initial heat rate.

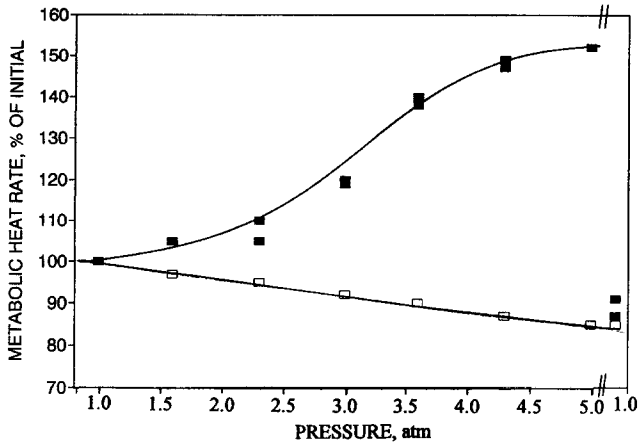

FIG. 3. The effect of increasing air pressure on the metabolic rate of mussel gill tissues. Two duplicate samples (■) were sealed in ampules, both with 50% O_2 and 50% N_2. The metabolic heat rates of these tissue samples were then examined after stepwise increases in ampule pressure with air. After the final measurements at 5 atm, pressure was returned to 1 atm and heat rate again measured as shown at the right portion of the graph. A third control sample (□) was examined simultaneously over the same time intervals with a constant 1 atm pressure containing 50% O_2 and 50% N_2. The measured values for the initial heat rates of samples (from which the reported percentages were calculated) in the experiment of Fig. 3 were 62 μW (4.6 μW/mg dry weight), 65 μW (4.6 μW/mg dry weight) and 75 μW (4.5 μW/mg dry weight) for the control and the two test samples, respectively.

The activity change of the unpressurized control tissue sample with time, shown as the lower curve in Fig. 3, is typical of most experiments. All samples exhibited similar slow losses of metabolic activity over the course of the experiment. Most experiments were terminated before the controls had decreased to 85% of their original activity.

The increase in heat rate of mussel gill tissue samples resulting from increasing air pressure begins to plateau above 4 atm at about 150% of the rate at 1 atm. Reduction of the pressure back to 1 atm showed that metabolic heat rate values return to the same value as the unpressurized sample at the end of the experiment (see points at lower right portion of the graph).

The importance of both Po_2 and total pressure to changes in metabolic rate is demonstrated in Fig. 4. The sample ampules containing mussel gill tissues were filled with either O_2, a 1:1 mixture of O_2 and N_2 or a 3:1 mixture of N_2 and O_2, and then pressurized with N_2. The data show metabolic heat rates increase with total pressure at constant Po_2. Metabolic rates are also strongly dependent on Po_2, larger increases in pressure being required to achieve the same level of activity at lower Po_2. The maximum heat rate also appears to depend on Po_2 within the pressure range examined. No measurable differences were observed in either the percent activity increase or the pressures required to induce

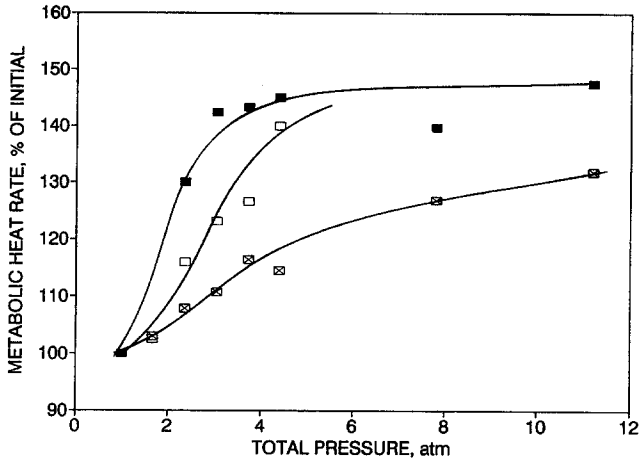

FIG. 4. Measurement of the effects of total pressure and the partial pressure of O_2 on metabolic heat rate of mussel gill tissue. Three samples of tissue were sealed in ampules with 1.0, 0.5, and 0.25 atm Po_2. The ampules were then pressurized with nitrogen and the heat rates measured. The percent of initial control metabolic rates are shown for ampules with Po_2 at 1 atm (■), 0.5 atm (□) and 0.25 atm (⊠).

the activity increase when He was used in place of N_2 as the pressurizing gas. Thus, leveling at high pressure was not dependent on a dissolution of N_2 in the tissue.

When pressure increase was generated by addition of pure O_2, metabolic rate increase again reflected the dependence on Po_2. Figure 5 compares metabolic rate increases for two samples, initially in air at 1 atm and then with pressures increased by addition of O_2 and N_2, respectively. Heat rate increases started at lower pressure with added O_2. The maximum heat rate increase leveled at ~150% of control sample for O_2 and to near 130% with N_2 additions.

The increased heat with oxygen and pressure exhibits saturation. Using the data in which the initial condition was

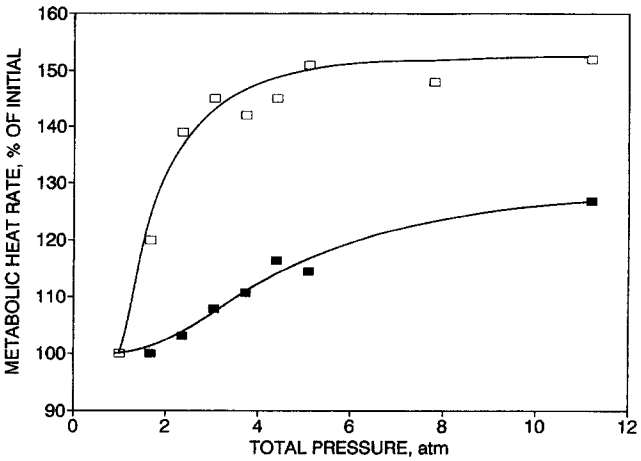

FIG. 5. Comparison of the effects of pressure increases with O_2 and N_2. Ampules containing mussel gill tissue were pressurized with either O_2 (□) or N2 (■).

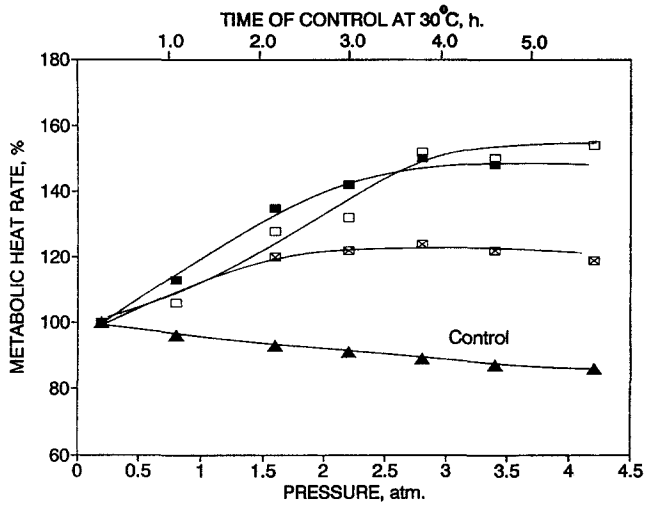

FIG. 6. Effects of temperature on pressure-induced increases in metabolic rates of mussel gill tissue. Tissue samples were sealed in ampules with 50% O_2 and 50% N_2 and then pressurized with air and metabolic heat rates determined at 25°C (■), 30°C (□) and 35°C (⊠). The control (▲) shows the decrease in metabolic heat rate for a control sample maintained at 1 atm and 30°C over the time course of the experiment.

air and the pressure change was provided by either nitrogen or oxygen, various simple saturation models were fit to the data. The simple saturation models in oxygen partial pressure, total pressure and a linear combination of these two were rejected because they did not describe the data as well as the following saturation model:

$$\text{Relative Heat} = B1 + B2(Po_2 \cdot P_{Total})/(B3 + Po_2 \cdot P_{Total}), \quad (1)$$

where $B1 = 100\% \pm 4\%$ (the normalizing value used in the data), $B2 = 58\% \pm 5\%$ (the maximum percent increase in heat rate) and $B3 = 50 \pm 2\%$ (the $Po_2 \cdot P_{Total}$ value at half maximal heat rate).

The effect of temperature on the pressure-induced increase of metabolic heat rates is shown in Fig. 6. The lower curve, labeled control, represents activity loss with time at 30°C without increased pressure. The percent increases for the 25 and 30°C curves are similar. The data suggest that higher pressures may be required to cause a given percent increase in metabolic rate at higher temperatures. The curves in Fig. 6 were normalized for differences in the rates of loss of activity in control samples at each temperature. Presentation as percent increase in metabolic rate due to pressure change focuses on pressure effects rather than the temperature dependence of metabolism, but the data also show a strong temperature effect on the rates, with a doubling about every 9°C (Q10 = 2.2) in the temperature range from 20 to 30°C.

The extent and time course of the return of tissue metabolic rates to their initial low-pressure values after release of higher pressures offers some insight into the mechanism of the pressure-induced rate increases. Experiments were run at 20, 25 and 30°C in which pressure was increased to 3 atm with O_2 to obtain the maximum heat rates. After measurement of these heat rates, the pressure was rapidly (30 sec) reduced back to 1 atm. The reduction of heat rates to near the starting low pressure values is plotted in Fig. 7 as the natural log of the metabolic rate vs time to show that the rate decrease followed first-order kinetics. The rate was strongly temperature dependent. At 30°C and above, the kinetics of decay to the low-pressure activity were too rapid to follow by our calorimetric methods. The rate of return is much slower at 25°C. At 20°C, the activity decay curve shows a delay before the first-order rate of decrease in activity becomes apparent.

Figure 8 presents O_2 and CO_2 concentrations obtained from analyses of headspace gas samples after 2 hours of metabolism by nearly equal weights of gill tissue samples but with different total pressures. Each gill tissue sample started with an intial Po_2 of 0.2 atm but at a different total pressure produced by the addition of N_2. Consistent with the observed heat rate increases, CO_2 was produced and O_2 consumed faster at high pressure than at low. The increased rates for O_2 consumption and CO_2 production due to increased pressure had the same magnitude with the accuracy of this experiment. Regression analysis of the change in percent oxygen over the 2-hr period against total pressure gave a slope of -1.1 ± 0.2 with $R^2 = 0.81$. The percent CO_2 regression line had a slope of $+ 1.5 \pm 0.3$ with $R^2 = 0.79$ and thus is not significantly different. Figure 8 therefore simply presents a plot of the regression line for the entire dataset. These data suggest that the respiratory quotient RQ

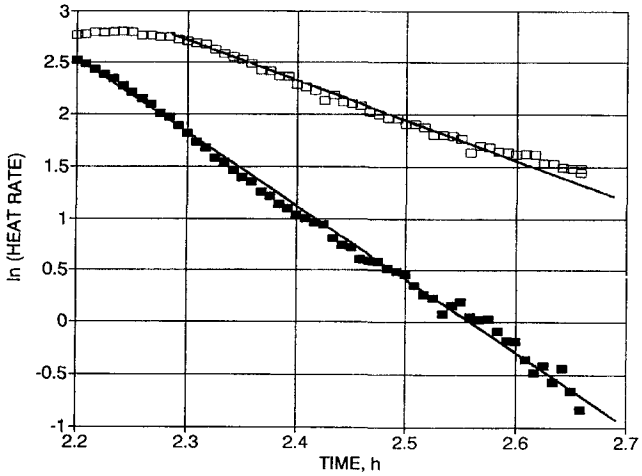

FIG. 7. Kinetics of decay of high-pressure activation after pressure release. □, rates at 20°C; ■, rates at 20°C. This experiment first measured metabolic heat rates of the tissues at 1 atm and then pressure was increased and activity remeasured. Finally, the decrease in metabolic rates was measured as pressure was returned to 1 atm. This measurement began at 2.2 hr after the start of this experiment.

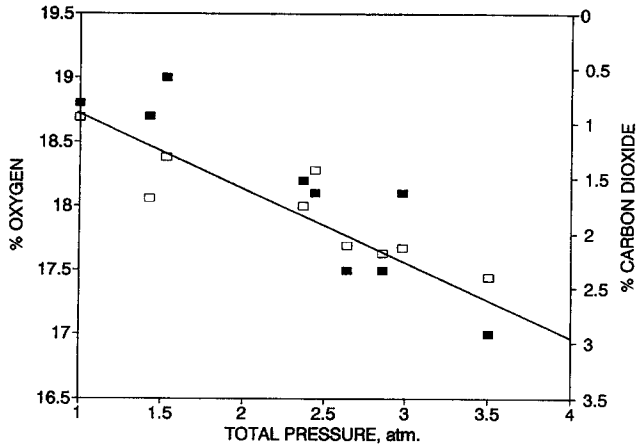

FIG. 8. O_2 (□) and CO_2 (■) in calorimeter ampules 2 hr after mussel gill tissue sections were sealed (under air) and pressurized to the indicated total pressure with N_2. The line fitting the points was obtained by regression analysis. Note CO_2 is plotted on the same scale as O_2 (but with increasing rather than decreasing partial pressure) to allow ready comparison of the rates.

does not change significantly with pressure. Both the rate of O_2 consumption and CO_2 production increased to ~160% of the initial 1 atm rate when the pressure was increased to 3.5 atm.

Mouse brain and lung tissues were also examined with increasing total pressure and Po_2. The effects of pressure were similar to those seen with mussel gill tissue samples but differed in the maximum percent increase in metabolic rates that could be obtined. The metabolic rate increases, as pressure on approximately equal weights of brain and lung tissue sections is increased by addition of air to the ampules, are shown in Fig. 9, A and B, respectively. Figure 9A shows that the rate of increase with brain tisue was generally proportional to the pressure (or the partial pressure of O_2, as the two were not separated in this experiment). Lung tissue has a lower specific heat rate than brain tissue and is not stimulated to the same extent by the combination of increased pressure and pressure of O_2. An increase to 120–125% of control was the maximum level reached. As with the mussel gill tissues, pressure-dependent increases in metabolic rates were notable at lower total pressure when O_2 rather than air or N_2 was used as the pressurizing gas.

DISCUSSION

The results clearly show that both increased Po_2 and total pressure can cause an increase in the metabolic rate of mussel gill and mouse brain tissues. Increased metabolic heat rate can result from an increase in the rate of metabolism with no changes in metabolic pathways or the ratios of fluxes through the various pathways, from alterations in cell metabolism that favor pathways such as oxidative reactions with increased levels of heat production, from loss of energy coupling efficienty through futile cycles or membrane leakage and from an increase of oxidative reactions that are not normally major contributors to cell metabolism.

The last possiblity does not seem likely because of the observed saturation of the heat rate increase at high Po_2 or total pressure. Also, high levels of nonbiological oxidative reactions probably would not allow the observed return to original activity levels after pressure is returned to ambient.

Loss of energy coupling cannot quantitatively account for the increase in heat rate. Total uncoupling increases the enthalpy change for metabolism by only about 10% (5). A substrate or product change alone also cannot account for increased heat rates because the heat produced per O_2 for all organic compounds is essentially constant (8).

Thus, we are left with explanations based on alterations in the flux ratios of the metabolic reactions or pressure-induced enhanced respiratory rates. With regards to alteration in flux ratios, both the rate of oxygen uptake and the rate of CO_2 production increase with pressure. These appear to change in an essentially constant ratio (i.e., RQ remains nearly constant), suggesting that the pressure increases cause no major shifts in the relative average oxidation states of substrates or products or the balance of aerobic vs anaerobic pathways.

The premise of a general increase in respiratory rates appears to account for all our results. One means of increasing the rate of existing metabolism is to increase the concentration of a limiting substrate. The obvious candidate for such a substrate in these studies is O_2. Several observations are consistent with the notion that O_2 may be limiting and that increased O_2 solubility in the tissues at higher Po_2 may be one cause for the observed rate increase. First, the pressure required to increase heat production rate shows a clear inverse dependence on Po_2 (Fig. 3). Higher pressures are required to achieve the same increase when Po_2 is low. Second, the greater water solubility of O_2 at low temperatures could account for the enhanced pressure effects on rate increases at low temperature. Third, the experiments tracking the return to normal metabolic activity after pressure release show first-order kinetics, consistent with the depletion of a vital substrate from the tissue or an outgassing of dissolved oxygen. Fourth, the observation of a heat rate increase to a maximum value independent of Po_2 but with a pressure dependence strongly influenced by Po_2 favors a conclusion that low concentrations of dissolved O_2 can limit tissue metabolic rates but at higher concentrations O_2 becomes nonlimiting. These are all consistent with the best fit of the heat rate $Po_2 \cdot P_{Total}$ data using a saturation model (eq. 1) including a term for interaction between P_{Total} and Po_2.

Although oxygen solubility effects appear important to the enhanced heat production rates, an additional pressure effect is also evident. Pressure increases cause the activity to increase even when Po_2 is maintained constant. This

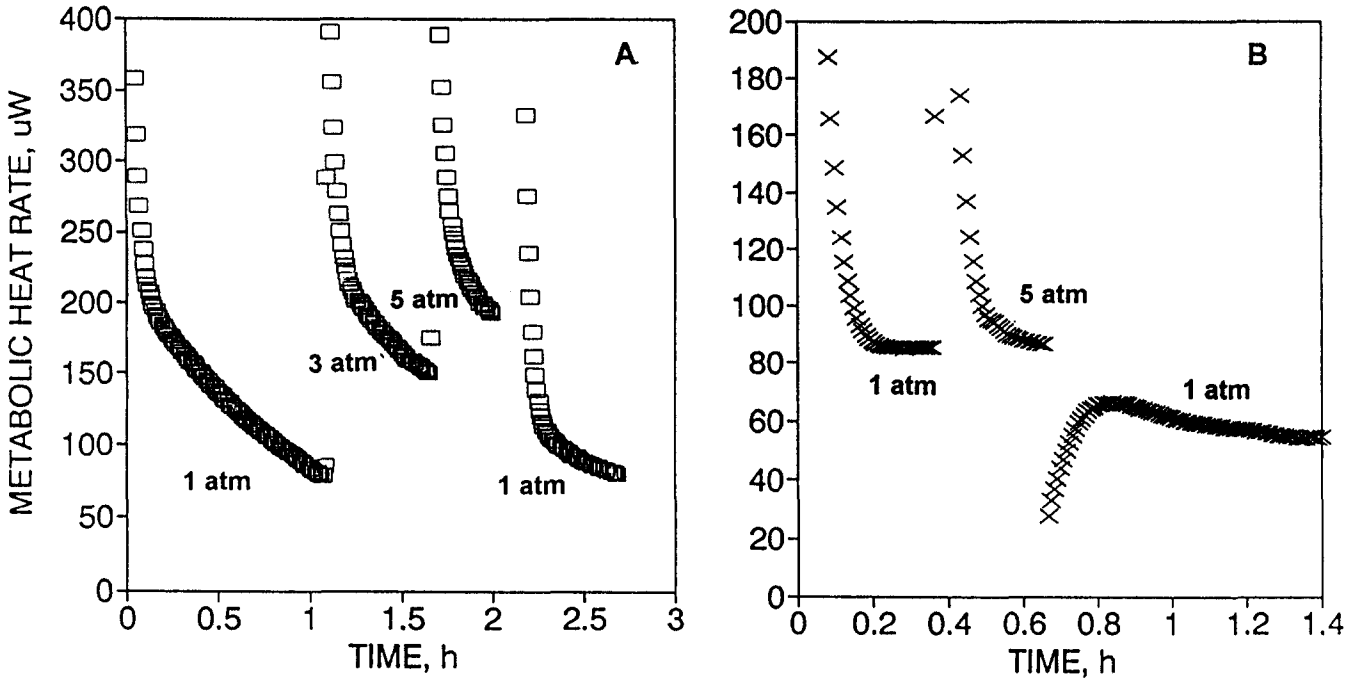

FIG. 9. Measurements of the effects of pressure on metabolic heat rates of mouse brain and lung tissues. (A) Mouse brain tissue sealed in an ampule with air and pressurized with air to 3 and 5 atm and then returned to 1 atm. (B) Mouse lung tissue in air, pressurized to 5 atm with air, and then returned to 1 atm.

must be a direct pressure effect on some component that influences the rate-limiting steps of metabolism. Pressure could influence activity by altering the structure of some rate-limiting component or of some essential complex structure in the cell to produce a more active form. Alternatively, pressure-induced changes could increase solubilities or alter partitioning of a rate-limiting component. Either of these possible mechanisms could give rise to the observed apparent saturation of the pressure effect. Nitrogen does not appear to play a chemical role in the increased metabolic rates as helium pressurization causes the same activity increase. Buildup of CO_2 also seems an unlikely factor to confound pressure effects, because activity increases were directly related to pressures but not to the total metabolic activity producing CO_2. In addition, reversibility of the pressure effects was independent of CO_2 buildup in the calorimeter ampules.

As indicated in Fig. 7, the kinetics of return to lower activity upon pressure reduction at 25°C was rapid and apparently first order. Lower temperatures enhance the solubility of O_2, increase the viscosity of cell interiors and slow the rate of gas dissolution and evolution. At 20°C, there is a marked lag between the time of pressure release and an observable decrease in metabolic activity. This may indicate a delayed decrease in the activity of some critical reaction or more likely indicates that the decrease in concentration of reactant only becomes rate limiting when its concentration is reduced below some value. Because outgassing of O_2 must begin immediately after lowering the pressure, the data of Fig. 7 suggest that O_2 may be dissolved at concentrations in excess of those required to maintain maximum velocity of oxidative reaction rates. O_2 concentration would then become kinetically limiting only after O_2 is depleted below some critical level by the rather slow (at 20°C) outgassing. At this time, metabolic rate then shows the previously observed first-order decrease in rate back to the rate at atmospheric pressure.

The pressure changes used here are very small when compared with the pressures commonly used in studies aimed at altering lipid or protein structures (16). The pressure effects causing metabolic activity changes must be more subtle than those involving a major volume change in such structures. Rather than causing a bulk structural change, small pressure increases could cause local structural alterations to give enhanced binding and/or changes in the relative solubilities of some rate-limiting molecules between lipid and aqueous phases. Additionally, small pressure changes could affect the distribution of molecules between conformations such as those in some flexible regions of proteins involved in substrate binding (11). For example, binding of O_2 to myoglobin is enhanced at high pressures, presumably due to a small change in conformation near the oxygen binding site on the heme. However, ΔV is only -3 cm^3/mol for myoglobin binding O_2 (10). Much higher pressures than those used here are required to significantly change the amount of bound O_2. Binding or local solubility changes of

this general nature but with relatively large ΔV values could, however, translate into significant local concentration and transport rate differences, even without major shifts in total concentrations. Our calorimetric methods appear to offer a general means for directly examining small pressure effects on biological reactions.

A large number of molecules regulating metabolic rates could be postulated to change their local concentrations in cells in response to pressure. O_2 could, for example, serve both as a pressure-dependent metabolic activator and as a substrate. If it is assumed that metabolic rate in the gill tissue was proportional to $[O_2]$ in tissues, then because $[O_2] = K\ Po_2$ by Henry's law, heat rate would increase as Po_2 increases. Increasing pressure with either O_2 or air would then increase observed rates. Still, with this model it is necessary to invoke an additional pressure-dependent change in solubility or binding of O_2 to account for our observations of increased metabolic activities when total pressure is increased while holding Po_2 constant. Kiesow (12) suggested that pressure may have significant effect on the hemoglobin–oxygen equilibrium in cells, although not in dilute hemoglobin solutions.

These observations on animal models may have important consequences if parallel changes in respiration with pressure occur in human tissues. The observed increased respiration rates caused by increasing both total pressure and Po_2 occur in pressure ranges commonly encountered by SCUBA divers (i.e., 1 to 5 atm) and could have a significant influence on metabolism. This becomes particularly important with the current increased use of enhanced oxygen mixtures for SCUBA diving. For example, a diver at 30 m using a 40%$_2$–60% N_2 gas mixture would be at 4 atm total pressure with a Po_2 of 1.6 atm. According to Figs. 2–5, this could cause a metabolic rate increase to near 150% of the control. A diver using air at this same depth would experience the same total pressure but a Po_2 of only 0.8 atm. This would increase metabolic rate to about 120% of control according to Fig. 3. Such pressure-related differences in metabolic rate may have undesirable consequences at the higher O_2 pressures. For example, there may be changed total metabolite consumption during a dive, changed rates of heat generation by the diver and possibly also changes in the levels of certain metabolic products such as lactate that serve as regulators of metabolic activity. All of these could have significant physiological effects on individuals and deserve futher study.

This research was supported in part by Dole Food Co. and The Agricultural Experiment Station, Cook College, Rutgers University.

References

1. Bennett, P.B.; Elliott, D.H. The physiology of medicine and diving. New York: Best Publishing; 1982.
2. Bennett, P.B.; Marquis, R.E. Basic and applied high pressure biology. Rochester, NY: University of Rochester Press; 1993.
3. Brauer, R.W.; Hogan, P.M.; Hugon, M.; McDonald, A.G.; Miller, K.W. Patterns of interactions of the effects of the light metabolically inert gases with those of hydrostatic pressure as such—a review. Undersea Biomed. Res. 9:353–395;1982.
4. Brauer, R.W.; Jordan, M.R.; Miller, C.G.; Johnson, E.D.; Dutcher, J.A.; Sheehan, M.E. Interaction of temperature and pressure in intact animals. In: Pequeaux, A.J.R.; Giles, R., (eds). High pressure effects. Berlin: Springer-Verlag. 1985:5–26.
5. Crabtree, B.; Taylor, D.J. Thermodynamics and metabolism. In: Jones, N.M., (ed). Biochemical thermodynamics. New York: Elsevier Scientific Publishing Co. 1979:333–378.
6. Criddle, R.S.; Breidenback, R.W.; Fontana, A.J.; Hansen, L.D. Reaction rates as a function of pressure, temperature, and concentration by heat conduction, differential scanning calorimetry. Thermochim. Acta 216:147–155;1993.
7. Criddle, R.S.; Fontana, A.J.; Rank, D.R.; Paige, D.; Hansen, L.D.; Breidenbach, R.W. Simultaneous measurement of metabolic heat rate, CO_2 production and O_2 consumption by microcalorimetry. Anal. Biochem. 194:413–417;1991.
8. Erickson, L.E. Energy requirements in biological systems. In: James, A.M., (ed). Thermal and energetic studies of cellular biological systems. Bristol: IOP publishing Ltd. 1987: 14–33.
9. Gessner, F. Hydrostratischer Druck und Pflanzenwachstum. In: Ruhland, W., (ed). Encyclopedia of plant physiology, Vol. XVI. Berlin: Springer-Verlag. 1961:668–690.
10. Hasinoff, B.B. Kinetic activation volumes of the binding of oxygen and carbon monoxide to hemoglobin and myoglobin studied on a high pressure laser flash photolysis apparatus. Biochemistry 13:3111–3117;1974.
11. Heremans, K. Pressure effects on phospholipids and proteins. In: Pequeaux, A.J.R.; Giles, R., (eds). High pressure effects. Berlin: Springer-Verlag. 1985:69–89.
12. Kiesow, L.A. Hyperbaric inert gases and the hemoglobin-oxygen equilibrium in red blood cells. Undersea Biomed. Res. I, 29–43;1974.
13. Lanphier, E.H.; Camporesi, E.M. Respiration and exercise. In: Bennett, P.B.; Elliott, D.H., (eds). The physiology and medicine of diving. London. Billiere Tindall. 1982:99–156.
14. Schlieper, C., High pressure effects on marine invertebrates and fishes. Marine Biol. 2:5–12;1968.
15. Schlieper, C. Comparative investigations on the pressure tolerance of marine invertebrates and fish. In: Symposia of the Society for Experimental Biology, No. XXVI, The effects of pressure on organisms. New York: Academic Press Inc. 1972: 197–207.
16. Suzuki, K.; Taniguchi, Y. Effect of pressure on biopolymers. In: Symposia of the Society for Experimental Biology, No. XXVI, The effects of pressure on organisms. New York: Academic Press Inc. 1972:103–124.